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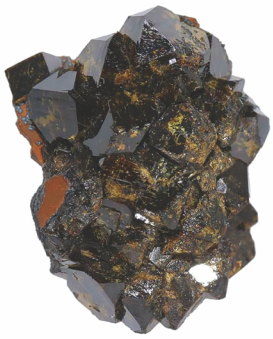

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Technical editor D. Nuzhin

Address:
220049, Knorin str., 17, Minsk
Phones: +375 17 322-16-77, 322-16-78
e-mail: lab@recipe.by

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Director N. Rabkova

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rural settlement Katynskoye, Avtoremzavod village, 1A, office 413
Phone: +7 4812 515923
e-mail: lab@recipe.by

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Ahmed A. Salim[1]✉, Ihsan AlSaimary[1], Amal A.K. Alsudany[2]
[1] College of Medicine, University of Basrah, Basrah, Iraq
[2] Basrah Health Directorate, Ministry of Health, Basrah, Iraq

CCL2, CCL5, and CXCL10 Chemokines Roles in Neuroinflammation Status of Subjects with Multiple Sclerosis

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Contacts: Ahmedsalihdr2008@yahoo.com

Abstract

Introduction. Multiple sclerosis (MS) is one of chronic autoimmune and neurological illness of the central nervous system (CNS). Young age peoples are the most common age grope of the onset of MS.
Purpose. To estimate the immunity and pathogenesis of MS relationship with chemokines (CCL2, CCL5, and CXCL10) in sera of patients with MS.
Materials and methods. This is case-control study, was conducted in Multiple Sclerosis Center in Basrah city at period from November 2021 to May 2022. All investigations of the measurement of CCL2, CCL5 and CXCL10 levels were done by using ELISA.
Results. The age of subjects ranged between 15–55 years. There was a predominant female gender (72.10%). The concentration of CCL2, CCL5 and CXCL10 chemokines showed greater levels among MS patients when compared with control group (P<0.0001).
Conclusion. Young age groups and female gender are prevalent in MS. The concentration of chemokines (CCL2, CCL5 and CXCL10) are at higher levels in multiple sclerosis. There is a positive association between CCL5 chemokine and age groups of normal persons.
Keywords: multiple sclerosis, CCL2, CCL5, CXCL10, immunity, pathogenesis, chemokines

■ INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune, inflammatory neurological disease of the central nervous system (CNS), disease onset is usually between 20–40 years of age [1]. MS is one of the most common non-traumatic causes of disability in young adults [2]. Infiltrating auto-reactive immune cells, in synergy with resident glial cells, will cause neuroinflammation and neurodegeneration, as characterized by demyelination, axonal loss, and finally irreversible damage to the central nervous system (CNS) [3]. MS was first

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described in 1868 by French neurologist Jean-Martin Charcot. Statistics all around the world show that women are affected with MS more than men, and generally, 2–3 million people worldwide are suffering from this disease. This disease like the other multifactorial diseases is influenced by genetic and environmental factors [4].

Despite the rapid progression in MS research over the decades, the underlying pathogenesis of the disease is still not well understood [5].

The diagnosis of MS depended of the 2017 revised McDonald Criteria with the establishment of dissemination of the relapses in space and time confirmed either clinically or radiologically and exclusion of MS mimics [6].

As a chronic autoimmune inflammatory disease, MS is especially characterized by demyelinating and neurodegeneration. A current consensus is that the infiltration, accumulation, and activation of myelin-specific T-lymphocytes and macrophages in the central nervous system are vital aspects of MS pathology [7].

No studies about chemokines of Multiple Sclerosis in Iraq, so the present study is found necessary as a first study to determine chemokines among patients with MS in Iraq – generally and in Basrah – especially.

■ PURPOSE OF THE STUDY

To estimate the immunity and pathogenesis of MS relationship with chemokines (CCL2, CCL5, and CXCL10) in sera of patients with MS.

■ MATERIALS AND METHODS

All the kits that used in the current study are Human C-C motif chemokine 2 ELISA Kit (MyBioSource test, ELISA; Catalog No: MBS2883889, USA), Human C-C motif chemokine 5, ELISA Kit (MyBioSource test, ELISA, Catalog No: MBS2886866, USA) and Human CXC-chemokine ligand 10 ELISA Kit (MyBioSource test, ELISA, Catalog No: MBS167142, USA)

Patients samples

A cases-control study was designed to collect blood sample from patients with multiple sclerosis from MS Center in Basrah Teaching hospital during November 2021 to May 2022. All patient's information which involved in the study were recorded in questionnaire. Both patients and control samples that investigated in this study have age ranged between 15 to 60 year. Most of MS patients suffering from fatigue, weakness, decrease in visual acute, urine incontinence, numbness of the extremities and ataxia. All cases were diagnosing and approve by MS committee of Basrah MS center.

Blood samples

Five-ml of venous blood was taken from 172 participants (86 for patients and 86 for control), 3ml collected in gel tube which subjected to centrifugation for 15 minutes at 1000x g for obtaining serum that used in immunological study (ELISA) in CCl2 and CCl5 chemokines while in CXCl10 centrifuge at 2000 RPM for 20 minutes, both sample were kept at –20 °C for preservation prior using.

Control group

A total of 86 individuals without any autoimmune disease, infectious disease or other disease they were regarded as control group.

Exclusion Criteria

All other disease that can produce clinical picture similar to MS like (Autoimmune disease and infectious disease).

Inclusion criteria

Any patient with signs and symptoms compatible with MS who met the 2017 Revised McDonald Criteria and approved by the MS committee at Basrah Teaching Hospital.

Demographical information

There were several demographical parameters have been taken in consideration to be involved in this study which includes: age, sex, marital status, occupation status, residency, smoking, obesity, BMI and family history.

Clinical examination

Clinical Symptoms was divided into essential symptomatic (fatigue, weakness, decrease in visual acute, urine incontinence, numbness of the extremities and ataxia with other symptoms (amnesia, sphincter disturbance, facial and oral paresthesia, speech difficulty and dysphagia).

Laboratory Investigation

All the following parameters were taken from patient report directly:

Biochemical parameters: (Glucose, Urea, Creatinine, Calcium, Triglyceride). 2 – Hormonal parameters: (Thyroid Stimulating Hormone (TSH), Triiodothyronine Test (T3), Thyroxine Test (T4)).

A – Hematology parameters: (Erythrocyte Sedimentation Rate (ESR), White Blood Cell (WBC), Red Blood Cell (RBC), Hemoglobin Test (Hb), Platelets Test (PLT)).

B – Differential WBC: (Neutrophils (NEU), Lymphocytes (LYM), Monocytes (MON), Eosinophils (EOS), Basophils (BAS)).

Other serological parameters: (Antinuclear Antibody Test(ANA), Anti-double stranded DNA (anti-dsDNA), Anti-phospholipid IgG, Anti-cardiolipin IgG, Rheumatoid factor (RH factor), C-Reactive Protein (CRP) Test, Venereal disease research laboratory test(VDRL), Brucella test.

Magnetic Resonance Imaging examination

Conventional magnetic resonance imagings of the brain with spinal cord have been done for the patients. The examination includes the following: – 1 – Axial T1 Weighted imaging. Axial T2 Weighted imaging Coronal or Axial FLAIR "Fluid attenuated inversion recovery" sequence for Free fluid suppression (e.g. CSF fluid suppression). Post-Contrast T1 Weighted images to demonstrate active lesions called enhanced, i.e., to detect new lesions not detected by native scans using a gadolinium-DTPA (diethylene triamine penta cetate) contrast agent, the dose was given through slow Intravenous route while the patient is still inside the MR Tunnel.

Scans were analyzed by specialist neurologists, for the number and site of hyperintense lesions at T2-weighted images and those of the enhancing lesions at T1-weighted images.

Serological Detection of CCL2, CCL5 and CXCL10 Chemokines by ELISA Technique

Sandwich ELISA test was used for detection the titer of CCL2, CCL5 and CXCL10 in serum samples for both patients and controls. By using MyBioSource ELISA kit composed of 96 well microplates. The procedure of this test was done according to kit manufacture instructions.

Statistical analysis

Statistical analysis was carried out by using SPSS (VER.23) and student's Fisher, Mann – Whitney, Kruskal – Wallis, chi-square, spearman's correlation, regression line and ROC curve was applied to find out the statistical differences between all variables. probability less than 0.05 is significant (P<0.05).

■ RESULTS

Figure 1 documented that most MS cases were recorded among females 62 (72.10%) vs. 24 (27.90%) for males (p=0.5).

Table 1 the distribution of age within MS patients showed that high number were within Group (thirty or younger) years counting 32 (37.2%) patients, while the lowest number of patients were found in group (fifty-one or old) years counting 6 (7.0%) patients from total study patients 86 (100.0%), Statistically the P-value=0.96 and differences were non-significant.

Sociological characteristics of patients and controls distribution

Table 2 distribution sociological characteristics of patients and controls, shows the documented that most MS cases were recorded among patients their occupation 29 (33.7%) worker versus 57 (66.3%) non worker, while residence in urban 40 (46.5%) versus 46 (53.5%) in rural, followed by marital status of patients 16 (18.6%) single versus 70 (81.4%) married, smoking 16 (18.6%) versus 70 (81.4%) nonsmoking, and obesity 8 (9.3%) versus 78 (90.7%) non obesity, the only variable which had significant statistical difference, when its distribution was compared among patients and controls was the occupation.

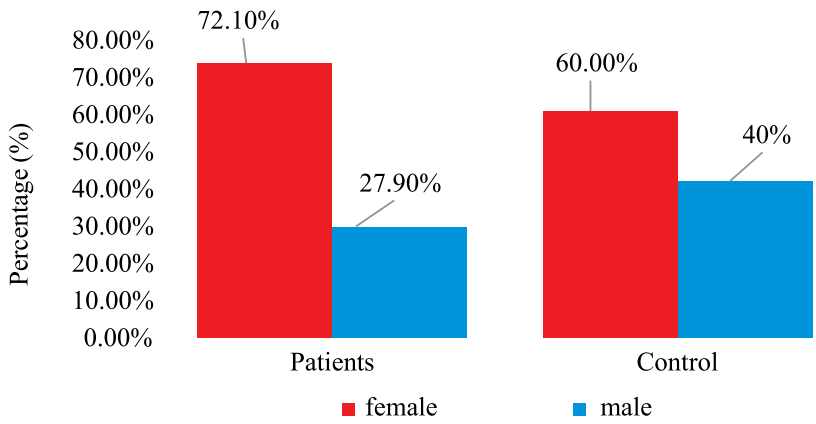

Fig. 1. Distribution of studied groups according to sex

Table 1
The distribution of age and sex in study groups

Sex	Age groups (years)	Category		Total	P-value*
		Case	Control		
Male	≤30	9	9	18	1.00
		37.5%	26.0%	31.0%	
	31–40	8	16	24	
		33.3%	48.0%	41.4%	
	41–50	5	9	14	
		20.8%	26.0%	24.1%	
	≥51	2	0	2	
		8.3%	0.0%	3.5%	
Female	≤30	23	17	40	0.73
		37.1%	33.0%	35.1%	
	31–40	17	9	26	
		27.4%	17.0%	22.8%	
	41–50	18	17	35	
		29.0%	33.0%	30.7%	
	≥51	4	9	13	
		6.5%	17.0%	11.4%	

Note: * Fisher's Exact Test.

Table 2
Sociological characteristics of patients and controls

Characteristic	Category		Total	P-value*
	Patient	Control		
Occupation				
Workers	29	77	106	0.001 odd ratio = 0.057
	33.7%	89.5%	61.6%	
Non-workers	57	9	66	
	66.3%	10.5%	38.4%	
Residence				
Urban	40	26	66	0.50
	46.5%	30.2%	38.4%	
Rural	46	60	106	
	53.5%	69.8%	61.6%	
Marital status				
Single	16	26	42	0.41
	18.6%	30.2%	24.4%	
Married	70	60	130	
	81.4%	69.8%	75.6%	
Smoking				
Yes	16	0	16	0.21
	18.6%	0.0%	9.3%	
No	70	86	156	
	81.4%	100.0%	90.7%	
Obesity				
Yes	8	0	8	0.59
	9.3%	0.0%	4.7%	
No	78	86	164	
	90.7%	100.0%	95.3%	

Note: * Fisher's Exact Test.

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Family history status

Table 3 documented that the number of cases which attacked with MS were only two (2.3%) within family history of MS from total study patients 86 (100.0%). Statistically the (P-value=0.48) and differences were non-significant.

Table 3
Comparison of the presence of MS family history among males and females

Family history of MS	Sex		Total	P-value*
	Male	Female		
Yes	1	1	2	0.48
	4.2%	1.6%	2.3%	
No	23	61	84	
	95.8%	98.4%	97.7%	

Note: * Fisher's Exact Test.

Table 4 the distribution of EDSS within MS patients showed that high number were within Group (5–8) counting 26 (30.2%) patients, while the lowest number of patients were found in group (0) counting 5 (5.8%) patients from total study patients 86 (100.0%), Statistically the (P-value=0.33) and differences were non – significant.

Table 4
Comparison of the grades of the classification system EDSS among males and females

Classification system EDSS	Sex		Total	P-value*
	Male	Female		
0	3	2	5	0.33
	12.5%	3.2%	5.8%	
1–1.5	4	12	16	
	16.7%	19.4%	18.6%	
2–2.5	2	11	13	
	8.3%	17.7%	15.1%	
3–4.5	3	14	17	
	12.5%	22.6%	19.8%	
5–8	10	16	26	
	41.7%	25.8%	30.2%	
8.5–9.5	2	7	9	
	8.3%	11.3%	10.5%	

Note: * Fisher's Exact Test.

Table 5 the distribution type of therapy within MS patients showed that high number were within Beta Interferone group counting 48 (55.8%) patients, followed by Finogolimod group counting 14 (16.3%), and no treatment group counting 14 (16.3%), then natalizumab group counting 7 (8.1%), while the lowest number of patients were found in MP group counting 3 (3.5%) patients from total study patients 86 (100.0%), Statistically the (P-value=0.8) and differences were non-significant.

Table 6 shown that the mean concentration of The chemokines was higher among MS patients (86), mean concentration values of CCL2 in MS patients were 12.69±14.52 Pg/ml, while in CCL5 1368.81±665.21 Pg/ml, and CXCL10 the mean concentration values in MS

Table 5
Comparison of the type of therapy used among males and females

Type of therapy	Sex		Total	P-value*
	Male	Female		
No treatment	3	11	14	0.8
	12.5%	17.7%	16.3%	
Finogolimod	3	11	14	
	12.5%	17.7%	16.3%	
Beta Interferone	16	32	48	
	66.7%	51.6%	55.8%	
Natalizumab	1	6	7	
	4.2%	9.7%	8.1%	
MP	1	2	3	
	4.2%	3.2%	3.5%	
Total	24	62	86	
	100.0%	100.0%	100.0%	

Note: * Fisher's Exact Test, ** MP – Methylprednisolone.

Table 6
Comparison of the chemokines serum levels in patients and controls

Variable	Patient	Control	P-value*
	Mean±SD	Mean±SD	
ccl2, Pg/ml	12.69±14.52	0.79±0.75	0.004
ccl5, Pg/ml	1367.81±665.21	15.33±4.76	0.0001
cxcl10, ng/L	64.71±93.45	4.36±3.80	0.0001

Note: * Mann – Whitney-U Test.

patients were 64.71±93.45 ng/L, statistically these differences were highly significant with P=0.0001.

Concentration of chemokines CCL2, CCL5 and CXCL10 according to various demographical factor

Table 7 documented that the mean levels of CCL2 (Pg/ml) among males and females of MS patients (8.75±11.45) (14.67±15.73), was higher than males and females of control group (1.23±0.87) (0.51±0.57) respectively, the mean levels of CCL5 (Pg/ml) among male and female of MS patients (1210.44±609.83) (1446.49±692.60), was higher than male and female of control group (14.52±4.76) (15.87±5.12) respectively, the mean levels of CXCL10 (ng/L) among males and females of MS patients (98.54±159.44) (47.79±19.59), was higher than male and female of control group (4.61±4.61) (4.19±3.63) respectively, in patient and control there are no significant statistical differences in the levels of chemokines when compared between males and females. As in other demographical factor showed the mean levels of chemokines of patients were higher than control group and no any significant difference in levels of chemokines in patients and controls when compared according to any various demographical factor.

Table 7
Concentration of chemokines CCL2, CCL5 and CXCL10 according to various demographical factor

			Chemokines Concentration					
			Patient			Control		
			ccl2	ccl5	cxcl10	ccl2	ccl5	cxcl10
			Pg/ml	Pg/ml	ng/L	Pg/ml	Pg/ml	ng/L
Sex	Male	Mean	8.75	1210.44	98.54	1.23	14.52	4.61
		SD	11.45	609.83	159.44	0.87	4.76	4.61
	Female	Mean	14.67	1446.49	47.79	0.51	15.87	4.19
		SD	15.73	692.60	19.59	0.57	5.12	3.63
	P-value		0.48	0.37	0.73	0.09	0.52	0.83
Residency	Centrally	Mean	12.44	1307.14	84.22	0.74	13.95	6.21
		SD	14.63	694.01	125.49	0.15	5.03	4.57
	Peripherally	Mean	12.99	1437.14	42.41	0.82	15.92	3.57
		SD	14.94	649.36	17.49	0.92	4.91	3.51
	P-value		0.83	0.94	0.11	0.82	0.73	0.31
Occupation	Worker	Mean	15.26	1352.49	46.95	0.88	15.64	3.98
		SD	15.75	781.42	19.76	0.75	4.94	3.82
	Non-worker	Mean	10.73	1379.53	78.29	0.07	12.51	7.82
		SD	13.66	586.29	122.81	0.03	2.93	9.02
	P-value		0.39	0.97	0.57	0.22	0.86	0.60
Marital status	Single	Mean	7.01	1222.58	97.04	0.91	15.04	4.93
		SD	11.38	708.83	151.07	0.51	7.39	3.64
	Married	Mean	15.99	1451.89	45.99	0.75	15.45	4.19
		SD	15.38	642.99	19.53	0.87	3.96	4.12
	P-value		0.06	0.38	0.36	0.73	0.73	0.91
** Smoking	Yes	Mean	4.84	1183.60	58.35			
		SD	6.49	540.98	20.91			
	No	Mean	14.26	1404.65	65.98	ND		
		SD	15.24	690.97	102.32			
	P-value		0.25	0.50	0.25			
** Obesity	Yes	Mean	.90	326.00	25.55			
		SD	0.3	126	6.1			
	No	Mean	13.10	1403.73	66.06	ND		
		SD	14.60	646.69	94.80			
	P-value		0.56	0.15	0.18			

Notes: * Mann – Whitney-U Test; ** In this study, non-smoking and non-obesity controls were selected so no statistic was done
ND – not determined.

Comparison of the chemokines levels according to EDSS classification system of patients

documented that the mean levels of CCL2 (Pg/ml) and CCL5 (Pg/ml) were high in group (3–4.5) patients counting (24.99) (1603.00) respectively and high mean levels of CXCL10 within group (5–8) patients counting (83.85), while lowest mean levels of CCL2 in group (8.5–9.5) counting (0.52) and lowest mean levels of CCL5 and CXCL10 within group (2–2.5) counting (1090.84) (35.14) respectively. No any significant statistical difference in the levels of chemokines when compared according to the EDSS classification system. As showed in figures 2–8 respectively.

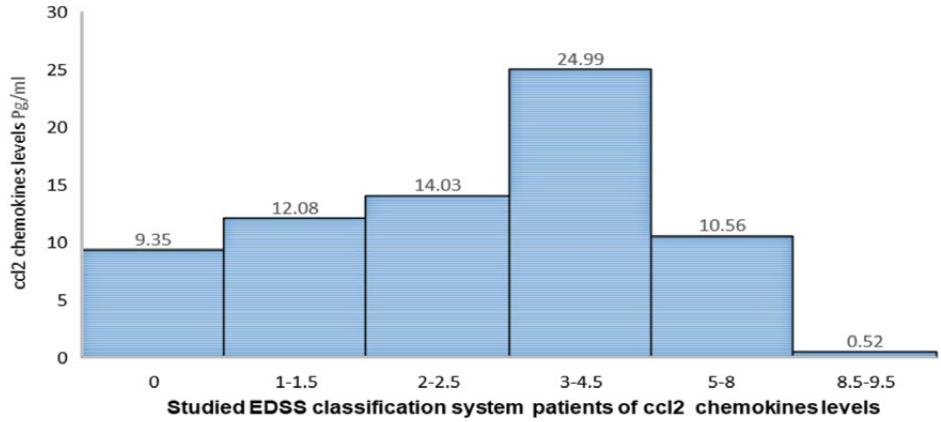


Fig. 2. Comparison of the CCL2 chemokines levels according to EDSS classification system of patients

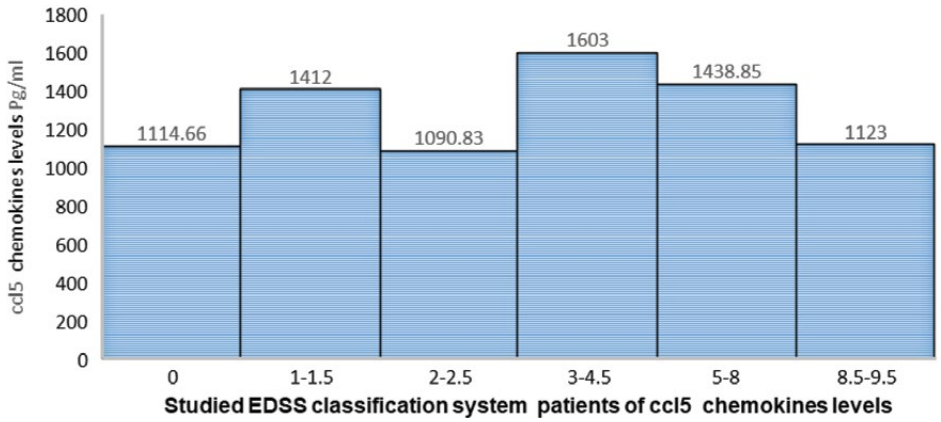


Fig. 3. Comparison of the CCL5 chemokines levels according to EDSS classification system of patients

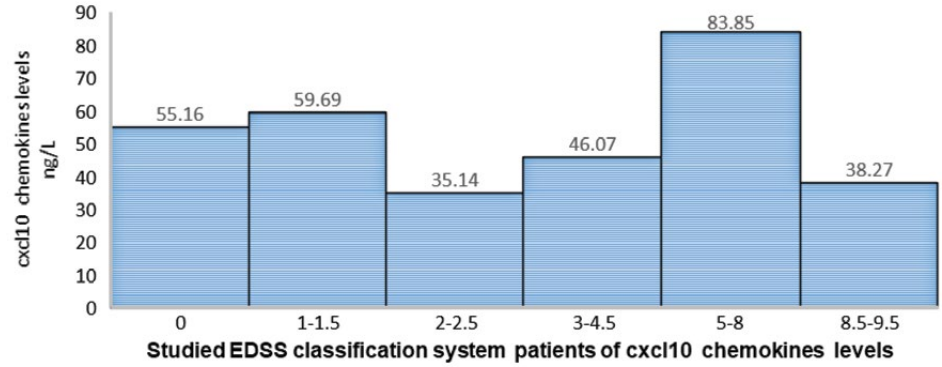


Fig. 4. Comparison of the CXCL10 chemokines levels according to EDSS classification system of patients

P value ccl2: .299, P value ccl5: .920, P value cxcl10: .633

Note: * Kruskal – Wallis Test.

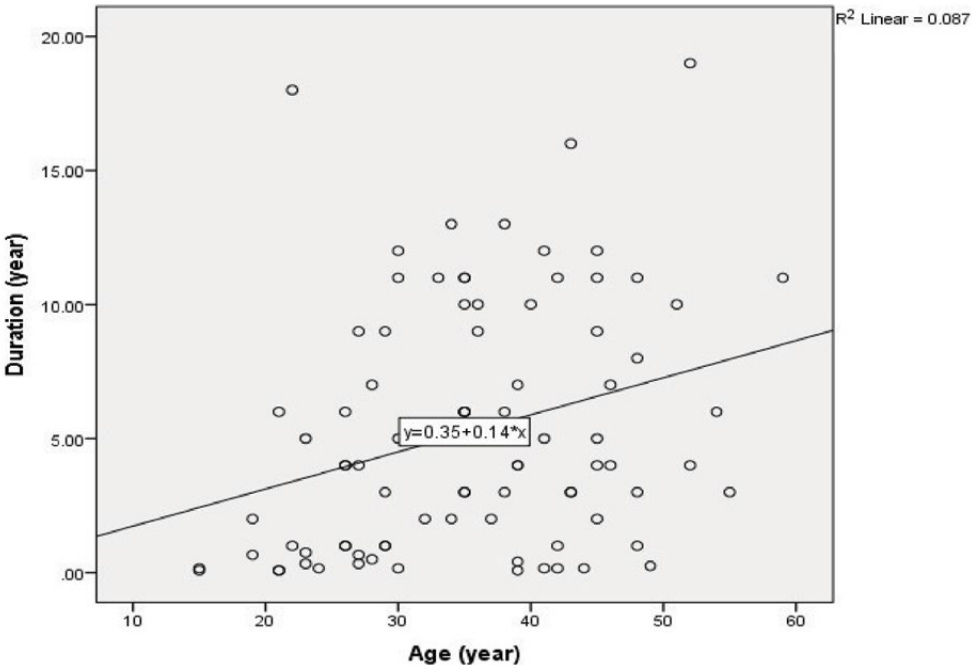
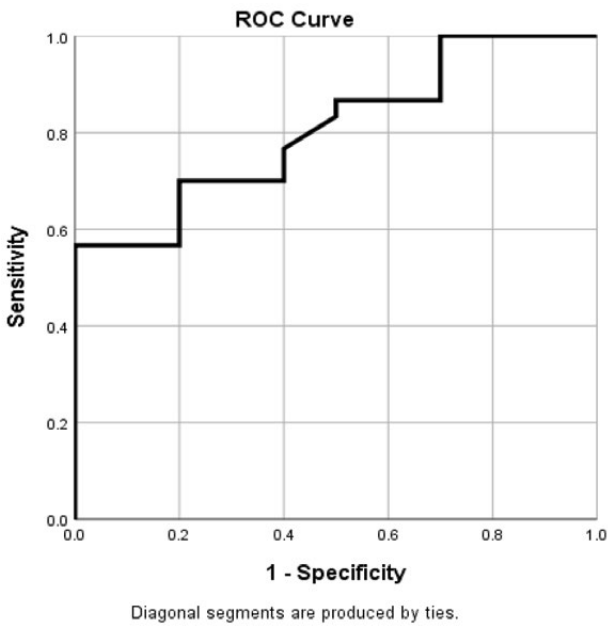


Fig. 5. Simple linear regression line of the correlation between age of the patients and duration of disease



ccl2 Area Under the Curve = 80.7%

Fig. 6. ROC Curve OF CCL2

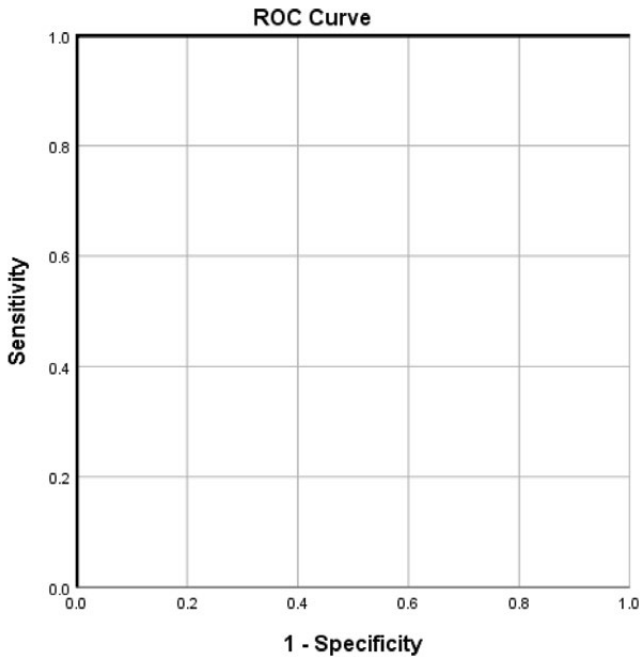


Fig. 7. ROC Curve OF CCL5

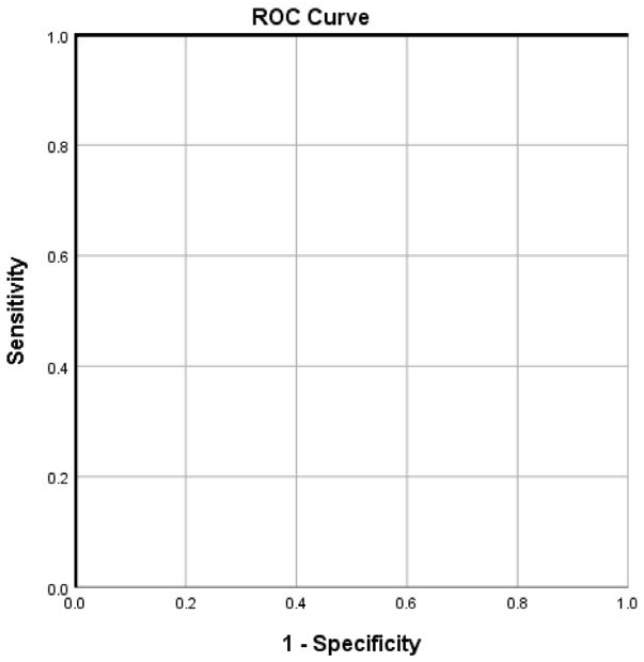


Fig. 8. ROC Curve OF CXCL10

DISCUSSION

In this investigation, the highest age group of patients with MS was (thirty or younger) year`s subgroups (37.2%) of total patients, which shows that this disease is mainly affecting young age group. while lowest cases of MS appeared at the age (fifty-one or older) were (7.0%) from total study cases. Similar finding was reported by Al-Araji and Mohammed [8] and Al-Shamssie [9] in their studies on Iraqi MS patients. Moreover, these findings are in agreement with nearby countries` studies, like from Lebanese, Kuwaiti, Jordanian, Libyan, Saudi, Palestinian, Omani and Iranian populations [10–12] as well as from other countries [13, 14]. Thus, the predisposing factor of first development of this disease has a peak between the third and fourth decades.

The current study of MS patients demonstrated increasing in the female to male ratio, which came in agreement with previous studies [15, 16]. In this study, the female to male ratio was 2.6:1, since 0.72% of the patients were females and 0.28% were males. These findings are agreed with the previous studies [17–19]. And that was in nearly agreement with Iraqi studies [20]. This female predominance which is a phenome – non shared with several other autoimmune diseases [18], may arises from the interaction between the gonadal hormones, genetic differences, immune system as well as different environmental exposures and modern lifestyle in men and women [21].

In this study most cases of MS recorded among non-worker groups 57 (66.3%) versus 29 (33.7%) for worker group according to results. which came in agreement with studies [22], where shown the people with multiple sclerosis suffer from an increased unemployment during the course of the disease. And other study [23] was the clear difference in employment rate in the various subtypes of MS. Patients with relapsing-remitting MS(RRMS) had higher employment rate than patients with secondary (SPMS) and primary progressive (PPMS). While control in this study worker 77 (89.5%) versus 9 (10.5%) non worker group, statistically the differences was significant, P-value (0.001) and ODD Ratio (0.057).

This investigation most number of cases which attacked with MS were residence in Basrah urban 40 (46.5%) versus 46 (53.5%) in Basrah rural, statistically the differences were non-significant compared with control group.

In this study documented that most MS cases were recorded among patients their marital status of patients 16(18.6%) single versus 70 (81.4%) married, statistically the differences were non-significant compared with control group. The result of this study is nearly identical with the Iraqi study of [24], where shown single patients (20%) versus (80%) married of MS but it was high significance differences compared with control group. In other study of Harrison et al, [25] in which it was shown married of MS higher percentage versus non married patients of MS, and was shown results for men and women combined, marital status was related to a person's acceptance of disability. Those who remained married had a higher acceptance of disability compared to those not consistently married; and all acceptance scores increased over time. At no time did the acceptance scores of the not consistently married reach the level of those who were consistently married. There was not, however, an interaction between marital status and acceptance. This indicates that being consistently married is associated with a higher level of acceptance over time.

In this study 16 (18.6%) of cases are smokers versus 70(81.4%) nonsmokers from total patients 86 (100.0%). Statistically the differences were non-significant compared with

control group. Makkawi et al. [26] and Healy et al. [27] not agree with this study by shown that Cigarette smokers are at higher risk of developing MS compared with No-smokers.

In this study 8 (9.3%) of cases are obesity versus 78 (90.7%) non obesity from total patients 86 (100.0%). Statistically the differences were non-significant compared with control group. Not agree with several studies have shown an increased risk of developing MS if you are obese [28, 29].

This indicates that smoking and obesity are predisposing factors and may increase the incidence of affected with MS, but they are not the main cause of multiple sclerosis, as there is a large proportion of people with multiple sclerosis who are not obese.

Other predisposing factor was family history status that affected with MS, in this study 2 (2.3%) of cases are presence of MS family history versus 84(97.7%) non presence of MS family history from total patients 86(100.0%). Statistically the differences were non-significant compared with male and female patients group. In other studies, Al-Wutayd et al. [30] was shown the MS risk was 5.8 times greater when there was family history of MS. One studies in Saudi Arabia that reported approximately 20% of MS patients have a family history of MS [31]. Two additional studies conducted in Kuwait reported that a larger number of patients had a family history of MS compared to controls [32, 33].

In this investigation most number of cases which attacked with MS were EDSS within Group (5–8) counting 26 (30.2%) patients, followed by Group (3–4.5) counting 17 (19.8%) patients, Group (1–1.5) counting 16 (18.6%) patients, Group (2–2.5) counting 13 (15.1%) patients and Group (8.5–9.5) counting 9 (10.5%) patients, while the lowest number of patients were found in group (0) counting 5 (5.8%) patients from total study patients 86 (100.0%). Statistically were non-significant compared with male and female patients group, nearly agree with other study [34] was shown highly in EDSS 4,0–6,0 and it was found in this study [35] with no significant difference was observed in EDSS for sex.

In this study the distribution type of MS within patients showed that high number were within RR type counting 77 (90.6%) patients, followed by SP type counting 9 (9.4%) patients from total study patients 86 (100.0%), Statistically the (P-value=0.72) and differences were non- significant compared with male and female patients group. This shows that Relapsing-Remitting type is the most common type of multiple sclerosis, which came in agreement with previous studies [36–38].

Recording to investigation in this study type of therapy within MS patients showed that high number were within Beta Interferone group counting 48(55.8%) patients, followed by Finogolimod group counting 14(16.3%), and no treatment group counting 14(16.3%), then natalizumab group counting 7(8.1%), while the lowest number of patients were found in MP group counting 3 (3.5%) patients from total study patients 86 (100.0%), Statistically the (P=0.8) and differences were non-significant compared with male and female patients group. Nearly agree with Iraqi study [20] showed that 84% (42 of 50) of MS patients were on Betaferon (Interferon beta-1b) subcutaneous injection and other MS patients 16% (8 of 50) were without treatment.

Several studies on chemokines in MS have been performed in recent years, and a subset of chemokines has been implicated in its pathogenesis. This hypothesis seems obvious as inflammation appears to be relevant in MS and chemokines are crucial for leukocyte recruitment into inflammatory sites [39, 40].

In this investigation was found the mean concentration of the CCL2 chemokine was higher among MS patients compared with control, mean concentration values of CCL2

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in MS patients were 12.69±14.52 Pg/ml. Statistically this difference was highly significant with (P=0.0001). That disagree with previous studied [40, 41] were showed CCL2 decrease in MS patients but increase after therapy compared with control.

Also no any significant difference in levels of CCL2 chemokines in patients with smoking, obesity and EDSS scale. That disagree with Yao et al., study [42] was showed found highly significant difference between concentration of CCL2 chemokines with smoking and obese.

In this study the mean levels of CCL2 (Pg/ml) was high in type RR counting (10.35) and No any significant statistical difference in the levels of CCL2 chemokines when compared according to type of MS. That agree with Bartosik-Psujek & Stelmasiak [41].

In this investigation that the mean levels of CCL2 (Pg/ml) was high in no treatment patients counting (23.47), while lowest mean levels of CCL2 in (MP) group counting (0.79). That agree with [41] study. But disagree with Szczuciński et al, [43] study was showed CCL2 decrease in no treatment MS patients but increase after therapy with MP. No any significant statistical difference in the levels of chemokines when compared according to type of therapy patients.

In this study No any significant statistical difference in the levels of CCL2 chemokines when compared according to clinical symptoms patients. That agree with Guerrero et al, [44] study.

CCL5 have role in pathogenesis of MS and may induce the recruitment of inflammatory cells in acute-stage MS [45]. In this investigation was found the mean concentration of the CCL5 chemokines was higher among MS patients compared with control, mean concentration values of CCL5 in MS patients were 1368.81±665.21 Pg/ml, statistically these differences were highly significant with (P=0.0001). That agree with Comini-Frota et al, [46] study. But disagree with previous study was showed the mean CCL5 in control (0.688±0.247) higher than MS patients [47].

In this study documented that the mean levels of CCL5 (Pg/ml) among male and female of MS patients (1210.44±609.83) (1446.49±692.60), was higher than male and female of control group (14.52±4.76) (15.87±5.12) respectively, in patient and control there are no significant statistical differences in the levels of CCL5 chemokines when compared between males and females. That agree with Ghafouri-Fard et al, [48] study, but disagree with Speirs and Tronson study [47] was showed concentration of CCL5 high in female and significant differences between sex and chemokines. As in other demographical factor showed the mean levels of CCL5 chemokines of patients were higher than control group and no any significant difference in levels of CCL5 chemokines in patients and controls when compared according to any various demographical factor. Also no any significant difference in levels of CCL5 chemokines in patients with smoking, obesity and EDSS scale. No previous studies interested studied in this demographical factor with CCL5 chemokine, so we cannot discuss the present study.

The mean levels of CCL5 (Pg/ml) was high in type RR-relapsing counting (1522.63) and No any significant statistical difference in the levels of CCL5 chemokines when compared according to type of MS, that agree with [42].

In this investigation that the mean levels of CCL5 (Pg/ml) was high in (natalizumab) group counting (1799.67). No previous studies interested studied in natalizumab with CCL5 chemokine. while lowest mean levels of CCL5 in group (Finogolimod) counting (973.24). No any significant statistical difference in the levels of chemokines when compared according to type of therapy patients, that agree with [45].

No any significant statistical difference in the levels of CCL5 chemokines when compared according to clinical symptoms patients, that disagree with [4] study.

Alternatively named as Interferon gamma-induced protein 10 (IP-10) or small-inducible cytokine B10, CXCL10 is produced by monocytes, endothelial cells and fibroblasts, and acts as a chemo-attractant for monocytes/macrophages, T cells, NK cells, and dendritic cells [49]. Treatment with IFN-β influences levels of this chemokine [50].

In this investigation was found the mean concentration of The CXCL10 chemokines was higher among MS patients compared with control, mean concentration values of CXCL10 in MS patients were 64.71±93.45 Ng/l. statistically these differences were highly significant with (P=0.001), that agree with [48]. But disagree with previous study showed the mean CXCL10 in control higher than MS patients [41].

In this study documented that the mean levels of CXCL10 (Ng/l) among male and female of MS patients (98.54±159.44) (47.79±19.59), was higher than male and female of control group (4.61±4.61) (4.19±3.63) respectively, in patient and control there are no significant statistical differences in the levels of chemokines when compared between males and females. That agree with [48]. But disagree with [49] study was showed concentration of CXCL10 high in female and significant differences between sex and chemokines. As in other demographical factor showed the mean levels of CXCL10 chemokines of patients were higher than control group and no any significant difference in levels of CXCL10 chemokines in patients and controls when compared according to any various demographical factor. No previous studies interested studied in demographical factor with CXCL10 chemokine, so we cannot discuss the present study.

Also no any significant difference in levels of CXCL10 chemokines in patients with smoking, obesity and EDSS scale. That disagree with [43] study was showed found significant difference between concentration of CXCL10 chemokines with smoking and obese. Correale and Farez [51] study was showed found significant difference between concentration of CXCL10 chemokines with smoking.

In this study the mean levels of CXCL10 (Ng/l) was high in type RR counting (50.38) and No any significant statistical difference in the levels of CXCL10 chemokines when compared according to type of MS. That agree with [42].

In this investigation that the mean levels of CXCL10 (Ng/l) was high in (Beta interferon) group counting (74.37), that agree with [48], while lowest mean levels of CXCL10 in group (Finogolimod) counting (42.27). No any significant statistical difference in the levels of chemokines when compared according to type of therapy patients. That agree with [45]. In this study No any significant statistical difference in the levels of CXCL10 chemokines when compared according to clinical symptoms patients. That agree with [44].

In ROC Curve of correlation between chemokines and MS were found sensitivity and specificity for CCL2 (80.7%) while for CCL5 and CXCL10 (100%). ROC Curve for CXCL10 in this study was agree with [52].

■ CONCLUSION

Age group 15–55 year has a greatest percentage of multiple sclerosis with females predominant. The concentration of CCL2, CCL5 and CXCL10 chemokines were elevated in the multiple sclerosis patients in comparison with controls, and there is positive correlation between ccl5 and age groups in control.

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Noor Hassoon Swayeh[1]✉, Haitham Mahmood Kadhim[2]
[1] Ashur University, Baghdad, Iraq
[2] College of Pharmacy, Al-Nahrain University, Baghdad, Iraq

Control of Glycemia and Dyslipidemia by Crude Methanolic Extracts of Iraqi Abelmoschus Esculentus and Corchorus Olitorius and Their Combination on Streptozocin-Induced Type 2 Diabetes Mellitus in Rats

Conflict of interest: nothing to declare.
Authors' contribution: Noor Hassoon Swayeh – conceptualization, data curation, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft and writing – review & editing; Haitham Mahmood Kadhim – conceptualization, validation, visualization, writing – original draft and writing – review & editing.
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Contacts: Noor.Hassoon@yahoo.com

Abstract

Introduction. Diabetes is a prevalent global disease, causing cardiovascular disease. Herbal medication like extracts of both Abelmoschus esculentus and Corchorus olitorius have anti-diabetic properties but can have negative effects. Historically, these plants can be a safe substitute for expensive pharmaceutical treatments.
Purpose. To investigate the active phytochemicals and the effects of Iraqi Abelmoschus esculentus pods, Corchorus olitorius leaves methanol extracts and their combination as herbal medicine on diabetes type 2 rats experimental model.
Materials and methods. The study involved 60 healthy albino male rats, randomly divided into six groups. Type II diabetes was induced in five groups, four groups received metformin, okra, albelmoschus, combination, respectively. Blood samples were collected for biochemical analysis, and pancreas was examined for histopathological examination.
Results. The methanolic extract of Abelmoschus esculentus and Corchorus olitorius and their combinations significantly reduced fasting blood glucose, glycated hemoglobin A1, insulin resistance index, total cholesterol, triglycerides, and low and very low-density lipoproteins in diabetic animals, and improve histopathological picture of pancreas.
Conclusion. The methanolic extract of Abelmoschus esculentus, Corchorus olitorius, and their combination enhances glycemic control, lipid control, reduces insulin resistance and high adiponectin levels.
Keywords: glycemia, dyslipidemia, crude methanolic extracts, abelmoschus esculentus, corchorus olitorius, streptozocin-induced type 2 diabetes mellitus

■ INTRODUCTION

Diabetes mellitus is a chronic clinical illness marked by elevated blood glucose levels due to decreased or nonexistent insulin secretion, frequently coupled with decreased

sensitivity to the insulin's function [1]. Hyperglycemia causes the production of free radicals, and oxidative stress is a major factor in cellular damage [2]. Since 90–95 percent of diabetics have type 2 diabetes, the prevalence of adult-onset diabetes and impaired glucose tolerance has dramatically increased globally in recent decades [3]. Chronically elevated blood glucose levels can lead to a range of difficulties by causing systemic vascular damage that can impact multiple tissues, including the heart, kidneys, eyes, and nerves [4]. Historically, natural products – especially those derived from plants – have been utilized to regulate health [5].

Although Abelmoschus esculentus is also known in English as okra, bamieh, bendi, and gumbo, it is most frequently known as lady's finger [6]. Phytochemical studies reveal numerous bioactive components in okra tissues, including minerals, carbohydrates, vitamins, pectin, mucilaginous compounds, and oil components [7–9]. Pharmacologically okra has many actions such as analgesic [10], anti-inflammatory [11], anti-diabetic and anti-hyperlipidemic [12], antioxidant [13], GIT protective [14], hepatoprotective [15], neuroprotective [16], anti-osteopenic [17], antiproliferative [18], wound healing [19], antimicrobial [20] and antiviral [21].

Additionally, Corchorus olitorius herbal extract, sometimes called bush okra, Jew's mallow, Nalta jute, and tossa jute [22], Jute, also known as "molokhiya" in Iraq. The leaves of this plant, rich in fatty acids, vitamins, minerals, and mucilaginous polysaccharides, possess anti-oxidant and anti-inflammatory properties [23], exhibits various properties including antinociceptive, anticonvulsive, antihypertensive, anticancer, antidiabetic, anti-obesity, gastroprotective, antimicrobial, and antiviral properties [24–26].

This study compares the effects of methanolic extracts from Iraqi Abelmoschus esculentus and Corchorus olitorius and their combination on rats with type II diabetes, comparing effects with metformin and controlling body weight and lipid profiles.

■ PURPOSE OF THE STUDY

To investigate the active phytochemicals and the effects of Iraqi Abelmoschus esculentus pods, Corchorus olitorius leaves methanol extracts and their combination as herbal medicine on diabetes type 2 rats experimental model.

■ MATERIALS AND METHODS

Chemicals

The insulin and adiponectin Elisa kits was sourced from Bioassay Technology Laboratory in China, while Streptozotocin (STZ) and Metformin (Met) were obtained from Sigma Aldrich in Germany.

Analysis of biochemicals

The study involved biochemical tests of triglycerides, total cholesterol, LDL, VLDL, HDL using auto-analyzer siemens-Germany, and fast blood glucose using Accu-Check Roche Diagnostics, while insulin resistance estimation by using the formula of calculating the homeostasis model assessment of insulin resistance (HOMAIR) [27]: HOMA-IR = Fasting glucose(mg/dl) × Fasting insulin (uU/mL)/405.

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Plant components and the method of extraction

Fresh fruits and leaves of Abelmoschus esculentus and C. olitorius, respectively were obtained from a Baghdad market, verified by the University of Baghdad's Department of Biology, ground into a powder, macerated three times in 60% methanol, and filtered [28], then dried by spray dryer to get final weight of 65.8 g (10.12 percent) and 209.7 (18.97) percent estimated of each Abelmoschus esculentus and C. olitorius respectively, as described by Norziah et al. (2015) [29]. The appropriate doses were reconstituted in distilled water just prior to oral administration (DW).

Initial phytochemical analysis of raw extract

The study assessed the alkaloids, flavonoids, steroids, anthraquinones, cardiac glycosides, and saponins in the methanol crude extracts of C. olitorius and Abelmoschus esculentus, respectively, using Harborne's standard procedures [30].

Diabetes induction

Fifty rats were fed by high-fat diet (HFD) derived from research diet with a total kcal value of 4.73 kcal/g (45 kcal percent energy as fat, 20 kcal percent protein, and 35 kcal percent carbohydrate) for four weeks, then fasted one night, diabetes were induced by a single intraperitoneal injection of streptozocin 35 mg/kg, which was prepared in 0.1M ice-cold citrate buffer at pH 4.57 [31]. In order to prevent hypoglycemia caused by drugs, animals were given a five percent glucose solution to drink throughout the night [32].

A standard pellet meal and the same volume of solvent (citrate buffer) as the IP injection were given to the control group. After five days of STZ induction, the animals' diabetes status was assessed; those with FBG levels of 200 mg/dl or more were classified as diabetics, retained on the HFD, and used in the study [33].

Animals and study design

From January 2023 to January 2024, the current experiment was carried out as an animal research study at Al-Nahrain University's biotechnology research center's animal house. Every study experiment was conducted in compliance with the procedure that the Al-Nahrain University College of Medicine's Institutional Review Board had approved. Forty male Albino Wister rats, estimated to be in good health, weighing 250–350 g at the age of 10–12 weeks, were housed in standard plastic cages with a 12-hour light/dark cycle, fresh water, and a standard diet of 22–25 °C (ad libitum). Before the trial started, the rats were allowed a week to become used to their new surroundings. The animals were split into six groups (Groups) of ten at random. Group1 serving as a normal control and receiving equivalent doses of used solvents, while the other five Groups induced diabetes 2, Group2 serving as a diabetic control given (DW) orally, Group3 receiving 100mg/kg metformin orally gavage, Group 4 C. olitorius (400 mg/kg), Group 5 received Abelmoschus esculentus (400mg/kg) and Group 6 received (200 mg/kg) from each C. olitorius and Abelmoschus esculentus. All groups are orally gavage, and therapy session lasted 28 days [34].

Sacrificing and sample collection

On day 29 of the trial, the rats were sacrified by diethyl ether anesthesia following an overnight fast. The pancreas was rinsed with 0.9 percent NaCl and preserved in 10%

formalin for sectioning and histological inspection, and the blood was taken straight from the heart for chemical analysis.

Statistical analysis

Quantitative data were expressed as the mean and standard deviation, and the mean difference between any two groups was found using a post hoc least significant difference test after one-way ANOVA was performed to examine the difference in mean of numeric variables between more than two groups. Using SPSS version 23, the statistical software for social sciences, p 0.05 was selected as the significant criteria [35].

■ RESULTS

The extraction recovery was computed as a yield percentage as shown in table 1 that represented the overall percentages of crude extracts of Abelmoschus esculentus dried pods powder and Corchorus olitorius dried leaves powder.

Anthraquinones, flavonoids, saponins, and steroids were shown to be positively correlated with aqueous methanol extracts of both herbal extracts, while cardio-active glycosides were found to be negatively correlated. While alkaloids are lacking in Corchorus olitorius extract, they are abundant in Abelmosschus esculentus extract as shown in table 2.

The study found a significant drop (P≤0.001) in body weight gain in diabetic animals induced by high-fat diet-HFD+STZ compared to the normal control group, while other treatment groups did not show significant differences. The findings indicated that there was no obvious variation between the four treatment groups when comparing the means of body weight gain, as shown in table 3.

The diabetic group showed significantly higher fasting blood glucose, HbA1c, and insulin resistance index compared to the normal control group (P≤0.001), while serum insulin levels significantly decreased. The study found a significant decrease (P≤0.001) in FBS, HbA1c, and HOMA-IR in metformin, okra, jute, and combination (A+C) treated groups compared to diabetic groups. The study found that metformin significantly (P≤0.001) decreased FBS and HOMA-IR levels in all treatment groups except okra, and HbA1c levels in jute (P≤0.05) compared to all other groups. The okra-treated group revealed a notable decline in HbA1c and HOMA-IR compared to the jute and combination groups (P≤0.001) and jute (P≤0.05). The combination group had noticeably lower HbA1c readings than the jute group (P≤0.05), as shown in table 4.

The HFD+STZ diabetic group showed significant differences in total cholesterol, triglycerides, low density lipoprotein, and very low-density lipoprotein, with a significant (P≤0.001) decrease in high density lipoprotein. The diabetic group showed a significant increase in serum total cholesterol, triglycerides, low density lipoprotein, and very low-density lipoprotein, while a significant (P≤0.001) decrease in serum high density lipoprotein was observed. Between the four treatment groups (metformin, okra, jute and combination (A+C)), this study's results demonstrated that okra group has significant higher mean level of high-density lipoprotein compared to other groups (P≤0.05) except for the combination at (P≤0.001). While other changes were not significant between them, as shown in table 5.

In comparison to section of normal control (fig. 1), section of diabetic group revealed pancreatitis which characterized by focal necrosis of pancreatic islet surrounded by

infiltrated lymphocytes, there were multiple focal hemorrhage and deterioration of pancreatic acini (fig. 2), histological sections of pancreas from all other groups showed normal appearance, as showed in fig. 3–6.

The mean serum adiponectin level was high significantly lower in the diabetes group (-ve) than in the normal control group (N) (11.84±4.10 vs. 33.96±3.66) at P≤0.001, also a significant decrease in serum adiponectin's levels observed when diabetic group compared with other four treatment groups (P≤0.05). When the means of the four treatment groups (metformin, okra, jute, and a combination (A+C)) were examined, metformin had a considerably smaller effect on adiponectin levels (18.50±9.20) than the jute group (P≤0.05), as shown in table 6.

Table 1
Yield obtained from methanol extraction of Abelmoschus esculentus and Corchorus olitorius

Herbs	Wt. of dried powder	Wt. of crude extract	Yield (%)
Abelmoschus esculentus pods	650 g	65.8 g	10.12%
Corchorus olitorius leaves	1105 g	209.7 g	18.97%

Table 2
Qualitative detection of some phytochemical compounds in Abelmoschus esculentus and Corchorus olitorius methanol extracts

Compound	Abelmoschus esculentus	Corchorus olitorius
Alkaloids	+	–
Anthraquinons	+	+
Cardio-active glycosides	–	–
Flavonoids	+	+
Saponins	+	+
Steroids	+	+

Notes: + – found, – – not found.

Table 3
Mean body weight and body weight change in the control, diabetic and study treatment groups

Type	Mean±SD Normal	Mean±SD Diabetic	Mean±SD Metformin	Mean±SD Okra	Mean±SD Jute	Mean±SD Okra+Jute
Wt. 1 (g)	288.2±40.74	228.2±21.45	233.4±25.39	237.4±18.27	291.6±23.63	309.2±24.23
Wt. 2 (g)	329.2±38.81	247.3±25.12	251.7±26.08	253.8±19.29	307.8±22.77	328.8±23.83
Body weight gain (g)	41±12.71 A	19.1±5.52 b	18.3±2.62 b	16.4±2.36 b	16.2±3.79 b	19.6±3.77 b

Notes: little letters are used to compare groups, similar letters indicate there is no difference, while different letters indicate substantial differences; SD – Standard deviation. Significance level at P≤0.001.

Table 4
Mean fasting blood glucose level, glycated hemoglobin A1c, serum insulin and HOMA-IR in control, diabetic and study treatment groups

Type	Mean±SD Normal	Mean±SD Diabetic	Mean±SD Metformin	Mean±SD Okra	Mean±SD Jute	Mean±SD Okra+Jute
FBS (mg/dl)	101.30±13.87 a*	408.90±72.33 b	140.50±13.53 c*	216.50±37.02 d*	271.50±105.16 d*	243.20±112.24 d*
H bA1c %	3.25±0.26 a*	4.98±0.46 b	3.62±0.46 c*e	3.23±0.17 c*	*4.23±0.55* d*	3.77±0.60 e*
S. insulin (mU/l)	6.60±0.87 a*	4.93±1.62 b	5.13±0.36 bc	5.41±0.47 bc	5.72±0.28 c	5.85± 0.76 c
HOMA-IR	1.47±0.23 a*	4.37±1.12 b	1.59±0.15 e*	2.62±0.61 c*	**3.42±1.24** d*	**3.06±1.13** cd*

Notes: little letters are used to compare groups, similar letters indicate there is no difference, while different letters indicate substantial differences; SD – Standard deviation; FBS – fasting blood sugar; HbA1c – glycated hemoglobin a1; HOMA-IR – insulin resistance index, Significance level at P≤0.05 – *, bold and italics represents high significant differ P≤0.001 compared to diabetic, metformin and okra respectively.

Table 5
Mean serum lipids in the normal control, diabetic and study treatment groups

Type	Mean±SD Normal	Mean±SD Diabetic	Mean±SD Metformin	Mean±SD Okra	Mean±SD Jute	Mean±SD Okra+Jute
S. total cholesterol (mg/dl)	65.97±6.40 a	123.01±23.77 b	69.49±4.24 c	77.63±9.83 c	73.05±9.14 c	74.65±9.71 c
S. triglycerides (mg/dl)	78.8±2.85 a	112.3±15.89 b	83.0±9.20 c	83.7±6.0 c	85.4±8.28 c	87.4±7.12 c
S. LDL (mg/dl)	19.29±8.71 a	85.49±23.88 b	32.11±4.93 c	35.52±5.71 c	36.07± 8.77 c	39.17±11.32 c
S. VLDL (mg/dl)	15.76±0.57 a	22.46±3.17 b	16.60±1.84 c	16.74±1.20 c	17.08±1.65 c	17.48±1.42 c
S. HDL (mg/dl)	30.92±4.34 a	15.06±4.11 b	20.78±4.87 c*	25.37±5.33 d	19.9±0.81 c*	19.2±1.68 c

Notes: little letters are used to compare groups, similar letters indicate there is no difference, while different letters indicate substantial differences; SD – Standard deviation; FBS – fasting blood sugar; HbA1c – glycated hemoglobin a1; HOMA-IR – insulin resistance index, Significance level at P≤0.05 – *, bold and italics represents high significant differ P≤0.001 compared to diabetic, metformin and okra respectively; SD – Standard deviation, LDL – Low density lipoprotein; VLDL – Very low density lipoprotein; HDL – High density lipoprotein.

Table 6
Mean serum adiponectin in the control, diabetic and study treatment groups.

Type	Mean±SD Normal	Mean±SD Diabetic	Mean±SD Metformin	Mean±SD Okra	Mean±SD Jute	Mean±SD Okra+Jute
S. adiopnectine (mg/dl)	33.96±3.66 a*	11.84±4.10 b	18.50±9.20 c	20.66±5.40 cd	24.24±4.17 d	21.16±6.13 cd

Notes: small letters for comparison among groups; similar letters for no difference; different letters for significant differences; SD – Standard deviation. Significance level at P≤0.05 – *.

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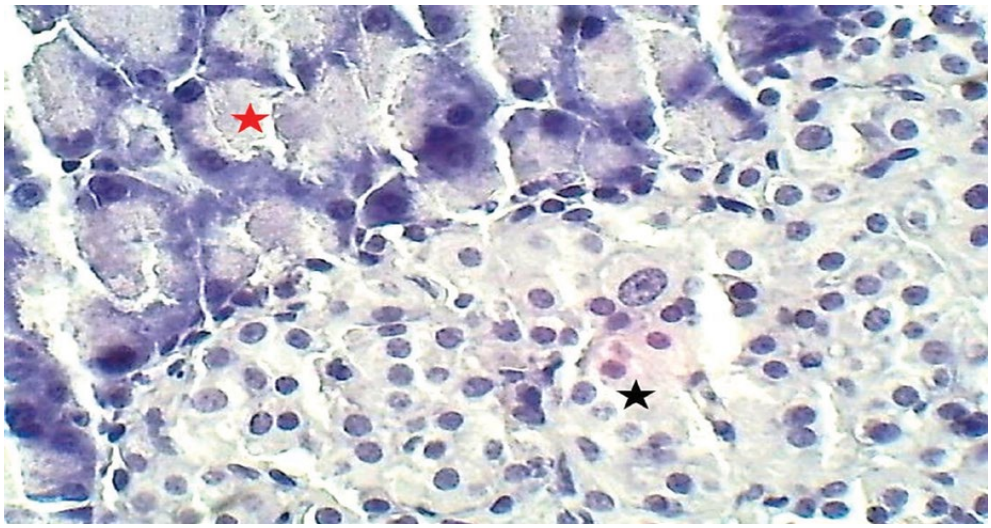

Fig. 1. Section of pancreas (normal control) shows: normal architecture of pancreatic acinus (red asterisk) & pancreatic islet (black asterisk). H&E stain. 40×

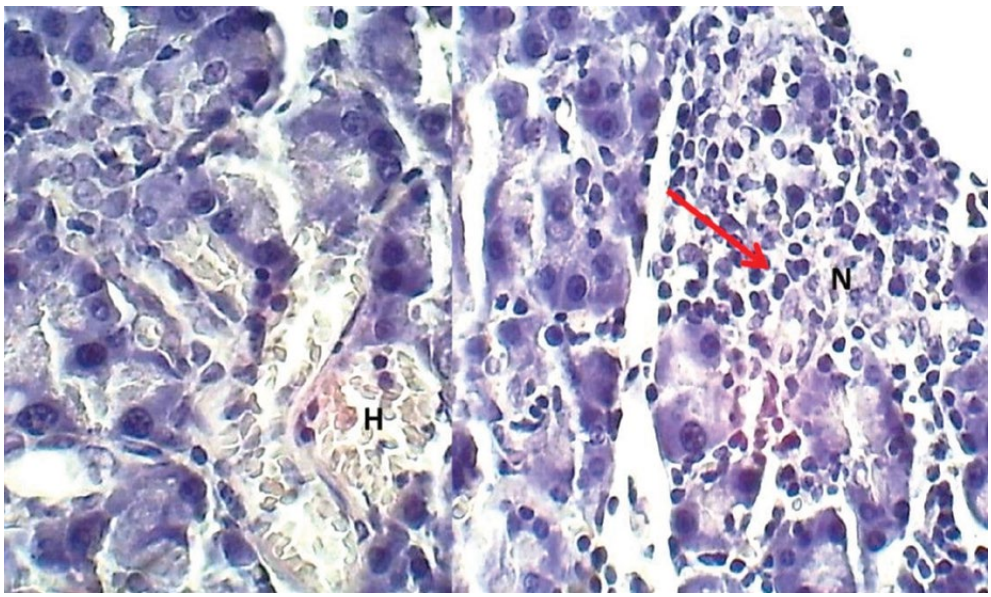

Fig. 2. Section of pancreas (diabetic control) shows: deterioration of acini with hemorrhagic focus (H) focal infiltration of mononuclear lymphocytes (red arrow), (N): necrosis. H&E stain. 40×

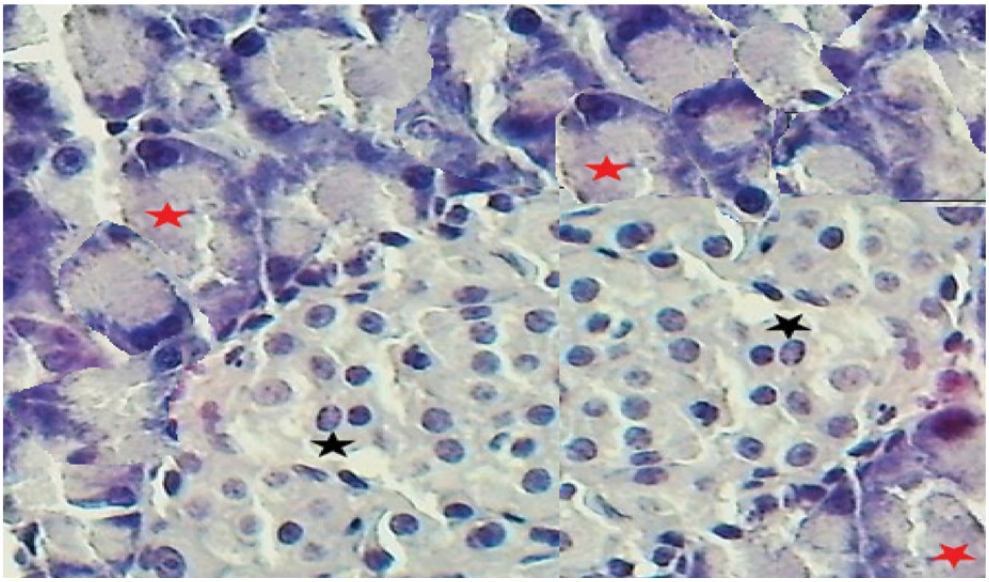

Fig. 3. Section of pancreas (metformin group) shows: normal architecture of pancreatic acinus (red asterisk) & pancreatic islet (black asterisk). H&E stain. 40×

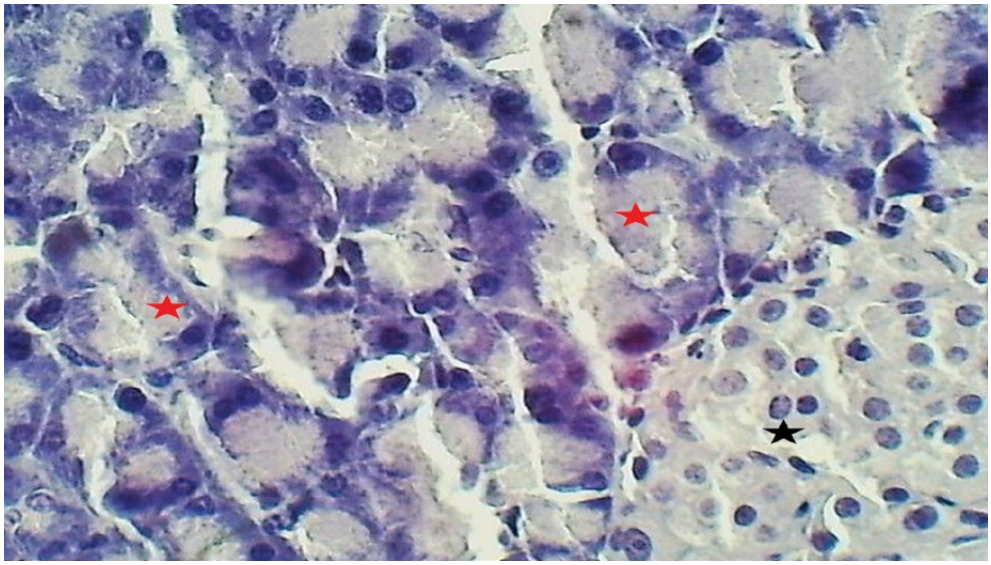

Fig. 4. Section of pancreas (okra group) shows: normal acinar cells (red asterisks) normal islet cells (black asterisks). H&E stain. 40×

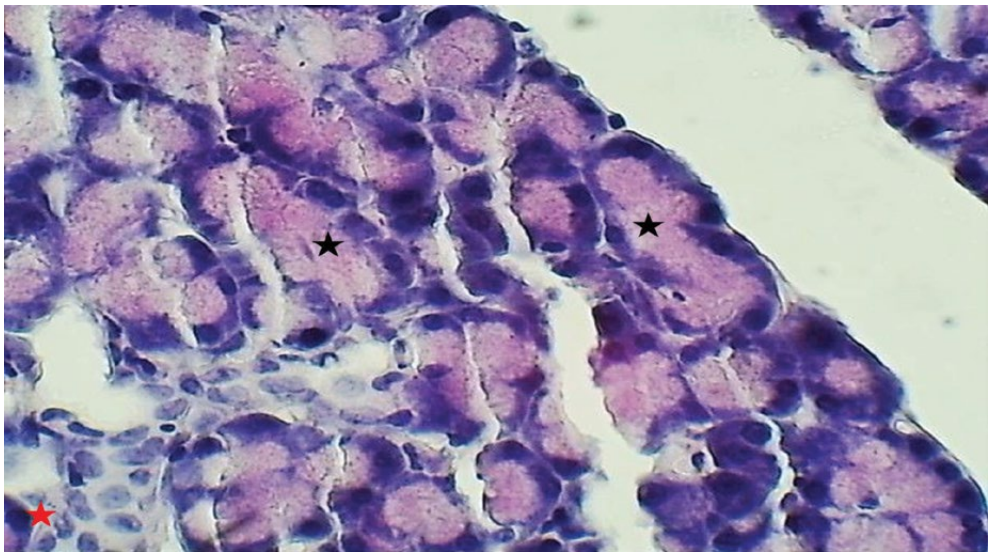

Fig. 5. Section of pancreas (jute group) shows: normal acinar cells (black asterisks) normal islet cells (red asterisks). H&E stain. 40×

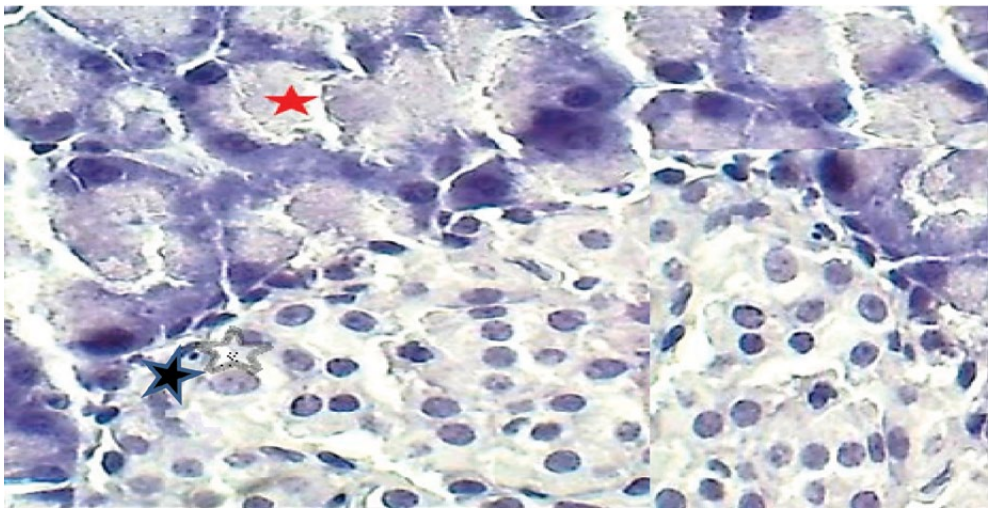

Fig. 6. Section of pancreas (combination group) shows: improved pancreatic architecture: black asterisk – pancreatic islet, red asterisk – pancreatic acinus. H&E stain. 40×

■ DISCUSSION

Diabetes mellitus was induced in animals after the administration of Streptozotocin (STZ) due to its cytotoxicity on pancreatic islet β-cells, additionally, intracellular

metabolism of STZ-produces nitric oxide free radicals, all these, produces a reduction in insulin level and leads to an alteration of glucose metabolism and utilization, resulting in hyperglycemia; all that related to the selective toxicity on β-cells due to alkylation of DNA [36]. High-fat diets (HFD) can cause hyperlipidemia, hyperglycemia, insulin resistance, and mitochondrial dysfunction when combined with low dose STZ, mimicking symptoms of human type II diabetes [37]. A previous study found that treating newly diagnosed T2DM-patients with metformin alone for three months reduced FBS and HbA1c in a manner comparable to the current study [38].

Abelmoschus esculentus has been shown in various studies to reduce FBG and HbA1c, relating to high flavonoid content (quercetin 3-o-gentiobioside and isoquercitrin) [36].

Diabetic rats fed with a methanolic extract of C. olitorius noticed drop their blood glucose and HbA1c levels [39, 40] by presence of phenolic compounds (such as caffeic acid, chlorogenic acid), as well as rutin in an extract from the leaves of C. olitorius has been shown to greatly inhibit α-glucosidase (modestly inhibit α-amylase) [41, 42].

Combination therapy has additive benefits, as demonstrated by the results of the current study. Previously, synergistic effects of both (quercitin and quinic acid) were found to improve hyperglycaemia, hyperlipidaemia, and insulin resistance in diabetic rats [43].

Diabetes induced by STZ in rats was previously found to dramatically increase TC, TG, LDL, VLDL, and reduce HDL levels when compared to normal control rats [36], and these findings are comparable to the current study. This is observed by our study, methanolic extracts of Abelmoschus esculentus, Corchorus olitorius and their combination were found to lower TC, TG, LDL, and VLDL levels and increase HDL values. Flavonoids have the potential to suppress pancreatic lipase function [44]. By effects of flavonoids extract of extracts [45, 46]. Quercitin derivatives and isoquercitin were the most prevalent flavonoids identified in Iraqi okra, the obtained results could be attributed to them [47–50].

Considering that caffeoylquinic acid derivatives were the predominant phenolics in Iraqi Corchorus olitorius. Combination of the two extracts give a comparable effect which indicates an additive effect which related to the phytochemicals present in both extracts.

Serum adiponectin levels were found to be significantly reduced in diabetics than in normal rats in the current study, and they changed for the better after treatment with each of metformin, okra, jute, and herbal combination extracts.

By suppressing hepatic gluconeogenesis, increasing skeletal muscle glucose transport, pancreatic insulin production, hepatic and skeletal muscles oxidation of fatty acids [51], control of inflammatory signals [52], adiponectin may reduce the risk of developing type 2 diabetes. In addition adiponectin serves as an antagonist of adipogenesis and regulates the lipid metabolism well [53]. By using of metformin has been linked to statistically significant increases in adiponectin in earlier research [52, 54, 55], which support the findings of the current study.

Adiponectin levels in C. olitorius extract-treated rats were greater than in HFD control rats as previously reported [56], and the current research observations support that. The primary components of the methanol extract from C. olitorius grown in Iraq include quinic acid and CQA derivatives, which may have a significant role in the observed impact. Chlorogenic acid component of C. olitorius may increase AMPK-phosphorylation, adiponectin and its receptors, while decreasing hepatic glucose-6-phosphatase expression [57].

The histopathological examination of diabetic rats revealed pathological changes in the pancreas, but rats treated with herbs (Abelmoschus esculintus, Corchorus olitorius, and their combination) in addition to standard drug (metformin) improved pathological changes caused by diabetes, which could be attributed to anti-hyperglycemic and anti-hyperlipidimic effects as biochemical analysis approved. The findings of this study corroborate with previous findings that okra alleviated histological damages to the pancreas, namely vacuolization and a reduced β-cell mass in diabetic rats [58].

Furthermore, it was discovered that combining (quercitin+sitagliptin) restored islet number, β-cell number and area, as well as restoring β-cell immunostaining strength in diabetic rats [59]. Abelmoschus esculentus subfractions, one containing a high concentration of quercetin glucosides and pentacyclic triterpene ester and compounds (contains high concentrations of carbohydrates and polysaccharides), might prevent palmitate-induced apoptosis of β-cells by hindering DPP-4 activity [60].

Because of the presence of several phytochemicals that may contribute to such pharmacological actions, extracts of C. olitorius leaves have been found to produce considerable improvement and enhance the number, size, and density of viable β-cells [61]. Previously, it was discovered that aqueous extract of C. olitorius intervention could restrict and antagonized the apoptotic signaling pathways and improved by [62] via the presence of a significant amount of phenolic chemicals and flavonoids in the extract, which may be responsible for the overall protective impact.

■ CONCLUSION

The Abelmoschus esculentus and Corchorus olitorius methanolic extracts contain alkaloids, anthraquinones, flavonoids, saponins, and steroids. These extracts are effective in managing hyperglycemia and lowering insulin resistance in type II diabetic rats. They also improve lipid profile, reduce the histopathological impact of pancreas in diabetic rats.

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Eman H. Rahi[1, 2]✉, Nameer A. Khudhair[1]
[1] College of Veterinary Medicine, University of Basrah, Basrah, Iraq
[2] College of Nursing, University of Basrah, Basrah, Iraq

Protective Effects of Tocotrienol Supplementation on Blood and Liver Functions in Rats Fed a High-Fat Diet

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Contacts: eman.rahi@uobasrah.edu.iq

Abstract

Introduction. Obesity has become one of the most serious worldwide health issues, affecting a sizable proportion of the world's population.
Purpose. To evaluate the protective role of tocotrienols produced from annatto oil on blood parameters and liver functions in rats fed a high-fat diet.
Materials and methods. Eighteen male rats were divided into three groups: the first group was fed normal chow as a control group, and the second and third groups were fed a high-fat diet to promote obesity in the presence or absence of tocotrienols (60 mg/kg for 12 weeks) and were examined for blood markers and biochemical and histopathological changes in the liver.
Results. The increase in liver weight of the high fat diet and high fat diet plus tocotrienol groups was significant at the final stage of the experiment ($P<0.05$) in comparison to the control group. On the other hand, males treated with HFD+ tocotrienol in the protective group showed significant ($P\leq0.05$) differences in the results of RBC and MCH. A significant ($P\leq0.05$) increase in WBC and neutrophils% in male rats treated with HFD alone compared with the control group and protective group.
Conclusion. Tocotrienol treatment increase MCH compared with control group but reduced WBC count and neutrophils% in male rats treated with high fat diet plus tocotrienols compared to the high fat diet alone group. It also led to an improvement in histological changes in liver of the group treated with tocotrienols. Tocotrienols reduce the effects of high-fat diet on the blood and liver.
Keywords: tocotrienols, HFD, Protective effects, blood, liver

INTRODUCTION

Over the past several years, obesity has become one of the biggest global health concerns, and it is now present in a significant fraction of the global population [1]. Obesity, which is characterized by an excessive buildup of body fat, significantly raises the risk of a number of illnesses, including type 2 diabetes, cardiovascular disease, and nonalcoholic fatty liver disease, and is associated with a shorter life span [2]. According to Prasad et al., obesity results from controlled systems' failure to maintain energy balance, a balance that is impacted by genetic [3], physiological, and/or environmental variables [4]. In order to address the calorie imbalance, managing obesity requires behavioral and lifestyle changes, such as calorie restriction and increased physical exercise [5]. However, several drugs, including as phentermine, lorcaserin, and orlistat, have been used to promote weight reduction but they come with adverse effects as a result of Alterations in people's lives, accompanied by decreased physical activity and dietary changes [6]. As bariatric surgery's success in weight control gains attention, it is becoming the go-to procedure when other treatments don't work [7]. In order to address the worldwide economic and social cost of obesity and metabolic syndrome, permanent changes in dietary and lifestyle norms are required, with a particular emphasis on recognizing metabolic disease and creating treatment options [8]. As a result, it's crucial to continue researching non-traditional methods of treating obesity, such as bioactive substances and functional foods [9]. Dietary therapy include omega-3 polyunsaturated acids [10], caffeine [11], astragaloside II [12], ginger [13] and vitamin E [14] are used as dietary treatments. Vitamin E contains two categories of biologically active chemicals known as tocopherols and tocotrienols. Each class has four isoforms (alpha (α), beta (β), gamma (γ), and delta (δ), each with its own biological function [15, 16]. Tocotrienols (T3s) are among the chemicals that have been widely researched for their metabolic effects. The tocotrienol group's medicinal potential originates from its nutritional characteristics as a dietary supplement. Animal studies, in vitro tests, and ongoing human trials have all found it to be safe at modest dosages for alleviating pathogenesis [17]. Tocotrienol may be found in a variety of foods, including rice bran, palm oil, and annatto oil [18]. Tocotrienol obtained from annatto seed is typical in that it includes 90% delta-tocotrienol, 10% gamma-tocotrienol, and no tocopherols [19]. Previous studies such as [19] and [20] have evaluated the effect of tocotrienol-rice bran and tocotrienol-rich fraction (TRF) of tocotrienols respectively on the blood picture and indicated that tocotrienols had no noticeable effect on hematological indices. To our knowledge, there is no study that included the effect of tocotrienols extracted from annatto on blood parameters in rats fed a high-fat diet.

PURPOSE OF THE STUDY

To evaluate the protective role of tocotrienols produced from annatto oil on blood parameters and liver functions in rats fed a high-fat diet.

MATERIALS AND METHODS

Experimental animals

The present study was carried out at the laboratory animal house in College of Veterinary Medicine, University of Basrah. Eighteen male rats weighting (80±25 gm) and

aged (3 months) were used for the present study. The animals were kept one week for acclimatization before the beginning of the experiments. All the experimental animals were maintained under optimum conditions (24±2 °C) also a 12/12-hours light/dark cycle throughout the study. The food and drinking water were administered ad libitum throughout the experimental period. Annatto tocotrienol 70% concentrate, 90% delta-tocotrienol and 10% gamma-tocotrienol produced by American River Nutrition Inc. (Hadley, MA, USA). To prepare a dose of 60 mg per kg according to [21], weigh 85.71 mg and diluted with olive oil to 1 ml. The substance was drenched orally to rats by gavage.

High fat diet and protocol of the study

A total of 18 adult male rats. Three groups of rats were distributed at random. Group 1 (the normal control) was given a diet low in fat. (LF 10% kcal from fat) D14031901 which included 67% carbohydrate, 4% fat, 19% protein. Group 2 was fed with HFD (HF 60% kcal from fat) D14031902 which have 26% carbohydrate, 35% fat, 26% pprotein for 12 weeks. Group 3 was fed with HFD plus tocotrienol 60 mg/kg of tocotrienol supplement dissolved in olive oil (1 ml/kg) [21].

Table 1
Formula diet were used in this study

Ingredient	gm	kcal	gm	kcal
Casein	200	800	200	800
L-Cystine	3	12	3	12
Corn Starch	506.2	2025	0	0
Maltodextrin	125	500	125	500
Sucrose	68.8	275	68.8	275
Cellulose	50	0	50	0
Soybean Oil	25	225	25	225
Beef fat	20	180	245	2205
Mineral & Vitamin Mix	20	40	20	40
Dicalcium Phosphate	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0
Potassium Citrate	16.5	0	16.5	0
Choline Bitartrate	2	0	2	0
Total	1055.00	4057	773.80	4057

Blood sample preparation

Rats were starved for 12–14 hours following the treatment period. Liver was removed, cleaned in purified water and weighed by digital scale after being anaesthetized with 1.9% exhaled diethyl ether, the jar's capacity was approximately 0.08 mL/L [22]. Blood samples (10 ml) were collected from each rat from cardiac puncture by using disposable syringe (10 ml). The samples were put into gel tube (8 ml), and centrifuged at 3000 rpm for 15 minutes for serum separation into clear and non-hemolyzed supernatant and divided into four parts to store in polyethylene Eppendorf tubes at –20 °C for further serological analysis. The remaining 2 ml of blood was deposited into EDTA containing tube for hematological analysis.

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Biochemical assays

The cobas c 311 an analyzer is an independent instrument used to obtain clinical chemistry profiles from rats' serum. This analyzer can determine sodium, potassium, and chloride levels in serum, plasma, and urine using ion-selective electrodes (ISE). Several markers have been studied and quantified including Aspartate Aminotransferase, Alkaline Phosphatase, Alanine Aminotransferase.

Bioassay for histopathology

The paraffin embedding method was used for histopathological examinations of the liver. To prevent autolysis, sectioned tissues were fixed in 10% neutral buffered formalin. Isopropyl alcohol and 50% ethanol were used to dehydrate and wash organ tissues, which were then stained in hematoxylin and eosin [23]. The stained sections were photographed with a light microscope at a magnification of 100. The histopathological examination was performed at the University of Basrah 's College of Veterinary Medicine.

Statistical analysis

The current studies' data had been analyzed in the computerized SPSS (Statistical Packages for the Social Sciences) V.13 programmed using univalent analysis of variance (ANOVA). The threshold of significance was set at $P<0.05$. The data was presented in the form of mean ± standard error. The least significant difference (LSD) test was used to compare groups [24].

■ RESULTS

The effect of the tocotrienol supplement on the liver weight of the male rats

The increase in liver weight of the high fat diet and high fat diet plus tocotrienol groups was significant at the final stage of the experiment ($P<0.05$) in comparison to the control group (Table 4).

The effect of tocotrienols supplement on RBC Counts, RBC Indices, WBC total counts, and percentage of differential count of WBC of male rats

The results of RBC, Hb, PCV%, MCV, MCH, and MCHC in Table 2 showed no significant ($P\leq0.05$) differences in males treated with a high diet in fat (HFD) compared with the control group. On the other hand, males treated with HFD+ tocotrienol in the protective group showed significant ($P\leq0.05$) differences in the results of RBC and MCH. Our results in Table(showed a significant ($P\leq0.05$) increase in WBC and neutrophils% in male rats treated with HFD alone compared with the control group and protective group, while there was no significant increase in lymphocytes%, basophils%, eosinophils%, or monocytes% in male rats treated with HFD comparation with anther groups in the protective experiment.

Effect of tocotrienol supplement on liver function

Table 4 shows a significant ($P\leq0.05$) rise in AST, ALT and ALP levels in the serum of HFD male rats compared to another groups.

Effect of tocotrienol supplement on liver histopathology in rats

As demonstrated in fig. 1, there were no histological abnormalities in the control group's liver that may influence liver function for each form, and the tissue looked to have

a normal structure, consisting of normal central veins, normal hepatocytes, and normal sinusoids. Pathological abnormalities such as significant pericentral venous vacuolation of hepatocytes were discovered in rats given a high-fat diet-induced obesity for 12 weeks (fig. 2). In contrast, the histopathological examination of rats livers that were fed a high-fat diet and supplemented with tocotrienol for 12 weeks showed hepatic changes less than rats fed a high-fat diet alone, such as early regenerated hepatocytes and normal sinusoids (fig. 3).

Table 2
Effect of tocotrienols supplement on RBC Counts and RBC Indices of male rats

Groups	RBC, $\times 10^6/\mu L$	Hb, g/dl	PCV,%	MCV, fl	MCH, pg	MCHC, %
Control	7.891±.249a	13.316±.393	41.166±1.063	52.266±.804	16.883±.328b	32.333±.2185
HFD	7.448±.173a	13.266±.321	40.400±.915	54.283±.665	17.816±.124ab	32.833±.282
HFD + tocotrienol	7.096±.272b	13.016±.510	38.900±1.679	54.383±.760	18.250±.588a	33.600±1.334
Signi.	*	NS	NS	NS	*	NS

Notes: small letter represents significant difference at P≤0.05. N.S. – non-significant. * significant.

Table 3
Effect of tocotrienols supplement WBC total counts and percentage of differential count of WBC of male rats

Groups	WBC$\times 10^3/\mu L$	NEUT, %	Lymph, %	Mono, %	% Eosino	% Baso
Control	9.440±.127b	2.400±.43b	6.550± .51	.373±.064	.071±.038	.135±.093
HFD	13.720±1.165a	4.101±.456a	8.900±1.444	.470±.121	.095±.073	.153±.057
HFD+ tocotrienol	9.325± .123b	1.843±.206b	7.265±.080	.200±.120	.073±.026	.115±.069
Signi.	*	*	NS	NS	NS	NS

Notes: small letter represents significant difference at P≤0.05. N.S. – non-significant. * significant.

Table 4
Effect of tocotrienols supplement on liver enzymes and relative weight of the liver of male rats

Groups	AST, U/L	ALT, U/L	ALP, U/L	Liver weigh / body weight
Control	142.000 ±10.475 b	34.833 ±1.249 b	218.666 ±13.691 b	.019±.000 b
HFD	264.000 ±62.505 a	47.333 ±6.520 a	303.833 ±16.543 a	.033±.001 a
HFD+ tocotrienol	161.500 ±11.898 ab	38.833 ±.749 a b	265.666 ±19.815 ab	.031±.002 a
Signi.	*	*	*	*

Notes: small letter represents significant difference at (P≤0.05). N.S. – non-significant. * significant.

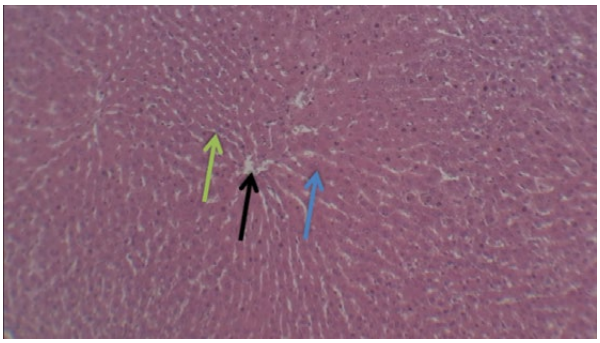

Fig. 1. Histological micrograph of liver of control group showed normal architecture of hepatic parenchyma, which consist of normal central vein (black arrow), normal hepatocytes (blue arrow), and normal sinusoids (red arrow). H&E stain. 100×

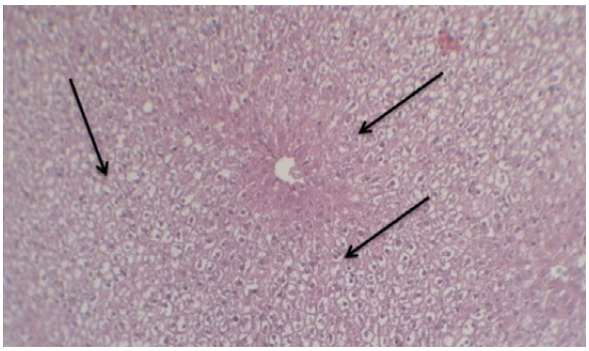

Fig. 2. Histological micrograph of liver of high fat diet group shows severe peri central vein vacuolation of hepatocytes (black arrow). H&E stain. 100×

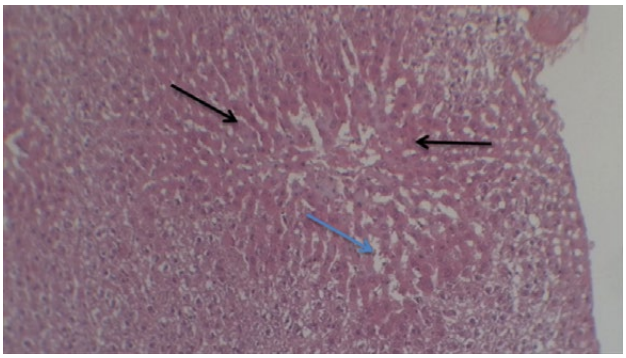

Fig. 3. Histological micrograph of liver of high fat diet plus tocotrienols group shows early regenerated hepatocytes (black arrow), normal sinusoids (blue arrow). H&E stain. 100×

■ DISCUSSION

Although an increase in adipocyte mass and number is a physical marker of obesity, the aspects of obesity that should be considered are adipose tissue malfunction and persistent low-grade inflammation [25]. These situations have unfavorable consequences, as an example changes in The immune system, metabolism of iron, and platelet activity, and they can also influence hematological parameters[26]. Anemia is characterized by a drop in either the total amount of hemoglobin or the number of RBCs [27]. A deficiency in iron is associated with inflammatory markers because obesity is characterized by a systemic low-grade inflammation [27]. Hepcidin expression in adipose tissue is powerfully induced by cytokines derived from adipose tissue such as IL-6, and and the IL-1 [25]. This elevation may reduce the amount of iron absorbed and reduce the efficiency of iron fortification [28, 29]. The obtained data (table 1) revealed that the examination of RBC-related parameters, such as RBC count, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) there was no significant difference in comparison to the control group. This could be attributed to changes in hematological parameters caused by a high fat diet, which may necessitate more prolonged exposure. These results were identical to the results for Shawky who used high fat diet for 6 weeks [30]. Despite the difference in the components of the diet, the percentage of fat used and, type of fat and duration of exposure. These results were inconsistent with the results of Alia et al. that found a subchronic high fat diet in mice may reduce the amount of RBC in the high fat diet group compared with the normal control group [27]. Obesity in individuals is linked to an increase in blood cell count, which could increase blood viscosity [31, 32]. Also, obese mice (since, after an a six-month high-fat diet) showed greater red blood cell and white blood cell levels, as well as an increase in packed cell volume PCV [33]. In this study the slight significant decrease in the red blood cell count in high fat diet plus tocotrienols, it did not indicate the presence of anemia especially with the significant increase in the MCH value. This was consistent with the results of Tasaki et al.'s study [34]. We may not be able to compare the results, especially since Tasaki et al.'s study [34] was at tocotrienols doses higher than the appropriate doses given in the study, in addition to the difference in the composition of tocotrienol used in his study and previous studies. On other hands, using tocotrienols with high-fat food reduces the number of red blood cells count and MCH, which in turn means improving the health condition resulting from reducing the viscosity that may arise from excessive formation of red blood cells.

When compared to the control group, the number of WBCs increased in the HFD obese inducement group. This rise in WBC in the treatment groups could be due to an inflammatory response in the bone marrow [35]. We further performed hematology analysis on a, monocyte, lymphocyte and granulocyte percentage table (3). The amount of neutrophil was tended to increase in HFD group as compared with other groups. Tocotrienol was tended to improve this condition in our experiment, and the role played by tocotrienols may be due to its properties as an antioxidant agent [36]. Previous research has shown that providing high-fat diets to specific animals can greatly increase the amount of WBC, lymphocyte subsets as well as increasing blood viscosity [37–39]. A high fat diet promotes fat buildup, increased inflammatory cytokine production, and macrophage infiltration, all of which enhance the course of liver disease [40]. In this study, relative weight of liver increase in HFD group compared with other groups and this result

disagreement with [41], who was noted no statistically significant difference in relative liver weight of rats fed with experimental diets (beef tallow), following an HFD resulted in a large increase in the liver enzymes aspartate aminotransferease (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) [42].

In this investigation, plasma enzymatic activity of ALT, AST and ALP were increased in the HFD group as compared with HFD plus tocotrienols and control groups, while level was statistically insignificant. These findings suggest that elevated plasma enzyme activity are linked to hepatocyte deterioration [43]. Thamer [44] indicated that feeding rats with a high-fat diet leads to histological liver changes characterized by the accumulation of fat droplets within the cytoplasm of parenchyma cells, along with inflammation and an increase in the number of Kupffer cells. Tocotrienol supplementation could prevent liver damage caused by a high-fat diet, according to the current research. It is probable that their antioxidant activity is responsible for their hepatoprotective impact. Non-enzymatic antioxidants such as vitamins C and E, as part of total antioxidant systems, may help to reduce oxidative damage [36]. This was confirmed by the noticeable improvement in the tissues of rats treated with tocotrienol in the current study. Which indicates that tocotrienols did reduce damage but not prevent it, that may arise from intake of high diet in fat. Our study agreed with study by Nakamura et al. that reported slight hepatocyte hypertrophy in male rats treated with tocotrienols at doses above 0.75% for 13 weeks it is part of a study of tocotrienol on rats at high doses [45].

■ CONCLUSION

There is an improved effect on blood parameters, liver enzymes, and histological composition resulting from tocotrienol treatment against the effects of a diet high in fat content.

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Sajad Haider Khairy✉, Afrah Abid Maktoof
College of Science, University of Thi-Qar, Thi-Qar, Iraq

Study of the Relationship Between Some Heavy Elements and Malondialdehyde (MDA) in the Blood of Workers in Al-Nassiriyah Streets

Conflict of interest: nothing to declare.
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Contacts: sajadh@sci.utq.edu.iq

Abstract

Introduction. Certain heavy metals, including Lead (Pb), Cadmium (Cd) and Copper (Cu) can have negative effects on human health even at low concentrations via affecting the antioxidant molecules Malondialdehyde (MDA).
Purpose. To evaluate the levels of Pb, Cd, Cu, and Zn and demonstrate their effect on MDA, antioxidant molecule, in the blood of workers.
Materials and methods. The present study was included 120 men aged 26–46 years and were divided into three groups (40 hawkers, 40 traffic police and 40 control). Pb, Cd, Cu, and Zn were measured in the serum of 120 participant men by using Flame Atomic Absorption Spectrophotometer (FAAS). Moreover, the levels of MDA were also measured using Buege and Aust method.
Results. A great increased in the levels of (Pb) and (Cd) in the hawkers as compared to other groups. In addition, Cu showed a significant increase in hawkers and traffic police groups compared with control group. In contrast, the concentration of (Zn) was increased in Control group compared with other groups traffic police group. However, the statistical analysis showed no significant differences between the study groups.
Conclusion. Hawkers and traffic police are more likely to have elevated serum levels of Pb and Cd. However, increased concentrations of Zn could protect against the oxidative stress in hawkers and traffic police groups.
Keywords: air pollution, heavy elements, oxidant, malondialdehyde (MDA), flame atomic absorption spectrophotometer (FAAS)

■ INTRODUCTION

Air pollution is a serious threat and widespread to the environment and is responsible for many harmful health effects. Due to the ease with which pollutants spread and the speed at which they move through the air from one location to another, air pollution is

one of the most prevalent environmental contamination issues [1]. This pollution may reflect anthropogenic sources emanating from road traffic that contains vehicle exhaust emissions and also tire and brake wear [2]. Urban environments are characterized by the presence of trace elements in the air, especially in areas experiencing high levels of traffic congestion and industrial activities [3]. All of Cars are considered a major source of pollution which included Lead (Pb) and other trace elements. These type harm the health of human and animals [4]. Any metallic element with a reasonably high density that is hazardous even in small concentrations is considered a heavy element. They are a class of elements and metalloids that have a specific density that is five times more than water, or more than 5 g/cm3. They are found naturally in the earth's crust and are persistent environmental pollutants that are incapable of being broken down or degraded [5]. Moreover, pollution of the natural environment with heavy metals is a global problem, as these pollutants pose a serious threat to the stability of the ecosystem and in particular the effects of cadmium and lead that are toxic and pose a major threat to human health [6]. Elements in biological systems have an interesting aspect in that some minerals important for health (such as copper) can become toxic under certain conditions. These metal ions can compete with critical ions for high-affinity metal-binding sites, resulting in structural changes and metal homeostasis disturbances [7]. It important to mention here that in addition to being a crucial cofactor for enzymes that catalyze metabolic processes, copper is a significant component of several other enzymes [8]. Moreover, it has been documented that Zinc has an essential anti-inflammatory role and its ability to reduce oxidative stress are given special consideration [9]. Furthermore, due to its usage in the construction and operation of numerous enzymes, such as the anti-inflammatory and antioxidant Cu/Zn superoxide dismutase, copper and zinc are involved in a wide range of biological activities [10, 11]. A recent study has found that Zn indeed is associated with decreased MDA levels [12]. Interaction of (Cd) and (Pb) with essential trace elements has been studied extensively in animals. According to these findings, heavy metals that are present in excess of typical levels becoming toxic and hazardous [13]. Humans can be exposed to heavy elements like (Pb) and (Cd) in polluted environments, which are considered to be toxic at low concentrations [14]. Free radicals are unstable molecules that can damage cells. Antioxidants are substances that protect cells from these molecules. Antioxidants can interact with free radicals to neutralize them and prevent them from causing harm [15]. Oxidative stress happens when the antioxidant defense system – which is made up of antioxidants – is overtaxed. Oxidative stress is believed to cause cellular and molecular damage, which further leads to tissue destruction when the antioxidant protection mechanism against ROS is compromised [16]. In addition, antioxidants are frequently reducing chemicals that oxidize to stop other oxidation reactions and eliminate free radical intermediates to inhibit chain reactions [15]. Malondialdehyde (MDA) is one of the end products of the lipid peroxidation produced by ROS formation and used as an indicator to estimate oxidative stress [17].

■ PURPOSE OF THE STUDY

To evaluate the levels of Pb, Cd, Cu, and Zn and demonstrate their effect on MDA, antioxidant molecule, in the blood of workers.

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■ MATERIALS AND METHODS

Study design

The present study was performed in Nasiriyah city,south of Iraq. The study included (120) participants with the age range 26–46 year. Samples were divided into three groups, 40 Hawkers, 40 Traffic Policeand 40 samples served as control group.

5 ml blood samples were harvested from participant people in this study. Blood samples were subjected to 5000 rpm centrifuge for 5 minute. Then, serum collected and kept at –20^{o}C till used.

Estimation of heavy elements

The levels of Pb, Cd, Cu and Zn elements were determined in the blood serum using Flame Atomic Absorption Spectrophotometer (FAAS. – Phoenix 986 AA. United Kingdom UK and according to manufacturer's instructions.

Determination of serum malondialdehyde (MDA) principle

Lipid peroxidation was measured using the thio bar bituric acid method and as previously described [18]. Briefly, Polyunsaturated fatty acids destruction results in (MDA). As a result of LPO reacting with thio bar bituric acid (TBA) in coexisting tri chloroacetic acid (TCA), a pink chromophore that absorbs at 535 nm is produced. The MDA concentrations were determined by utilizing the molar extinction coefficient of MDA (E MDA), which is equivalent to 1.56×105 mol-1. cm-1.

Procedure

A thio bar bituric acid (TBA) solution was used to measure the levels of serum MDA spectrophotometrically and according to previous study [19]. The following were added to a 150µl serum sample: 1 milliliter of 17.5 percent trichloroacetic acid (TCA) and 1 milliliter of 0.66 percent TBA were well combined using a vortex, incubated for 15 minutes in boiling water, and then allowed to cool. One milliliter of 70% TCA was added, and the mixture was let to rest at room temperature for twenty minutes. After centrifuging for fifteen minutes at 2000 rpm, the supernatant was removed and subjected to spectrophotometric scanning at (532nm).

$$\text{MDA } \frac{\mu\text{mol}}{\text{L}} = \frac{\text{Absorbance at 532 nm}}{\text{L} \times \text{E}^{\circ}} \times \text{D} \times 10^{6}.$$

The concentration of MDA calculated as follow:

L: Light Path (1cm).
E_°: Extinction coefficient (1.56×105 mol-1. cm-1).
D: Dilution factor = 1 ml Vol. Used in ref./0.15 = 6.7.

Statistical analysis

The statistical analysis was performed using SPSS version 26. The data of present study were analyzed using independent sample t test, One Way ANOV and LSD The statistical significant between groups was at p. value <0.05.

■ RESULTS

Age criteria was considered in the present study. Our results showed no significant differences in mean of age between the groups. Moreover, the results also showed no significant differences between hawker and traffic police in regard to service period. In contrast, our findings documented a significant difference between groups according smoking criteria at p<0.05, as shown in the table.

Demographic characteristic of studied groups

Demographic		Hawker	Police-man	Control	p. value
		Mean±S.D.			
Age		36.6±9.28	39.2±5.44	37.5±6.57	0.281
Service period		14.9±8.17	13.9±5.57	0.0±0.0	0.504
Smoking	Yes	21 (52.5)	19 (47.5)	4 (10)	< 0.01
	No	19 (47.5)	21 (52.5)	36 (90)	

The current study showed a great increase in the Pb levels in hawkers and traffic police as compared to control group and corresponding to mean±SE (0.21±0.04, 0.28±0.09 and 0.16±0.05, respectively) (Fig. 1A). In addition, the statistical analysis showed a significant elevated the Pb levels in traffic police comparing to hawkers (Fig. 1A). Moreover, we found

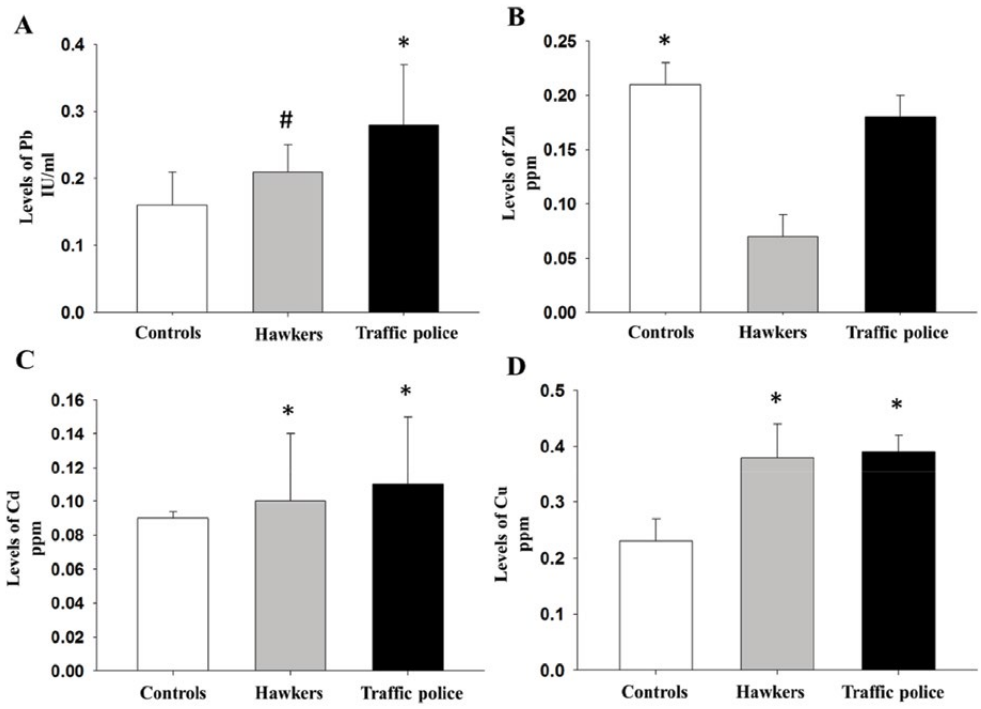

Fig. 1. Levels of Heavy elements in Hawkers, Traffic police and control group. Serum levels of (A) Pb, (B) Zn, (C) Cd and (D) Cu. White box represents healthy control group. Gray box represents hawkers. Black box represents traffic police. * P<0.05 versus control and # P<0.05 versus traffic police

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that the levels of Cd and Cu substantially increased at p. value <0.05 in hawkers and traffic police in related to control group (Fig. 1C and D) However, the present results found that the level of Zn increased significantly in control group comparing to hawkers and traffic police (Fig. 1B).

Malondialdehyde analysis

Next, it was important to examine the levels of MDA between the study groups. The statistical analysis showed no significant differences in the levels of MDA between study groups at p. value <0.05 (Fig. 2).

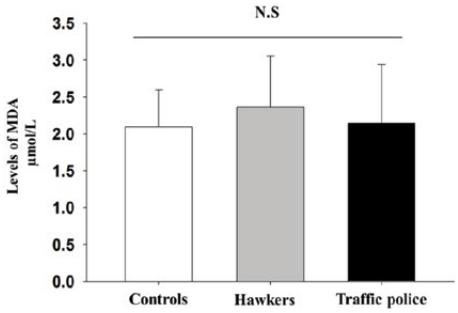

Fig. 2. MDA concentration in hawkers, traffic police and control group. Serum levels of MDA. White box represents healthy control group. Gray box represents hawkers. Black box represents traffic police. N.S = no significant between the groups

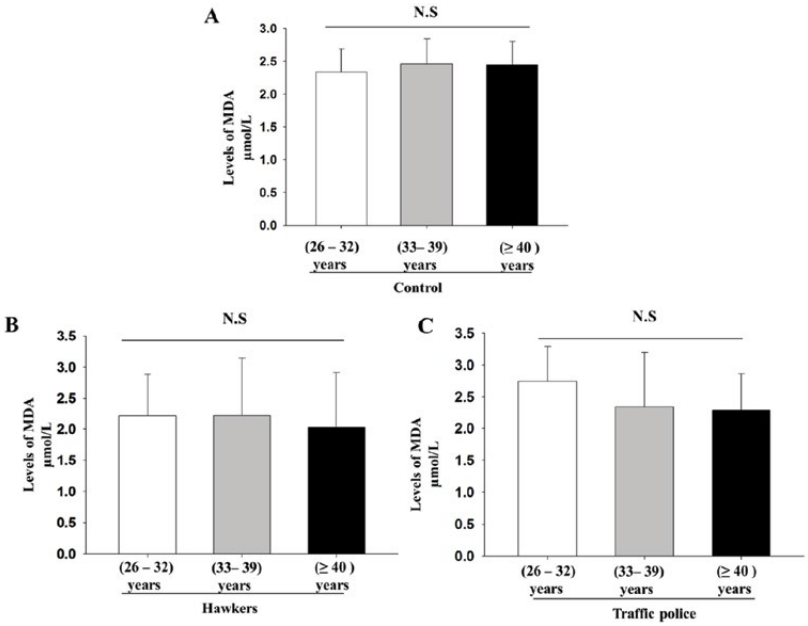

Fig. 3. Malondialdehyde levels between the groups according to the age factor. Serum levels of MDA for age group (A) (26–32) year, (B) (33–39) year and (C) (≥40) year, in control, hawkers and traffic police, respectively. Gray box represents hawkers. Black box represents traffic police. N.S = no significant between the groups

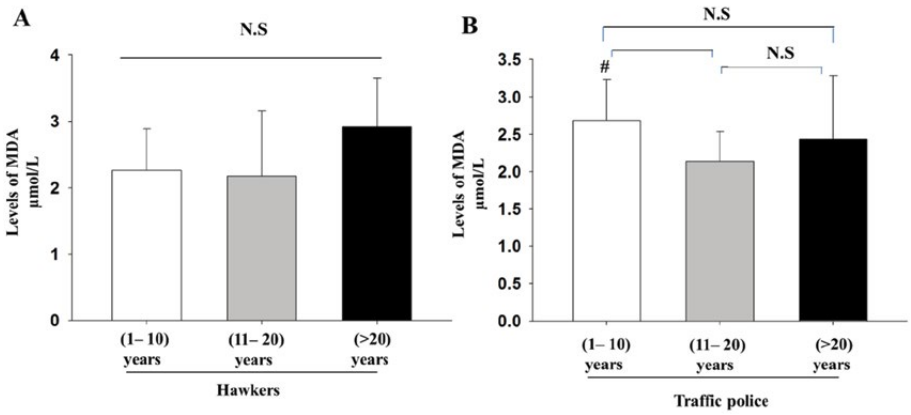

Fig. 4. Malondialdehyde levels between the hawkers and traffic police according work duration. Serum levels of MDA for work period (A) (1–10) years, (B) (11–20) years and (C) (>20) year, in hawkers and traffic police, respectively. Gray box represents hawkers. Black box represents traffic police. # P<0.05 versus (11–20) years group. N.S = no significant between the groups

It was important to examine the levels of MDA in the study groups according to the age criteria. We found that the levels of MDA showed no significant differences between age groups in control, hawkers and traffic police (Fig. 3A, B and C).

In addition to age factor, we also examined the work period for the study groups. The levels of MDA showed no significant differences among the work period in the hawkers group (Fig. 4A). However, the statistical analysis showed a significant increase in the levels of MDA in the work period (1–10 years) as compare to (11–20 and >20 years, respectively) in the traffic police group (Fig. 4B).

■ DISCUSSION

The current findings demonstrated that hawkers and traffic police are more likely to expose to heavy metals such as Pb, Cd and Cu. The study also showed that levels of Zn increased in hawkers and traffic police. Moreover, our findings did not found a significant difference in the levels of MDA between the groups. Thus, increased levels of Zn could protect against the oxidative stress in hawkers and traffic police groups.

The current investigations shoed that in comparison to the control group, the Pb concentration in the hawkers group was higher (0.28 ppm), with a significant difference (LSD=0.031). Additionally, the concentration of Pb in the traffic police group was higher (LSD=0.031) than that of the control group (0.21 ppm) (Fig. 1). This suggests that because hawkers labor during the day and their workplaces are in close proximity to lead fumes, they are continuously exposed to vehicle emissions. Police traffic is exposed to lead during the half-day work session. The findings align with the research conducted by Tchounwou, Paul et al. (2016), which demonstrated that elevated levels of Pb in the bloodstream of laborers are a consequence of their occupational practices and inadequate safeguards against environmental contaminants, thereby augmenting their susceptibility to hazardous substances [20]. In line with Jalood's (2020) research on industrial foundation workers, workers in automobile repair workshops had significantly higher blood serum lead concentrations than those of the other study groups [21]. This is likely because

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welding exposes workers to lead fumes and dust. The concentration of trace elements varies depending on the workplace, according to almost all worker studies. Similarly, Altaay et al. (2018) discovered in their research, workers in The Industrial and traffic Areas in Baghdad city, that the blood serum Pb content of auto repair shop employees and traffic police officers was considerably greater than that of the other groups [22]. Pb is found in healthy individuals' serum in concentrations of less than 0.001 ppm, according to a 1988 study by Lyengar and Wolttlez. However, the WHO has lately suggested that Pb is dangerous at all levels [23].

Moreover, in comparison to the control group, the hawkers group had a significantly higher concentration of Cd (0.11 ppm) (LSD=0.019) (Fig. 1). However, the results did not show any appreciable distinctions between the traffic police and other groups. This could be the result of Cd exposure at work, as the respiratory system absorbs 15–30% of the total Cd in occupational exposure settings, compared to less than 0.05 µg/L in clear areas for the general public. In polluted areas, Cd levels can reach unusually high levels, up to 30 times higher than in areas free of pollutants [24]. The International Cadmium Association (ICdA) states that the anticipated levels of Cd in suspended. The present results were in line with the majority of studies [18, 21] that found a link between occupational exposure and mercury (Cd). They showed that there were notable distinctions between those who lived far from pollution sources and those who were exposed to pollution at work. Once absorbed through the lungs, Cd is excreted in equal amounts as urine and feces. Cd builds up in the body as a result of excessive exposure, and this has a variety of negative consequences on the body, such as oxidative stress, apoptosis, and DNA damage. The main organs affected by Cd poisoning are the kidneys, the lungs, and the bones [25].

In addition, (Fig. 2D) also showed that hawkers and traffic police had higher concentrations of copper element than the control group (0.39 ppm and 0.38 ppm), with a significant difference (LSD=0.024). The three elements that are most prevalent in the body are copper, iron, and zinc. Ceruloplasmin is a co-factor in the oxidation process, and Cu is a component of cytochrome oxidases, cytochrome oxidases, and dopamine. Approximately 65–70% of cu can be bioavailable through diet, depending on its molecular structure and interactions with other minerals, among other things. It is mostly removed by bile and has a half-life of 13–33 days in bioavailable meals. Cu is a structural element or cofactor of enzymatic antioxidants like glutathione [26]. Previous research discovered that all workers' blood concentrations of Cu increased when compared to the control group [18, 27]. In actuality, elevated amounts of Cu can raise the production of harmful ROS, which may raise the risk of oxidative stress illnesses even though copper is an essential component of the human body. Moreover, consuming large amounts of Cu can result in nausea, diarrhea, and vomiting. Breathing high quantities of Cu can also irritate the throat and nose. Excessive use of Cu can harm the liver and kidneys and could result in death [28], however, found that all study groups had lower Cu concentrations than the control group. Low blood copper levels have been linked in studies by Kil, Min Seong et al. (2013) to anemia, hair loss, coronary heart disease, frailty, and bone necrosis [29]. Furthermore, our findings demonstrated a significant increase (LSD=0.019) in the zinc concentration in the control group as compared to other hawkers and traffic police (Fig. 1C). Numerous earlier research concurred with this conclusion [18, 30]. Zinc is crucial for human health; deficits in zinc can lead to serious

clinical problems as well as enzymes necessary for intracellular functions. Because zinc participates in some activities as an enzyme cofactor, it is comparable to copper in the body [31]. Additionally, a number of studies showed that workers' blood serum had a greater Zn concentration than that of the control group [32, 33]. Significant antioxidant activity of zinc has been demonstrated in the lungs and other body parts. It plays a part in immunological processes such inflammation and oxidative stress and is essential to immune function case control [34].

The current investigations concentrated on MDA as a biomarker of lipid peroxidation; as shown in Fig. 2 that the present results did not reveal any noteworthy distinctions between the study and control groups. These findings are consistent with previous study found that increasing copper concentrations cause a decrease in peroxidation levels [35]. MDA takes into account the most detrimental consequences of oxidative stress. It is used as a marker of oxidative damage and is a result of polyunsaturated fatty acid lipid peroxidation [36]. Increased formation of reactive oxygen species (ROS) causes a rise in lipid oxidation and MDA production, which is an excellent biomarker of oxidation. High MDA concentrations show increased lipid peroxidation as a result of prolonged exposure to organic solvents and trace elements. Similarly, previous study found that trace metals like zinc, magnesium, and copper were formed as cofactors of antioxidant enzymes and were constantly used to build antioxidant enzymes that have a role in the detoxification of reactive oxygen species [37].

Fig. 3 from the current study show that there were no discernible differences between the age groups in any of the study groups. These findings are at odds with those of Al-Azzawi (2022), who found substantial variations in study groups and control groups based on age groupings. MDA may change the structure and function of proteins, DNA, and other biomolecules through interaction [38]. Moreover, we also examined the levels of MDA based on work duration and the results indicated a substantial increase in the traffic police group between the years of 1–10 and 11–20 (LSD=0.024). However, the hawkers group did not demonstrate any significant variations across categories. This study's findings concur with those of Medina-Navarro et al. (1997), who examined the impact of air pollution on a population in Mexico City. MDA levels were measured in the first and sixteenth weeks; in the sixteenth week, a decrease in MDA was seen. He ascribed the causes to the human organism's capacity for adaptation [39]. Additionally, the study's findings concur with those of Khajehnasiri et al. (2013), who discovered that workers' MDA levels increased during work periods [40].

We also looked at the link between MDA levels in the groups of smokers and non-smokers in this study, as shown in table. However, there were no discernible changes between the study groups according to the data. This data is consistent with a study conducted in Mosul City, Iraq, by Luay and Ahmed (2014) about antioxidants and some biochemical parameters in workers exposed to pollutants from petroleum stations [41].

■ CONCLUSION

Based on the study's findings, we can say that, in comparison to the control group, there was no increase in the incidence of oxidative stress among the study groups' occupational exposure to heavy metal levels in Nasiriyah's streets. This could be because the research groups' concentrations of the elements (copper, zinc) were higher.

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Iqbal Aziz Ameen[1]✉, Hayder Fadhil Okab[2]
[1] College of Education for Girls, Al-Shatrah University, Thi-Qar, Iraq
[2] Thi-Qar Health Directorate, Thi-Qar, Iraq

Evaluation of Immune and Inflammatory Response in Diabetic Foot Patients and Patients Presenting Diabetes Only in Thi-Qar Province / Iraq

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Contacts: al-kalibyiqbal@utq.edu.iq

Abstract

Introduction. In the group of diabetes complications, diabetic foot is a key contributor to the disease, so it is essential to raise public understanding of basic health care practices, including self-care for diabetics' feet.
Purpose. To study the immunological reactions of diabetic patients.
Materials and methods. The current study involved 30 patients 15 with diabetic mellitus and 15 with diabetic foot and 15 as control group, the study was conducted on patients attending the outpatient clinics for the treatment of diabetes patients in Thi-Qar Governorate/ southern of Iraq. All groups were tested for FBS, HbA1c by spectrophotometer, and immune parameters were estimated by cobas for CRP, ESR manually and IL-6 by ELISA.
Results. The BMI was significant increase in both diabetic groups, while age not-recorded significant difference. In metabolic the FBS increased in DM2 patients only, HbA1c increase, ESR, CRP and IL-6 significantly in Diabetic**.** BMI recorded a non-significant difference in all studies parameters according gender, with except ESR was recorded significant increase in both male and female patients with diabetic foot, additionally, patients with diabetic feet who are both male and female show a large rise in IL-6. Significant CRP increases were seen in diabetic patients with illnesses lasting longer than five years.
Conclusion. Diabetes and diabetic foot are chronic diseases that have invaded the planet, and treatment protocols for diabetic foot have not achieved anything on their own, so following health safety methods and periodic examinations for ages beyond 30 years, as an early detection of diabetes is the most appropriate method, especially in developing countries.
Keywords: diabetic, inflammatory response, IL-6, diabetic foot, spectrophotometer

INTRODUCTION

Diabetes mellitus considered one of the most common chronic non-communicable diseases and that distributed worldwide. By 2025, the WHO predicts that over 60% of diabetics would reside in developing Asian nations [1]. Diabetes complications are now a significant global public health issue [2]. It is marked by numerous, persistent problems that frequently result in cardiovascular disease, blindness, kidney failure, stroke, amputations, and nerve damage and impact almost every organ in the human body [3]. Both diabetics with type 1 and type 2-foot issues experience foot problems, and it is thought that the patient risk of having a foot ulcer may be higher than 30%. Patients over 60 years old and men are more likely than women to develop ulcers [4]. Diabetic patients that develop foot ulcers are associated with many risk factors, peripheral arterial disease (PAD) is present in approximately half of all patients with foot ulcers and is an important predictor of outcome [5]. Asia and sub-Saharan Africa, foot problems, particularly foot ulcers, are a major public health problem among individuals with diabetes and are significant contributors to longer hospitalizations and patient mortality in these regions [6]. He most frequents reason for prolonged hospitalization and amputation of limbs is diabetes foot infection resulting in gangrene. Furthermore, within five years of the initial amputation, 28–51% of diabetics who had lower limbs amputated will get a second amputation [7]. As a complicated condition, obesity is frequently associated by insulin resistance, elevated oxidative stress, and increased expression of inflammatory markers. More than 300 million people are considered obese by the Global Obesity Task Force, and the majority of these individuals get diabetes [8].

With no evidence that biomarkers or imaging approaches can reliably detect the existence of bone infection or monitor treatment efficacy, diagnosing diabetic foot and monitoring infection approval remain hard. Although most guidelines consider bone culture and histology to be the gold standard for diagnosing DFO, they are rarely used in clinical practice [9]. The best laboratory test currently available for identifying and tracking DFO has been suggested to be the erythrocyte sedimentation rate (ESR) [10]. The relevance of C-reactive protein (CRP), procalcitonin (PCT), and cytokines for the diagnosis and monitoring of osteomyelitis in diabetic foot has only been studied in a few numbers of studies [11]. Unfortunately, it is challenging to compare the data because of the poor quality of the available studies, the small number of patients, and the inconsistent use of reference tests to confirm DFO [12]. The most crucial sort of treatment given to a patient is primary care; it is the first line of defense and frequently serves as emergency first aid. Primary care can be given by a medical professional via the herbal route or by the patient himself as self-care. In any instance, it is essential that the person making the assessment is knowledgeable and skilled in order to provide the necessary management and care of the ailment at hand.

PURPOSE OF THE STUDY

To study the immunological reactions of diabetic patients

MATERIALS AND METHODS

Sample collection

The current study included 45 participants (30 patients, 15 with diabetes only, 15 with diabetic foot, and 15 as a control group). The study was conducted in the outpatient clinics

for diabetic patients in Thi-Qar governorate for the period from December 2022 to Marich 2022. Each patient and member of the control group had three milliliters of venous blood drawn. The samples were divided into two parts, the first of which was used to calculate the ESR.

It was left at room temperature for about 30 minutes to allow the blood to coagulate before being centrifuged at 4000 rpm. The serum was then collected and crimped until it was needed. The parameters were estimated as follows, FBS, HbA1c, CRP were estimated by Cobes instrument and IL-6 was estimated by ELISA.

Statistical analysis

By using SPSS version 26, the data for this study were analyzed using independent t-test, one-way ANOVA, and LSD with a p-value less than 0.05.

■ RESULTS

Table 1 shown the BMI recorded a significant increase in both diabetic groups, while age not-recorded significant difference. In metabolic the FBS increased in DM2 patients only, HbA1c increase, ESR, CRP and IL-6 significantly in Diabetic foot compared with other groups at p. value <0.05, as shown in table 3–1.

Table 1
BMI, age and other metabolic and immune parameters in DM2 groups with control

Statistic Groups	DM only	D-Foot	Control	p. value	LSD
	Mean±SD				
BMI, Kg/M^2	30.3±3.9^a	29.4±7.6^a	25.3±3.3^b	0.032	3.9
Age Year	47.6±7.4^a	52.0±8.9^a	48.6±9.4^a	0.335	Non-sig
FBS, mg/dl	219.6±49.7^a	177.8±48.6^b	88.0±11.5^c	0.000	30.0
HbA1c, mg/dl	8.04±1.1^b	9.81±2.3^a	5.05±0.2^c	0.000	1.1
ESR, mm/1H	14.8±3.1^b	21.5±5.6^a	10.7±1.4^c	0.000	2.8
CRP, mg/dl	5.38±1.4^b	6.86±2.0^a	3.89±0.6^c	0.000	1.1
IL-6, ng/L	10.6±2.3^b	17.6±3.2^a	6.93±1.6^c	0.000	1.8

Note: the similar small letters indicate that there are non-significant differences, while different letters indicate the presence of significant differences at p. value <0.05.

Table 2
BMI, age and other metabolic and immune parameters according to gender

Statistic Groups	Male		Female		p. value	LSD
	DM only	D-Foot	DM only	D-Foot		
	Mean±SD					
BMI	27.3±1.6^a	27.6±7.6^a	31.8±3.8^a	31.1±9.0^a	0.362	Non-sig
Age	49.2±7.3^a	54.0±9.6^a	46.8±7.7^a	50.3±8.5^a	0.394	Non-sig
FBS	192.8±23.6^a	182.8±56.2^a	233.0±54.7^a	173.4±44.4^a	0.072	Non-sig
HbA1c	7.72±0.6^a	9.75±2.6^a	8.20±1.3^a	9.85±2.2^a	0.104	Non-sig
ESR	15.2±2.9^b	21.0±7.4^a	14.7±3.3^b	22.0±3.9^a	0.007	3.5
CRP	4.44±0.6^a	7.05±2.1^a	5.85±1.5^a	6.70±2.1^a	0.086	Non-sig
IL-6	9.70±1.5^c	15.7±2.2^b	11.0±2.6^c	19.3±3.0^a	0.000	1.8

Note: the groups were classified in to 5 male and 10 female with diabetic only, 7 female and 8 males with diabetic foot.

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Table 3
BMI, age and other metabolic and immune parameters according disease period

Disease Period	Diabetic only		p. value	Diabetic Foot		p. value
	<5 years	>5 years		<5 years	>5 years	
	Mean±SD			Mean±SD		
FBS	218.7±41.1	220.5±61.5	0.947	197.5±38.3	155.3±51.9	0.094
HbA1c	8.11±0.9	7.95±1.3	0.803	9.18±2.3	10.5±2.2	0.283
ESR	15.2±2.3	14.4±3.9	0.628	19.7±4.3	23.5±5.2	0.206
CRP	4.35±0.7	6.55±1.1	0.001	7.03±2.6	6.67±1.2	0.737
IL-6	10.3±2.7	10.9±1.9	0.613	16.7±2.1	18.7±4.0	0.243

Note: the groups were classified in to 8 less than 5 year and 7 more than 5 years in both group.

Table 2 shown the BMI recorded a non-significant difference in all studies parameters according gender, with except ESR was recorded significant increase in both male and female patients with diabetic foot, also the IL-6 record significant increase in both male and female patients with diabetic foot at p. value <0.05.

Table 3 recoded a non-significant difference in all studies parameters according disease period, with except CRP was recorded significant increase in diabetic patients with disease more than 5 year compare with patients with disease period less than 5 year at p. value <0.05.

■ DISCUSSION

The current study recorded the diabetic patients have high BMI than control group, and FBS increase significantly in patients with DM_2, while HbA1c, CRP, ESR and IL-6 increase in diabetic foot compared with diabetic and control group.

According to gender the result noted the ESR and IL-6 increase in both male and female of diabetic foot. While the study not recorded a significant difference according to disease period with except CRP was increased with disease period in diabetic patients. The finding of Renero [13], was like study results, their study investigated the diabetic patients have high BMI than healthy patients, and diabetic women is high BMI than men.

Moore et al. [14], recorded in diabetic patients, the physiological process of plantar skin temperature regulation is affected somewhere within its pathways as from the free nerve to the integumentary structure of the hypothalamus, and/or from the hypothalamus up to stimuli to conserve or dissipate heat.

In my opinion the patients with type 2 diabetes, patients may be developed diabetic foot due to damage to the peripheral vessels and nerves, which results in irregular thermoregulation of both feet. Several studies showed the Obesity induce development of chronic inflammation. Obese patients have excess adipose tissue and hypertrophic adipocytes lead to high concentration of fibrinogen, CRP and other acute phase proteins including as IL-6 and other pro-inflammatory cytokine in blood circulation. The increased pro-inflammatory cytokines stimulate an endothelial vascular response.

There is enhanced production of adhesion molecules together with adipokine lead to induced chemokines, which stimulate the recruitment of macrophages to adipose tissue, and the result local inflammation enhances local insulin resistance [15, 16]. The study of Costa et al. [17], recorded the mean age was more than mean age in the current study.

The study of Setacci et al. [18]. In a study involving more than 11 hospitals in various regions of Argentina, it was discovered that diabetic patients had a significant amputation rate of 32.5%. It was also discovered that infections were the most frequent direct causes of amputation, with peripheral arterial disease (PAD) serving as the major contributing factor, and that the mean age was higher than in our study at 63 years. The likelihood of lower limb amputations in diabetic patients with diabetic feet has been linked to aging [19], and a specific risk factor for in-hospital mortality.

These elderly people usually have peripheral neuropathy, progressive atherosclerosis with peripheral arterial disease, carotid and coronary artery disease, and chronic renal failure [20, 21]. The recent study performed by Casadei et al. [22], their study involved diabetic patients with foot ulcer, and their study concluded the HbA1c was increased in diabetic foot compared with other diabetic groups, also recorded the diabetic foot were more incidence in men than women. Several studies recorded the peripheral neuropathy in diabetic foot is often associated with other complications caused by diabetes.

The quality of life of these individuals might be impacted by a variety of diabetic foot syndrome-related factors, including gait alterations, psychological concerns, and even disruptions; In addition, men are more likely to have diabetic foot weakness than women are, although the frequency of this condition varies by geographic area [23, 24]. This study also included health education and knowledge of the danger posed by diabetic feet and how to maintain the feet in the event of ulcers. The recent study of Appil et al. [25], recorded the education through family empowerment, family assistance, and family intervention in the management of individuals with diabetes has been shown to improve health status and glycemic control.

The study of Aura Victoria et al. [26], it was noted that after 14 days of therapy, CRP, ESR, PCT, and IL-6 levels were significantly lower in patients without foot osteomyelitis than in patients with osteomyelitis but remained elevated in patients with osteomyelitis. However, the study did not reach significant differences in reducing biomarkers parameters. The study concluded that the best approach to prevent foot disease from developing is to take care of the feet and adhere to medical advice.

Preoperative testing for ESR and CRP was used to evaluate 499 patients who underwent revision total hip arthroplasty in retrospective research by Ghanem et al. [27]. ESR and CRP readings were considerably higher in patients with PJI, with optimal cutoff values of 31 mm/hr for ESR and 20.5 mg/L for CRPs.

■ CONCLUSION

Diabetes and diabetic foot are chronic diseases that have invaded the planet, and treatment protocols for diabetic foot have not achieved anything on their own, so following health safety methods and periodic examinations for ages beyond 30 years, as an early detection of diabetes is the most appropriate method, especially in developing countries.

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Sara S. Mahdi[1]✉, Huda S. Abdulghani[2]
[1] College of Pharmacy, Ashur University, Baghdad, Iraq
[2] College of Engineering, University of Baghdad, Baghdad, Iraq

Clinical Study to Investigate Serum Vitamin D3 and Ferritin Levels in Iraqi Adult Obese Women

Conflict of interest: nothing to declare.
Authors' contribution: Sara S. Mahdi – conceptualization, data curation, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft and writing – review & editing; Huda S. Abdulghani – conceptualization, data curation, validation, visualization, writing – original draft and writing – review & editing.
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Contacts: Sara.salah@au.edu.iq

Abstract

Purpose. To reveal serum ferritin and vitamin D3 level and their agreement or disagreement with different previous research to evaluate the current evidence linking vitamin D3 status and ferritin with obesity.
Materials and methods. Fifty Iraqi women are divided into two groups: group 1 with obese (25) and group 2 (25) normal weight were accepted for the study. The participants were classified as obese (BMI ≥30.0 kg/m^2) and normal weight (BMI = 18.5 to 24.9 kg/m^2) group. The informed clinical data, including general information, anthropometric data, body composition and blood analysis results, were obtained from the participants. Analysis showed a significant alteration in all the parameters in obese adults.
Results. The correlation of ferritin and vitamin D level with body mass index showed a negative correlation ($p<0.01$) respectively. Level of serum vitamin D3: The mean ± SD of serum vit. D3 (ng/ml) of obese was (10.5±5.902 ng/ml) that indicated a highly significant decrease ($p<0.01$) in comparison to that level in normal group (38.71±12.627 ng/ml).
Conclusion. Estimation of serum ferritin level will be an important early indicator for the risk of developing metabolic disorders in young adults.
Keywords: obese women, iron deficiency, ferritin, vitamin D, obesity

■ INTRODUCTION

Obesity is a serious public health problem. In Iraq, the prevalence of overweight and obesity were 61.1% and 76% [1, 2]. Worldwide, among adults, the prevalence of obesity (body mass index (BMI) (30 kg/m^2) is 10.8% among men and 14.9% among women [3].

Obesity and iron deficiency are global health problems affecting billions of people worldwide [4, 5]. The first report of a potential connection between people with obesity and iron status appeared in the early 1960s [6]. One of the most important components of cellular or organismal iron homeostasis is iron storage. Intracellular iron storage function

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is carried out by ferritin, which is structurally composed of 24 subunits of light (FTL) and heavy chains (FTH) that form a nano-cage complex to hold up to 4500 iron atoms [7]. Ferritin sequesters excess intracellular iron and stores it in a redox-inactive form for future use in conditions of deficiency or high demand. Cellular and systemic ferritin levels are not only crucial indicators of iron status but are also important markers of inflammatory [8]. Ferritin could be an important early indicator for the risk of developing adipose tissue dysfunction in obese individuals [9]. The association between obesity and iron deficiency. The mechanisms responsible for this relationship remains undefined [10].

Obesity is now frequently cited as a cause of vitamin D3 deficiency [11, 12] which may in fact lead to other co-morbidities, such as diabetes and CVD, conditions commonly linked to obesity [13].

Overall, a large amount of evidence supports an inverse (albeit sometimes weak) association between adiposity and vitamin D status in adults [14].

Indeed, obesity and vitamin D3 deficiency have concomitantly reached epidemic levels worldwide and research linking the two has grown extensively over the last number of years. It is reasonable to suggest that this nutritional inadequacy may be driven by the increasing obesity rates within the general population, and may therefore improve with a reversal of the latter [14].

■ PURPOSE OF THE STUDY

To reveal serum ferritin and vitamin D3 level and their agreement or disagreement with different previous research to evaluate the current evidence linking vitamin D3 status and ferritin with obesity.

■ MATERIALS AND METHODS

Fifty Iraqi women are divided into two groups: group 1 with obese (25) and group 2 (25) normal weight were accepted for the study. The participants were classified as obese (BMI ≥30.0 kg/m^2) and normal weight (BMI = 18.5 to 24.9 kg/m^2) group. The informed clinical data, including general information, anthropometric data, body composition and blood analysis results, were obtained from the participants. Patients with hereditary anemia were exclusion. Serum vitamin D [25(OH)D3] and ferritin were measured. Statistical analyses were performed using the SPSS Statistics for Windows version 24.0.

Diagnostic measurement using kit manufactured by cobas e 411 analyzers for immunoassay tests (ElectroChemiLuminescence (ECL) technology for immunoassay analysis).

■ RESULTS

The results presented in this paper based on the analysis of 50 patients divided into two main groups depending on anthropometric measurements as shown in Table 1.

Table 1
Information of the studied groups

Groups	No.
Obese women	25
A woman of normal weight	25

The result presented in Table 2 indicate highly significant ($p<0.01$) differences of Ferritin, Vitamin D3 and BMI between studied groups.

Table 2
Mean±SD levels of studied parameters

Parameter	Group	Mean	SD	P value
BMI	Obese women	38.18	8.811	0.0001
	A woman of normal weight	21.33	1.897	
Vit D3	Obese women	10.5	5.902	0.0001
	A woman of normal weight	38.71	12.627	
Ferritin	Obese women	7.86	3.811	0.0001
	A woman of normal weight	46.74	24.08	

Anthropometric measurements

BMI are presented in Fig. 1 showed a highly significant increase ($p<0.01$) between studied groups.

Level of serum vitamin D3: The mean ± SD of serum vit. D3 (ng/ml) of obese was (10.5±5.902 ng/ml) that indicated a highly significant decrease ($p<0.01$) in comparison to that level in normal group (38.71±12.627 ng/ml), as shown in Fig. 2.

Level of serum ferritin: The mean ± SD of serum ferritin (ng/ml) of obese was (7.86±3.811 ng/ml) that indicated a highly significant decrease ($p<0.01$) in comparison to that level in normal group (46.74±24.08 ng/ml), as shown in Fig. 3.

As shown in Table 3 ferritin results showed a significant ($p<0.05$) negative correlation with BMI between studied groups (Fig. 4).

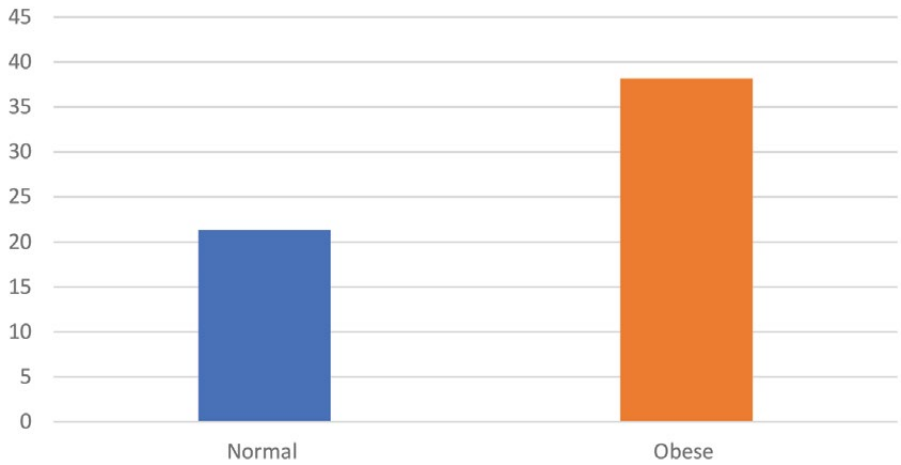

Fig. 1. Mean values ± SD of BMI of obese and normal weight women

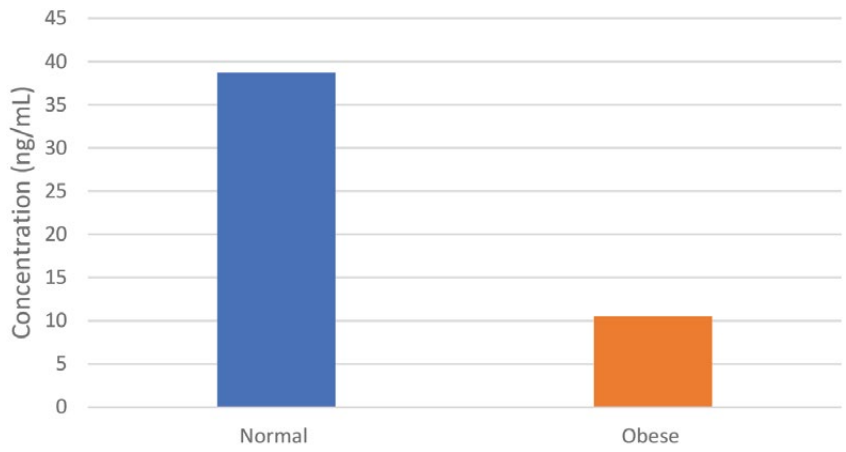

Fig. 2. Mean values ± SD of vitamin D3 of obese and normal weight women

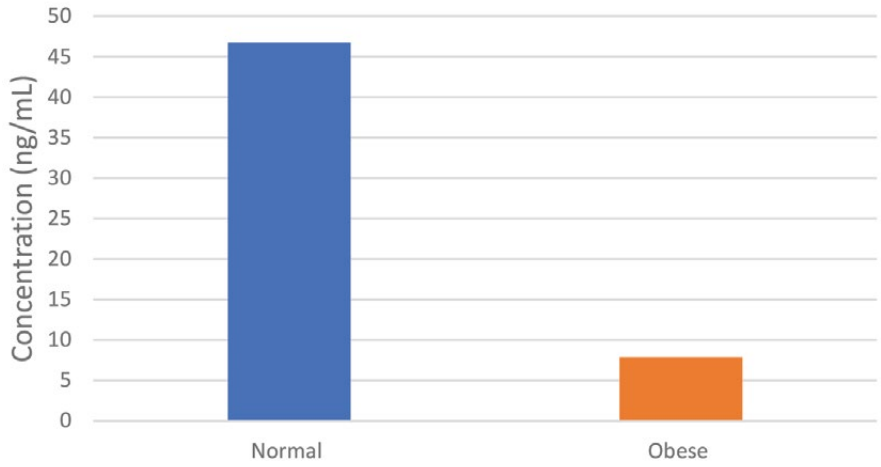

Fig. 3. Mean values ± SD of ferritin of obese and normal weight women

Table 3
Pearson correlation analysis of BMI, Ferritin and Vitamin D3 in all studied groups

		BMI
Vitamin D	Pearson Correlation	0.000
	Sig. (2-tailed)	1.000
Ferritin	Pearson Correlation	-.456-*
	Sig. (2-tailed)	0.022

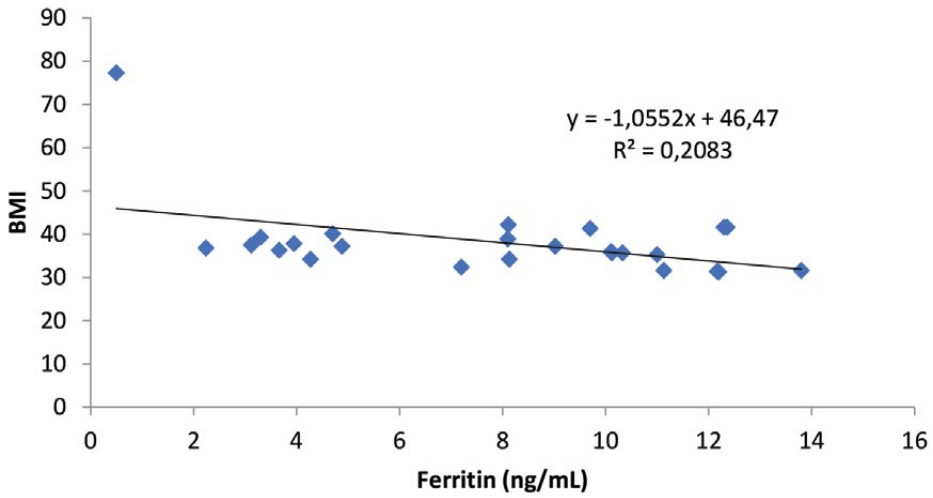

Fig. 4. The correlation between serum ferritin and BMI in all studied groups

■ DISCUSSION

Obesity represents one of the great modern health challenges defined as an excessive fat accumulation in adipose tissue [15], high prevalence of obesity was observed and associated with risk factors [16] for many chronic diseases, such as cardiovascular diseases, diabetes, and certain cancers or iron deficiency anemia [17], the association between obesity and iron deficiency may be due to increased hepcidin levels, which is a small peptide hormone found in adipose tissue and inhibit iron absorption in the proximal small intestine [18].

As shown in Table 2 BMI showed a highly significant increase ($p<0.01$) between Obese women group and woman of normal weight group, similar finding was observed with Wazir M.K., et al., who "showed significant differences of BMI between studied groups" [19].

Table 2 showed the level of serum vit. D3 in obese group that indicated a highly significant decrease ($p<0.01$) in comparison to that level in normal group, this result agree with J Christopher Gallagher et al., who showed "Women with BMI <25 kg/m (2) develop much higher levels of serum 25OHD compared to those with BMI of >25 kg/m (2) [20]. While Sharon H Chou et al., study showed There were no effects of vitamin D3 on weight and BMI in normal and obese [21].

Table 2 showed the level of serum ferritin in obese that indicated a highly significant decrease ($p<0.01$) in comparison to that level in normal group, The result was in disagreement with Sixtus Aguree et al., and Abidullah Khan et al., those reported that Mean serum ferritin were higher in women with obesity than those with normal weight [18, 22]. However, agree with Ekhlas Jabbar Kadhim, who showed there's Iron-deficiency anemia in obese women [23]. The result agreed with agree Hanna S-Kulpa et al., study that showed in the obese women an inverse correlation was observed between iron status and obesity [24].

CONCLUSION

According to the results of this study, the level of serum ferritin in obese that indicated a highly significant decrease, so there's a negative correlation between ferritin and BMI.

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Osama Karim Abde Sada[1], Esraa Rashid Hamed[1], Iman Ibrahim Awad[1], Hanan Hasan Ali[2], Basil A. Abbas[3]✉, Adel J. Hussein[3]
[1] College of Applied Medical Sciences, University of Karbala, Iraq
[2] College of Health and Sciences, North Carolina Central University, Durham, United States of America
[3] College of Veterinary Medicine, University of Basrah, Basrah, Iraq

Overview of Lead Poisoning and Smoking

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Contacts: basil.abbas@uobasrah.edu.iq

Abstract

Introduction. Lead is a toxic metal that occurs naturally in the earth's crust but has become widespread due to human actions such as using it in pipes, paint, and pesticides.
Purpose. To collect recent information about lead poisoning by smoking.
Materials and methods. Data were collected to give a viewpoint of lead poisoning which can enter drinking water through deteriorating pipes and can also be found in food.
Results. Lead accumulates in the body and can cause various health effects, especially in children who are more at risk due to their behavior and exposure in playgrounds. Lead poisoning can result in neurological damage, developmental delays, and learning disabilities. The brain is the most sensitive organ to lead, and lead is a cumulative toxic substance that can be stored in the body over time.
Conclusion. Lead exposure is a major public health problem in many parts of the world including Iraq.
Keywords: lead, toxicity, pipes, paint, pesticides, poisoning, smoking

■ INTRODUCTION

Epidemiological research has focused particularly on tobacco smoking, which has been studied more extensively than any other form of consumption. In the twentieth century, 100 million people died as a result of the practice of smoking, and thus smoking is the largest epidemic that causes death worldwide. The relationship of smoking to lung cancer is the strongest, both in public perception and in pathology. Among male smokers, the lifetime risk of developing lung cancer is 17.2%. Among female smokers, the risk is 11.6%.

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This risk is much lower in non-smokers: 1.3% in men and 1.4% in women. A person's risk of developing a disease directly increases with the length of time the person continues to smoke as well as the amount of smoked. However, if someone stops smoking, these chances gradually decrease as the damage to their body is repaired. One year after quitting smoking, the risk of developing heart disease is half that of continuing to smoke [1].

■ NICOTINE

Nicotine is a chemical found in the tobacco plant, and eating it is somewhat addictive, eating some vegetables and fruits helps to clean them. The center of nicotine in smoke is located and binds to tar particles when they enter the body through the lungs. The nicotine reaches the bloodstream and from there to the brain. Nicotine helps release the commercial substance dopamine that causes feelings of happiness, joy and excitement. Genotene also has a similar effect to drugs, after taking it, its effect disappears, the body needs another dose of it to feel the excitement again, knowing that citrus and green leafy vegetables are food by eating to cleanse the body of nicotine [2].

■ THE RISK OF LUNG CANCER

The main risks of tobacco use include many forms of cancer, particularly lung cancer kidney cancer, laryngeal and head and neck cancer, bladder cancer, esophageal cancer pancreatic cancer and stomach cancer. Studies have demonstrated a relationship between tobacco smoke, including secondhand smoke, and cervical cancer in women. There is some evidence to suggest a small increase in the risk of developing myeloid leukemia, squamous cell carcinoma, liver cancer, colorectal cancer, gallbladder, adrenal, small bowel, and various childhood cancers. The possible relationship between breast cancer and tobacco remains uncertain [3]. Smoking can be linked to all types of lung cancer. Small cell lung cancer (SCLC) is most closely associated with nearly 100% of cases that occur in smokers [4].

■ CARDIOVASCULAR DISEASE

Inhaling tobacco smoke causes several immediate responses within the heart and blood vessels. Within one minute your heart rate begins to rise, increasing by up to 30 percent during the first ten minutes of smoking. The carbon monoxide in tobacco smoke exerts negative effects by reducing the blood's ability to carry oxygen [5].

Smoking also increases the chance of developing heart disease, stroke, atherosclerosis, and peripheral vascular disease. According to a study by an international team of researchers, people under the age of 40 are five times more likely to have a heart attack if they smoke [4].

■ SMOKING HAZARDS

Smoking has many damages that reflect on a person in all aspects of his life, as it has health, religious, social, environmental, and economic harms.

Type of cancer	**Non-smokers**	**Smokers**
Laryngeal cancer	5%	95%
Lung cancer	7%	93%
Oral cancer	18%	82%
Tongue cancer	17%	83%

■ LEAD POISONING

Lead is a chemical element that is considered a heavy metal due to the relatively high density and highly relative atomic weight. Lead has a half-life of thirty days in blood unless it is deposited into the bones due to accumulation the half-life then changes to a range between 20 to 30 years. Lead is also considered a flexible metalloid which consists of metal and nonmetal properties [6]. Lead has a wide range of uses by mankind due to its flexibility and many forms of applications [7]. Lead is used as a shield against nuclear reactors as well as radiation rays due to its high density [8]. According to Singh and colleagues, lead is considered as a contaminant due to its metalloid properties which causes damaging effects human health and the environmental; exposure of plants to heavy metals such as lead causes damage to the cells of plants as well as disturbance of the cellular homeostasis.

Evidence of lead presence in the blood is detected by the exchangeable component on the body, erythrocyte, and hemoglobin. Due to its half-life in the body, the concentration of lead normally reflects the time of exposure [9]. Moreover, there is a difference between the absorption and metabolisms on lead that is based on age difference in children. In adult example, the respiratory tract route is responsible to up to 70% of lead absorption and the size of the particulate matter of lead plays an important role as well in influencing the absorption [10].

■ COMMON SOURCES AND EXPOSURE OF LEAD

Lead is considered a rare element and represents less than 1% of the earth crust and most of the lead is accumulated through recycling [11]. Exposure to lead is through various sources such as air, water, and soil and through use in many industrial manufacturing in cosmetics, ceramics and even jewelry making [12]. The exposure of lead causes many adverse health issues. There is a low concentration of lead in the body even though it's unhealthy; however, in the US there are laws that prohibit lead use to minimize exposure. The exposure to lead could be acute or chronic depending on the period and concentration of exposure [13–15]. According to Stewart and colleagues, lower concentration of exposure to lead does indeed have harmful effects to NS because chronic exposure lower concentration does accumulate over time [15].

■ LONG-TERM TRENDS AND KNOWN INCIDENCE

Although environmental lead poisoning is a major concern, many of the lead poisoning incidence cases resulted from exposure within the homes. For instance, Gordon and colleagues reported a case of a 44 year old painter who had been having symptoms of depression, abdominal cramps, nausea, arthralgia and mild mental impairment. Before the symptoms show, he had been working in an old Georgian building to remove close 8 layers of old paints. The blood work done by his physician showed a high level of lead in his blood which is what caused the symptoms he was having since me safety measures were taken during his work periods. In addition, a 22 year old painter was working at the same site but his exposure was over 12 weeks, but the level of the lead exposure was close to the first case. Despite the removal from the exposure site, both cases showed longer time for the lead level in blood to decrease even with intensive treatment [16]. Moreover, prior to the mid-60s, a mortality analysis showed an increase in numbers of death due

chronic disease and cerebral hemorrhage among smelter workers who were poisoned by the lead exposure [17]. In England and Australia, hypertension and chronic nephritis are common among workers in the lead industry depending on the duration of exposure although it has not been accepted in the US during that time. Furthermore, many cases of whiskey lead poisoning were reported due to the fact that the whiskey was made in part that were lead soldered [18].

■ LEAD TRANSPORT AND THE ENVIRONMENTAL FATE

Although lead is a naturally occurring chemical, the majority percentage of lead in the environment is caused because of activities done by human industrial consumption. The solubility of lead depends on the pH, solidity, and salinity; and it's highly soluble in acidic water. Lead typically precipitates in soil and sediment, and it's highly obtained in the upper layers which prevents it from reaching the sub-soil, and groundwater. Lead creates a risk factor of accumulation in Human and plants but not in aquatic organisms. Lead has the ability to be absorbed into the soil easily and transformed into the environment in the form of other lead isotopes (ATSDR). According to the EPA, lead travels through air, water, and soil, and in the air, it stays in the atmosphere for 10 days, and its presence is persistent. Since lead is released into the environment in small particles, the most important factor in determining the transport of lead is the size of the particulates [19]. Also, the fate of the lead particulate in soil is determined by the solubility of the solid form of it; and that is dependent of the pH of the soil, type of soil, minerals present in the soil, organic material in the soil, and even the size of the lead particulate . The movement of lead into the soil and groundwater is very slow under normal environmental conditions . Furthermore, the bioavailability of lead in the soil is not high due to the high ability of lead absorption in organic matters found in the soil, and the increase in the bioavailability will result in decrease in the Ph and other organic matter in the soil. For instance, there was low accumulation of lead in plants that were grown in soil that has been contaminated by soil [20].

■ TOXICOLOGICAL EFFECT OF LEAD ON HUMAN HEALTH

The exposure of human to lead at the environmental level was low but due to the increase in industrial use it became relatively high [21]. Although lead has many practical uses, continuous exposure will result in damage at the biological level as well as cause neurological damage to the nervous system [22]. As mentioned earlier, the half-life of lead is different depending on if the exposure is in the blood or the bones. Due to the half-life, lead toxicity is measured by blood and bones depending on the exposure period [6]. The direct effects are classified into either morphological effect which deal with nervous system malformation from embryo formation through childhood, or pharmacological effects which are caused by the use of lead as a pharmaceuticals substitute. The direct morphological effects of lead cause a range of interference to many cellular functions such as disruptions of key cellular differentiation in addition to disruption of neural migration [23]. Furthermore, exposure to lead disrupts synaptic formation causing a reduction of neural sialic acid that is produced by the NS plus causing differentiation of the glial cells at earlier stages [24]. Lead toxicity causes encephalopathy which is a brain disease and malformation and severe exposure result in loss of muscle control, attention

deficit, memory loss, and hallucination [25]. Adults are exposed to lead from various sites in the workplace unlike children are exposed in various environmental sites. For instance, adults who work at lead mines, printing industry, paints manufactories and battery manufacturing are at a high risk of exposure although the exposure occurs at a lower concentration over longer periods of time hence chronic exposure [25].

■ TOXICOLOGICAL EFFECT OF LEAD ON THE WILDLIFE

The adverse effect of lead exposure does not include just humans but the wildlife is also affected. For instance, in recent years many studies have focused on the exposure of lead that resulted from the release by rifles that are used on many hunting outdoor activities [26]. Several of those studies focused on gut piles, ground squirrels, prairie dogs, and several deer species which indicated that the lead exposure is causing a danger for hunting animals [27]. Furthermore, these studies suggested that there is a tremendous increase in the blood pressure level due to the lead exposure that was acquired during hunting games [28]. In addition, in the US and Great Plains, two studies were conducted and both found an increased incidence that suggests hunting games are a significant source of lead exposure in eagles. These studies found a positive correlation between lead exposure from the rifles and adverse effect in eagles suggesting ammunition as the source [29]. Keep in mind that several studies have raised concerns for human health from lead based ammunition, and these fragments of lead are also found in meat packages [30].

■ CONCLUSION

Lead poisoning is a serious health concern that can have both short-term and long-term effects on individuals, particularly young children and pregnant women. It occurs when lead accumulates in the body over time, usually through exposure to contaminated water, soil, or household items. Short-term effects of lead poisoning can include abdominal pain, constipation, and headaches, while long-term effects can include neurological damage, developmental delays, and learning disabilities. The most effective way to prevent lead poisoning is to minimize exposure to lead sources, such as lead-based paint and contaminated soil or water. This can be achieved through regular testing of household water and soil, as well as the removal or encapsulation of lead-based paint. Overall, lead poisoning is a preventable and treatable condition, but it requires awareness and action from individuals, families, and communities to reduce the risk of exposure and protect public health.

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Muna H. AL-Saeed
College of Veterinary Medicine, University of Basrah, Basrah, Iraq

Evaluation of Hematological and Biochemical Values in Local Buffalo in Basrah Governorate

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Contacts: muna.mahmood@uobasrah.edu.iq

Abstract

Introduction. Serum biochemical and hematological reference values are used to establish normality and to diagnose disease and physiological alterations.
Purpose. Up till now there is no reference values for different blood biochemical and hematological constituents in buffaloes, consequently the current study purposed to establish those values in male and female buffalo (Bubalus bubalis).
Materials and methods. Animals were examined at buffalo farms that belong to Basrah Governorate, Iraq. Based on selection criteria, a total of 20 clinically healthy female and male buffaloes (1-2 years old) were included in the study. Whole blood samples for hematological analysis and serum samples for biochemical analysis.
Results. The result showed significant (P≤0.05) increase in all blood parameters in male buffalo compared with female buffalo except lymphocyte %. The results showed significant (P≤ 0.05) decrease in lymphocyte % in male buffalo compared with female buffalo.
Conclusion. The established values will be a useful guide for interpreting serum biochemical and hematologic data in male and female buffalo.
Keywords: haematology, buffalo, Basrah governorate, meat, blood

■ INTRODUCTION

The buffalo (Bubalus bubalis) originally Asian animals and the majority of them are distributed in tropical and subtropical Asia. The buffaloes have been classified according to their appearance, wallowing habits and uses into river and swamp buffaloes. The latter are used for drought power and are found in countries like the Indian sub-continent and the mediterranean countries [1]. The water buffalo can surpass the cattle genus Bos in its ability to adapt to the hot climates and swampy lands [2]; therefore, water buffaloes have special importance in milk and meat production in the valley of the River. The major part of the laboratory diagnostic tests is the measurement of serum biochemical and hematological variables that are used to establish normality and to diagnose disease and physiological alterations [3].

Several reviews specified some factors that must be established when using reference values, as the characterization of the reference population in respect to age and sex, the

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inclusion and exclusion criteria that used for selecting or excluding animals from the reference sample group, the physiological and environmental conditions under which the reference population was studied and the samples collected from the reference sample group. Textbook reference intervals produced by European or United States Veterinary Laboratories are often based on animals living under good husbandry conditions in temperate climates. However, those reference sample groups may differ from those of the developing countries. Reasons for potential differences could include genetic factors, nutritional quality and quantity, water availability, sweat electrolyte losses, parasitism and hot climate [4].

■ PURPOSE OF THE STUDY

Up till now there is no reference values for different blood biochemical and hematological constituents in buffaloes, consequently the current study purposed to establish those values in male and female buffalo (Bubalus bubalis).

■ MATERIALS AND METHODS

Samples were collected at 8.00 am prior to feeding. Two blood samples were collected from the jugular vein into vacutainer tubes from all buffaloes under study; the first blood sample was collected in plain vacutainer tube and used for obtaining serum. The second blood sample was collected in vacutainer tubes containing EDTA as anticoagulant and used for hematological analysis. Samples were transported in ice tank directly after collection to the research laboratory of Physiology, Pharmacology and Biochemistry in College of Veterinary Medicine, University of Basrah.

Blood samples were used in measured for:

The haematological parameters viz. RBC, Hb, PCV, WBC and DLC and cortisol and glucose levels of buffalo were studied by collecting blood samples with the help of disposable syringes by jugular vein. RBC and WBC were counted with the help of improved Neubauer haemocytometer [5]. DLC was counted by methodology adopted by [6]. Hb was estimated by using Sahli haemometer [7]. PCV was determined by centrifugation method. Samples were prepared (blood serum) or analyzed (Whole blood. Blood samples in plain tubes were centrifuged at 3000 rpm for 15 minutes, after which serum was harvested according to standard methods [8], and then divided into 4 equal parts in eppendorf tubes, stored at –20 °C, and were used for measuring serum biochemical constituents. Samples that showed hemolysis were excluded from the study. Serum samples were analyzed within a maximum period of two weeks. Biochemical analysis Serum biochemical variables were measured using UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea), reagents and chemicals were supplied with the purchased commercial kits, different methods used for analysis of biochemical analytes for the estimation of cortisol and glucose blood was collected in plastic Eppendrof tubes. After centrifugation, blood plasma was removed and the samples were then analyzed for measuring the levels of glucose, lipid profile, TSH, T_3, T_4, cortisol and testosterone by ELISA Kit.

Statistical analysis

The results obtained were analyzed. The results obtained were analyzed statistically by t-test by SPSS software for determining the significance of change from control [9].

■ RESULTS

Mean value of hematological parameters in local male and female buffalo

The results observed in the Table 1 that haematological parameters in male and female buffalo. The result showed significant (P≤0.05) increase in all blood parameters in male buffalo compared with female buffalo except lymphocyte %. The results showed significant (P≤0.05) decrease in lymphocyte % in male buffalo compared with female buffalo.

Mean value of glucose and hormonal parameters in local male and female buffalo

The results observed in Table 2 that the results of biochemical and hormonal parameters in local male and female buffalo. The results showed significant (P≤0.05) increase in concentrations of T_3, T_4, testosterone, T-Ch, LDL, VLDL in male buffalo compared with female buffalo while the results showed (P≤0.05) decrease in concentrations of glucose, TSH, cortisol in male buffalo compared with female buffalo. But the results showed non-significant change (P>0.05) in concentrations of triglyceride and HDL in male buffalo compared with female buffalo.

Table 1
Mean ± SD value of hematological parameters in Basrah city, N=10

Groups / Parameters	Buffalo Sex	
	Male	Female
RBC (×106/cmm)	6.83±0.15a	4.79±0.18b
WBC(×103/cmm)	12.23±0.27a	10.02±0.11b
Hb (mg/dl)	16.6±0.23a	14.9±0.37b
PCV %	50.3±0.12a	40.2±0.13b
Neutrophiles %	50.18±0.61a	40.3±0.20 b
Eosinophiles %	5.2±0.29 a	3.1±0.36b
Basophiles %	1.5±0.25a	0.4±0.39b
Lymphocyte %	33.2±0.02b	48.8±0.16a
Monocyte %	10.3±0.22a	8.1±0.1b

Notes: N = number of buffalo. Small letters denote differences between groups, P≤0.05.

Table 2
Mean ± SD values of glucose, lipid profile and hormonal parameters in local buffalo in Basrah city, N=10

Groups / Parameters	Buffalo Sex	
	Male	Female
Glucose (mg/dl)	110±18.06 b	143±31.08 a
TSH (µlU/ml)	2.05±0.10 b	3.60±0.11 a
T3 (ng/ml)	1.91±0.08 a	1.42±0.51 b
T4 (µg/dl)	11.08±4.72 a	7.40±1.60 b
Testosterone (ng/ml)	0.78±0.011 a	0.42±0.015 b
Cortisol (ng/ml)	4.47±0.016 b	7.86±0.012 a
T-Ch (mg/dl)	170±6.28 a	145±3.30 b
Triglycired (mg/dl)	55.87±5.19 NS	50.53±2.11
HDL-Ch (mg/dl)	32.44±7.12 NS	28.77±1.17
LDL-Ch (mg/dl)	19.04±2.81 a	16.83±1.66 b
VLDL-Ch (mg/dl)	10.33±0.21 a	5.67±0.18 b

Notes: N = number of male and female buffalo. Small letters denote differences between groups, P≤0.05.

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■ DISCUSSION

Buffaloes (Bubalus bubalis) subjected to study were selected from farms to ensure that they received periodical clinical examination and their productive and reproductive status were regularly checked and recorded. Also, the physiological condition of the reference sample population was defined. In the present study, mean values for body temperature were 38.35±0.33 °C and 37.7–39.01 °C respectively. Generally, the observed body temperature agreed with that reported by [9] and was in-accordance with values reported by [10].

Large species differences in lipoproteins profiles and the percentage of total cholesterol and triglycerides carried by each lipoprotein class were recorded in different animals. Whereas in human and pigs, the majority of cholesterol is transported as LDL-C, in cattle, cholesterol is equally divided between LDL-C and HDL-C, while in sheep and horses, the majority of cholesterol circulates as HDL-C [11]. Mean values of serum total cholesterol, HDL-C, LDL-C and VLDL-C estimated in the present study (Table 2) were lower than findings of previous studies in adult buffaloes [12, 13]. The present study revealed that serum LDL-C levels were lower than serum HDL-C, which disagreed with the findings of equal distribution for the two components in blood of adult buffaloes that reported by [14], this may give indication that the distribution of HDL-C and LDL-C is not equal in buffalo's heifers. Total cholesterol reported in this study was lower than levels of recorded by[15]. [16] reported that serum cholesterol level was not affected by the feeding system but it shows an increasing trend after puberty. The authors added that the higher concentration of cholesterol with the progress in age is probably a physiological adjustment to meet growth requirements. Mean value for triglycerides obtained from this study was lower than that reported by[17], who reported that serum triglycerides was of in adult water buffaloes. Ina previous study, mean serum glucose were in heifers[18], which agreed with mean glucose level from the present study. At present, the complete blood cell count can be performed using an automated hematology analyzer, which can increase the throughput of the test. The current study is the first one that provided a reference values for these new indices in buffalo heifers. Reference limits of hematological analytes developed in the present study (Table 2), were slightly differed from those developed by [19]. Mean hematological values from this study were higher than RBCs count, HCT and lower than Hgb reported by [20]. Total WBCs count from the present study was higher than WBCs count reported by [21]. Also, differential leucocytes counts recorded by [22] were slightly different from that obtained from the current study.

■ CONCLUSION

The established values will be a useful guide for interpreting serum biochemical and hematologic data in male and female buffalo. Raise all blood parameters in male buffalo compared with female buffalo except lymphocyte %. Raise in concentrations of T_3, T_4, testosterone, T-Ch, LDL and VLDL in male buffalo compared with female buffalo while a decrement in concentrations of glucose, TSH, and cortisol.

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Amal F. Ghanim, Ali B. Al-Deewan, Basil A. Abbas✉
College of Veterinary Medicine, University of Basrah, Basrah, Iraq

DNA Sequencing and Phylogenetic Analysis of Escherichia Coli O157:H7 Isolated from Stool Samples Causing Public Health Threat in Basrah (Iraq)

Conflict of interest: nothing to declare.
Authors' contribution: Amal F. Ghanim – conceptualization, data curation, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft and writing – review & editing; Ali B. Al-Deewan – conceptualization, data curation, investigation, methodology, project administration, resources, software, validation, visualization, writing – original draft and writing – review & editing; Basil A. Abbas – conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, software, validation, visualization, writing – original draft and writing – review & editing.
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Submitted: 03.03.2024
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Contacts: basil.abbas@uobasrah.edu.iq

Abstract

Introduction. Presence of Escherichia coli O157:H7 in high rate lead to public health threat to the investigated community.
Purpose. To analyze stool samples for the presence of Escherichia coli.
Materials and methods. 100 samples were collected and detected for presence of Escherichia coli. Conventional diagnosis, Vitek-2 test and 16S rRNA were used for bacterial identification. DNA sequencing and Phylogenetic analysis indicate different relationships with other strains previously isolated from Iraq and other countries. Escherichia coli O104:H4 and Escherichia coli O145:H28 were also isolated during the study.
Results. Showed that from 100 human sample there were 87 positive samples. Out of them 30% are of Escherichia coli O157:H7.
Conclusion. Escherichia coli is frequently isolated from clinical samples and can cause a serious diseases. The phylogenetic tree analysis of E. coli isolates indicated the link between the different strains.
Keywords: sequencing, phylogenetic, escherichia coli, stool, pathogenesis

■ INTRODUCTION

Among the intestinal pathogens there are six well-described categories: enteropathogenic E. coli (EPEC), enterohaemorrhagic E. coli (EHEC), enterotoxigenic E. coli (ETEC), enteroaggregative E. coli (EAEC), enteroinvasive E. coli (EIEC) and (DAEC) diffusely adherent E. coli [1]. The various pathotypes of E. coli tend to be clonal groups that are characterized by shared O (lipopolysaccharide, LPS) and H (flagellar) antigens that define Serogroups (O antigen only) or Serotypes (O and H antigens) [1, 2]. Pathogenic E. coli strains use a multi-step scheme of pathogenesis that is similar to that used by other mucosal pathogens, which consists of colonization of a mucosal site, evasion of host defenses, multiplication and host damage. Most of the pathogenic E.

coli strains remain extracellular, but EIEC is a true intracellular pathogen that is capable of invading and replicating within epithelial cells and macrophages. Other E. coli strains might be internalized by epithelial cells at low levels, but do not seem to replicate intra cellularly. Pathogenic E. coli strains possess specific adherence factors that allow them to colonize sites that E. coli does not normally inhabit, such as the small intestine and the urethra. Most frequently these adhesins form distinct morphological structures called fimbriae (also called pili) or fibrillae, which can belong to one of several different classes [2].

Indeed, a variety of molecular methods, including 16S rRNA sequencing and MLST, fail to distinguish Shigella from E. coli [3], while core genome based phylogenetic approaches cluster Shigella and E. coli strains together [4], suggesting that they represent a single species genetically. As such, although it remains a unique genus largely for clinical reasons, Shigella has been determined to be phylogenetically E. coli, and is essentially an E. coli pathovar [5].

■ PURPOSE OF THE STUDY

To analyze stool samples for the presence of Escherichia coli.

■ MATERIALS AND METHODS

Bacterial isolation

A total of one hundred stool samples were obtained from patients visiting diagnostic laboratory and residing in various locations within the city of Basrah, Iraq. Standard techniques were used to isolate and identify the E. coli present in the samples. The faeces samples were introduced into tubes containing newly made Tryptone Soya Broth (TSP) supplemented with Vancomycin. The tubes were then placed in an aerobic incubator at 37 °C for overnight incubation. Subsequently, the samples were transferred onto MacConkey agar plates and allowed to grow for 24 hours at the same temperature of 37 °C. The colonies that underwent lactose fermentation were immediately transferred onto Eosin methylene blue (EMB) agar and incubated at 37 °C for 24 to 48 hours. Colonies exhibiting a metallic appearance were collected thereafter [6, 7].

Bacterial identification

Vitek-2 and the PCR method using 16s rRNA were used to identify the isolated bacterial colonies. The primers used for 16S rRNA were F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and R: 5′-GGTTACCTTGTTACGACTT-3′ [8].

Bacterial DNA extraction and PCR analysis

Table 1 lists the primers used in this investigation (Miller et al., 2013). The primers were dissolved in distilled water to get a stock solution with a final concentration of 100 pmol/µl. This stock solution was maintained at –20 °C. To create a working primer suspension with a concentration of 10 pmol/µl, 10 µl of the stock solution was mixed with 90 µl of distilled water, resulting in a final volume of 100 µl.

Phylogenetic analysis

Using the UPGMA method, the evolutionary past was inferred [9].

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Latex agglutination test for E. coli O157:H7

This test was used for more specific identification of E. coli O157:H7 by using commercial kit (Wellcolex E. coli O157:H7, Remel) to detect the somatic antigen O157 and flagellar antigen H7.

■ RESULTS

Isolation and identification

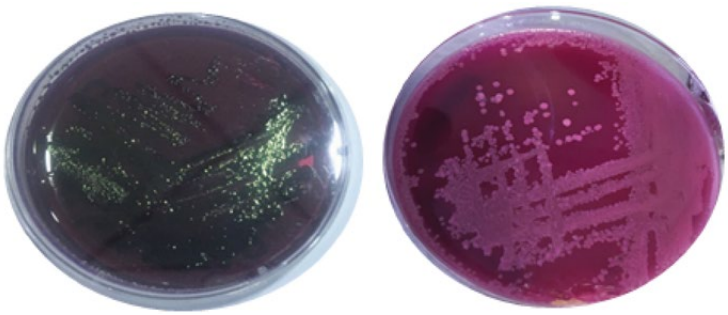
In order to obtain Escherichia spp., samples were collected from several medical laboratories in Basrah province. One hundred samples were collected between 10 Jan. 2022 to 1 March 2023. 50 Stool samples collected directly from patients admitted to clinics.

Primarily identification were done on TSB with vancomycin and EMB (Eosin Methylene Blue) medium agar plate as in Fig. 1.

A total 100 samples were summarized in table (1) showed that from 100 stool samples there were 87 positive with percentage of 87%.

VITEK 2 Kit

Vitek kit was used in order to obtain the exact identification of bacterial isolates. The results were listed in the table (2) showed only 80 percent were confirmed as E. coli.



A B

Fig. 1. The shiny-green colony of E. coli bacteria on EMB media (A). The pink colony of E. coli bacteria on TS agar (B)

Table 1
Total number of samples used for isolation of E. coli

No.	No. of Samples	No. positive	Percentage of positive samples	Escherichia coli O157:H7 No (%)
1	100	87	87%	35 (40%)

Table 2
The results of vitek test for E.coli identification

No	Samples	No. of isolate	No. of E.coli isolates	Percentage of positive samples	No. of other types	Unidentified Organism
1	Human	87	70	80%	17	–

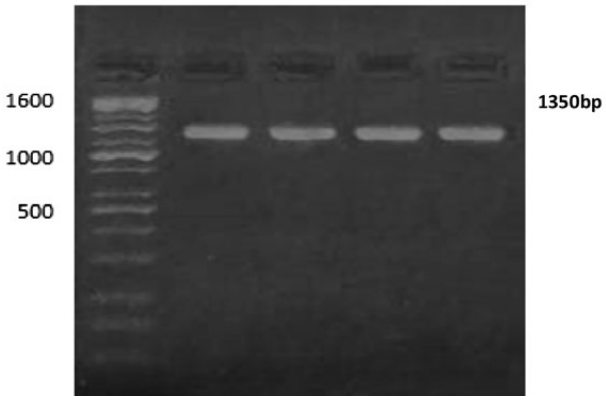


Fig. 2. PCR product the band size 1350 bp. The product was electrophoresis on 1.5% agarose at 5 volt/cm². 1x TBE buffer for 1:30 hours. M: DNA ladder (100)

Detection of bacteria by PCR technique

DNA Extraction: The DNA extracted from several bacterial strains using the kit mentions in Methods.

PCR for 16S rRNA gene: All strains showed a band of 1350 bp when E.coli strain were selected for16S rRNA gene detection (Fig. 2).

DNA Sequencing

The PCR products of 16S rRNA gene of three strains of E. coli were sequenced. The results of sequencing were analyzed using Blast (https://www.ncbi.nlm.nih.gov/).

Table3
Type of substitution of nucleotides in sequenced DNA.

16S ribosomal RNA gene							
No.	**Type of substitution**	**Location**	**Nucleo-tide**	**Sequence ID with compare**	**Sequence ID with Submissions**	**Source**	**Identities**
1	Transition	2831071	T/C	ID: CP024992.1	ID: ON680704.1	Escherichia coli O104:H4	99%
	Transversion	2831714	G/C				
	Transition	2831762	A/G				
	Transition	2831765	A/G				
	Transversion	2831771	A/T				
2	Transversion	228345	A/T	ID: AP019703.1	ID: ON680705.1	Escherichia coli O145:H28	99%
	Transition	228569	A/G				
	Transversion	228798	G/T				
	Transversion	228825	G/T				
	Transition	228840	G/A				
	Transversion	228869	G/T				
	Transition	594783	G/A				
3	Transversion	555482	T/G	ID: CP038398.1	ID: ON680708.1	Escherichia coli O157:H7	99%
	Transition	555483	C/T				
	Transition	555486	T/C				
	Transversion	556081	G/C				

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The results showed that all investigated strains belong to E. coli. The detail results presented in table 3.

The results in table have indicates that the investigated E. coli belong to three groups Escherichia coli O104:H4, Escherichia coli O145:H28 and Escherichia coli O157:H7. The strains were submitted and registered at ncbi IDs: ON680704.1; ON680705.1; ON680708.1. The table also include there were many mutation occurred inside the DNA in both type transition or transversion mutations. Number of transition and transvertion mutations were vary in types and numbers through the strains in composition to sequences ID present in database.

Phylogenetic tree analysis

The phylogenetic tree analysis of E. coli isolates indicated the link between the different strains. In the current study three strains were analyzed for phylogenetic in comprises in between the strains and strains isolated worldwide. Results indicate no fixed relation with exact country strains (fig. 3).

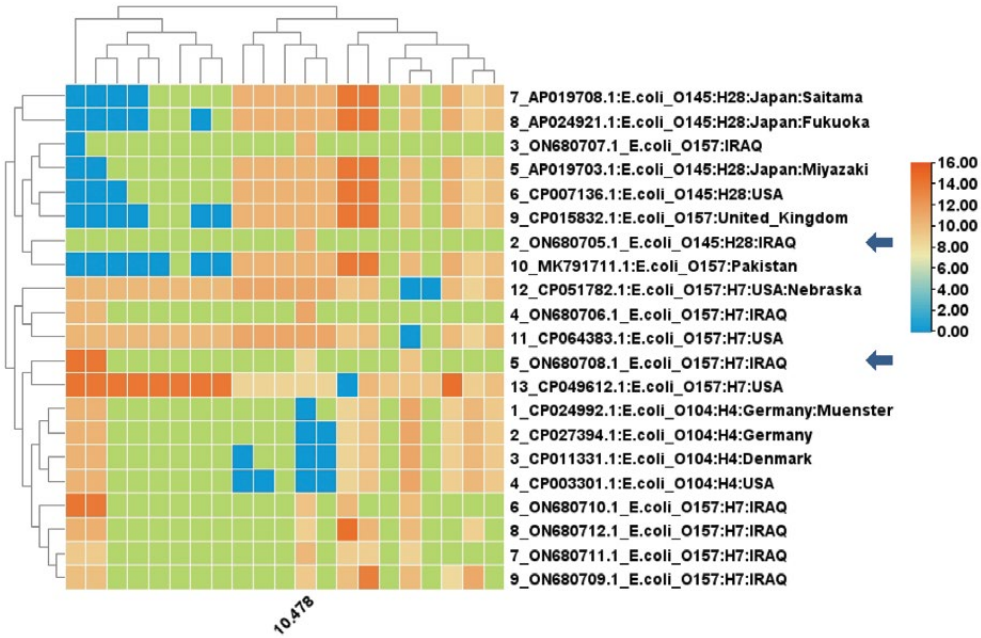

Fig. 3. Evolutionary relationships of the isolated strains of E. coli (ON680705.1; ON680708.1)

■ DISCUSSION

Using EMB and MacConkey Agar showed 87 strains of E. coli out of 100 samples. This indicate that these test is a good aid for primary identification. E. coli is nonspore-forming and beta hemolytic. On MacConkey Agar, it usually ferments lactose or produce pink colonies with surrounding areas of precipitated bile salts. It also presents with a green

sheen on EMB agar. E. coli strain will produce indole from tryptophan; it does not produce hydrogen sulfide, urease, and cannot use citrate as sole carbon source [10].

The classical screening medium for E. coli O157:H7 is sorbitol MacConkey agar. This method exploits the fact that E. coli O157:H7, unlike 90% of E. coli isolates does not ferment sorbitol rapidly [11]. Other studies reported that sorbitol MacConkey agar medium is a useful, rapid, and reliable screening aid for the detection E. coli O157:H7, but it is not generally useful of EHEC strains of serotypes other than E. coli O157:H7 [12].

The percentage of frequency of E. coli isolated in previous study was 134 (59.6%) out of 225 stool samples, 67 (29.8%) out of 225 beef samples and 55 (24.4%) out of 225 raw milk samples. The rate of E. coli isolated from raw milk in the present study was 24.4 % which agrees with the result obtained by others [13, 14], which were 27.91%, 23.2% and 25% respectively. Other studies such as Soomro et al. [15] mentioned much higher rate of E. coli isolates in raw milk (57%).

The occurrence of NSFEC in children stool, beef and raw milk samples which detected by conventional microbiological methods were 38.9%, 67.4% and 48.6% respectively. The present occurrence of NSFEC in children stool sample was higher than the result of obtained by Isibor et al., (2013) who reported 15.6%. It is also higher than the finding of A'iaz [16] and Dhanashree and Mallya [17] who detected 57.5% and 73.9%.

Escherichia coli O157:H7 is frequently isolated from different samples including food in addition to stool samples [18–20]. In this study 30% of isolates were Escherichia coli O157:H7. This agreed with previous results from food samples [21].

Phylogenic analysis indicate a variety of relationships of present isolates of E. coli with local and isolates of worldwide. Phylogenetic analysis of Escherichia coli (E. coli) has provided crucial insights into the genetic diversity and evolutionary relationships within this bacterial species. Notably, E. coli is categorized into four main phylogenetic groups: A, B1, B2, and D [22].

■ CONCLUSION

Escherichia coli is frequently isolated from clinical samples and can cause a serious diseases. The phylogenetic tree analysis of E. coli isolates indicated the link between the different strains.

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Duaa Abdullah Al-Musawi✉, Shaymaa Jabbar Raisan
College of Education for Pure Sciences, University of Basrah, Basrah, Iraq

Relationship of Cytokines in Patients with Food Allergy Caused by Ovalbumin Protein

Conflict of interest: nothing to declare.
Authors' contribution: Duaa Abdullah Al-Musawi – conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, visualization, writing – original draft and writing – review & editing; Shaymaa Jabbar Raisan – conceptualization, data curation, methodology, project administration, resources, supervision, writing – original draft and writing – review & editing.
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Contacts: pgs.duaa.abdullah@uobasrah.edu.iq

Abstract

Introduction. Food allergies are a major health problem. Cytokines play an important role in food allergies. Ovalbumin represents the majority of proteins found in egg whites, has been used commercially in many experimental models of food allergies.
Purpose. To determine the role of cytokines 4, 9 in patients with food allergies caused by egg white protein ovalbumin using the ELISA technique.
Materials and methods. A total of 88 venous blood samples were collected, comprising 70 patient samples and 18 control samples, across different age groups and selected randomly. The study utilized a questionnaire form to gather necessary information during blood collection. The ELISA test was used to detect both IgE specialized against the egg white antigen, ovalbumin, and interleukins 4, 9, directed toward the egg white antigens in the serum of the people under study. Direct ELISA test was used to measure the level of IL-4, IL-9 in the serum of the people under study, using a special kit prepared by Bioassay Technology Laboratory/China.
Results. The results revealed that the percentage of females sensitive to the egg white antigen ovalbumin was higher compared to males, amounting to 7.2%. When distributing those susceptible according to age, it was noted that the first age group (≤18) exhibited the highest sensitivity to the egg white antigen ovalbumin. An increase in the concentration of interleukin 4 in those sensitive to the egg white antigen ovalbumin. The highest percentage of those sensitive individuals exhibited a concentration of ng/L ovalbumin ≥40 (11.4%) compared to control samples (9.1%). An increase in the concentration of interleukin 9 in those sensitive to the egg white antigen ovalbumin. The highest concentration recorded in ovalbumin ≥200 pg/ml was 12.9% compared to control samples (0%).
Conclusion. Interleukins 4, 9 had a high concentration in patients suffering from food allergies, indicating their vital role in the immune response. Their concentration was also high in patients allergic to the egg white antigen ovotransferrin.
Keywords: allergy, cytokine, interleukin, protein, ovalbumin

INTRODUCTION

Food allergies comprise the majority of allergies today, with the US Centers for Disease Control and Prevention (CDC) estimating in 2012 that 5.6% of children (infants to 18 years) have a food allergy [1]. Several studies indicate that two main cytokines are interleukin 4 (IL-4) and IL-13 [2]. They are powerful mediators of the inducible and effector phases of the immune response. Recent studies suggest a pivotal role for IL-9 in promoting intestinal mastocytosis and inducing experimental food allergy [3].

IL-4 is a differentiation factor that converts CD4+ cells to a Th2 phenotype, a growth factor for B cells, an inducer of IgE isotype class transition, as well as an activator of chemotaxis [4]. Increased expression of IL-4 in the epidermis contributes to inflammatory activity, leading to the appearance of itching and edema within the dermis. IL-4 can cause repression of genes filaggrin, loricrin, responsible for skin barrier function [5]. In addition, IL-4 stimulates normal keratinocytes, resulting in increased serine protein activity, which promotes skin desquamation and increases transepidermal water loss [6].

Ovalbumin represents the majority of the proteins in egg whites (54% w/w). It consists of 385 amino acids and has a molecular weight of 45 kDa [7]. The protein is commercially available and has been widely used in experimental models of food allergy [8].

PURPOSE OF THE STUDY

To determine the role of cytokines 4, 9 in patients with food allergies caused by egg white protein ovalbumin using the ELISA technique.

MATERIALS AND METHODS

Sample collection

A total of 88 venous blood samples were collected, comprising 70 patient samples and 18 control samples (58 samples from females and 30 samples from males), across different age groups and selected randomly. These samples were obtained following clinical examination by a specialist doctor from private laboratories and from Al-Hussein Teaching Hospital in Thi-Qar Governorate, after obtaining official approvals from the health institution and the patients. The study utilized a questionnaire form to gather necessary information during blood collection.

Enzyme linked immunosorbent assay (ELISA)

In the current study, the ELISA test was used to detect both IgE specialized against the egg white antigen, ovalbumin, and interleukins 4, 9, directed toward the egg white antigens in the serum of the people under study.

Ovalbumin specific IgE protein determination

The Indirect ELISA test was used to measure the level of ovalbumin in the serum of the people under study using a special kit prepared by Bioassay Technology Laboratory/ China.

Estimation of interleukin IL-4, IL-9

The Direct ELISA test was used to measure the level of IL-4, IL-9 in the serum of the people under study, using a special kit prepared by Bioassay Technology Laboratory/ China.

■ RESULTS

IgE ELISA test for egg white antigen (ovalbumin)

The results of this study, as shown in Table 1, revealed that the percentage of females sensitive to the egg white antigen ovalbumin was higher compared to males, amounting to 7.2%. When distributing those susceptible according to age, it was noted that the first age group (≤18) exhibited the highest sensitivity to the egg white antigen ovalbumin.

Table 1
Results of the IgE ELISA Test for the Egg White Antigen Ovalbumin

Sex	Examined patient samples, N=70	Specific IgE	
		allergic	Non-allergic
		N (%)	N (%)
Males	(32.9) 23	4 (5.7)	19 (27.1)
Females	(67.1) 47	5 (7.2)	42 (60)
Total (%)	(100) 70	9 (12.9)	61 (87.1)

IL-4 level in allergic and non-allergic subjects to egg white antigen ovalbumin

The results of this study, as shown in Table 2, revealed an increase in the concentration of interleukin 4 in those sensitive to the egg white antigen ovalbumin. The highest percentage of those sensitive individuals exhibited a concentration of ng/L ovalbumin ≥40 (11.4%) compared to control samples (9.1%).

Table 2
Demonstrates the relationship between the level of IL-4 in those sensitive to the egg white antigen ovalbumin and those who are not sensitive

IL-4 concentration	Examined patient samples N=70		Control samples N=18
	allergic	Non-allergic	
	N (%)	N (%)	
IL-4 <40	1 (1.4)	6 (8.6)	10 (11.4)
IL-4 ≥40	8 (11.4)	55 (78.6)	8 (9.1)
Total (%)	9 (12.9)	61 (87.1)	18 (20.5)

IL-9 level in allergic and non-allergic individuals to egg white antigen ovalbumin

The results of this study, as shown in Table 3, revealed an increase in the concentration of interleukin 9 in those sensitive to the egg white antigen ovalbumin. The highest concentration recorded in ovalbumin ≥200 pg/ml was 12.9% compared to control samples (0%).

Table 3
Demonstrates the relationship between the level of IL-9 in those sensitive to the egg white antigen ovalbumin and those who are not sensitive

IL-9 concentration	Examined patient samples, N=70		Control samples, N=18
	allergic	Non-allergic	
	N (%)	N (%)	
Ovotransferrin <200	2 (2.9)	45 (64.2)	18 (20.5)
Ovotransferrin ≥200	7 (10)	16 (22.9)	0
Total (%)	9 (12.9)	61 (87.1)	18 (20.5)

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DISCUSSION

The results of our study revealed that the percentage of females suffering from food allergies was higher than that of males, at a rate of 38.6%. This effect may be attributed to the fact that patients who suffer from food allergies either have a history of some allergic diseases (such as hay fever, atopic bronchial asthma) or have close relatives (such as parents, siblings, or grandparents) who suffer from these diseases. The predisposition to atopy is believed to be controlled by specific genes, each encoding the potential to manifest a particular trait of atopy [9].

A study conducted by [10] revealed that the highest frequency of food allergies occurred among allergy patients aged 13–30 years compared to those aged 31–52 years. Specifically, the prevalence of food allergy was 12.35% among individuals aged 13–30 years and 10% among those aged 31–52 years.

Our study showed an increase in the E antibody in those sensitive to the egg white antigen Ovalbumin with a percentage of 12.9%. This finding is consistent with recent studies. The results of the study by [11] revealed statistically significant differences between the number of patients who had specific immunoglobulin for food allergies (Specific IgE) and healthy individuals whose food allergy results were negative for eggs. The quantitative measurements of specific IgE antibodies to both egg white and oocyte membrane, along with evaluation against the proposed positive and negative decision points for specific IgE, would be useful in the diagnosis of egg allergy [12].

There are various local studies on food allergies, such as [13] recent studies recorded an increase in the rate of IgE in patients with food allergies by 76.67% [14]. The results of a previous study showed that the total IgE for food allergy patients was higher at 72.9% [15].

The results of the current study documented an increase in the concentration of interleukin 4 in those sensitive to egg white antigens. The highest percentage of these individuals exhibited a concentration of ng/L ovalbumin ≥ 40 (11.4%) compared to control samples (9.1%).

There are many studies on cytokines, each with different biological roles [16, 17]. Indicated in their results that IL-4 is a valuable guide to understanding the molecular mechanisms of the IL-4-mediated anti-hepatitis B virus response [18]. Therefore, it is necessary in inflammation.

The results of the current study revealed a high concentration of interleukin 9 in those sensitive to egg white antigens. The highest concentration recorded was Ovalbumin ≥200 pg/ml (12.9%) compared to control samples (0%). Additionally, the highest concentration of Ovomucoid ≤200 pg/ml was 4.3% compared to control samples (0%).

The stimulation of PBMCs with Egg White Protein revealed increased expression of genes associated with allergic inflammation as well as increased secretion of IL-9 in the group of children with egg allergy reactive to cooked egg white [19].

CONCLUSION

In conclusion, we found that interleukins 4, 9 had a high concentration in patients suffering from food allergies, indicating their vital role in the immune response. Their concentration was also high in patients allergic to the egg white antigen ovotransferrin.

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Ali Mazin Talib Alanbaky✉, Mohammed Hussain Al-Mayahy
Ashur University, Baghdad, Iraq

Comparative Effects of Selective A2A Adenosine Receptor Agonist Regadenoson and B-Sitosterol in Experimental Induced Wound Healing in Mice

Conflict of interest: nothing to declare.
Authors' contribution: Ali Mazin Talib – conceptualization, data curation, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft and writing – review & editing; Mohammed Hussain Al-Mayahy – conceptualization, data curation, project administration, resources, software, supervision, validation, visualization, writing – original draft and writing – review & editing.
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Contacts: ali.mazentalib@au.edu.iq

Abstract

Introduction. Wound healing is a multi-phase and organized dynamic process to repair damaged tissue. Adenosine (A2A) receptor agonists were found to have a role in wound healing process such as increase collagen and angiogenesis and re-epithelization.
Purpose. To evaluate the healing effect of regadenoson ointment in experimentally induced wounds in mice.
Materials and methods. Regadenoson ointments (0.25% and 0.5% w/w) were prepared by dispersing regadenoson in castor oil followed by the addition of vasaline base to obtain the final ointment preparation. In vivo study: Sixty female albino mice were enrolled in this study and they divided into five groups (N=12/group). Group I: normal control. Group II: induced control which has received vasaline base only. Group III were treated topically with β-sitosterol ointment (0.25% w/w) as a positive control. Group IV and V were treated topically with regadenoson ointment (0.25% and 0.5% w/w) respectively. These products were applied once daily for ten days.
Results. Regadenoson ointment produced a highly significant reduction in wound size in comparison with vasaline base ($P \leq 0.001$). In a histological study, re-epithelization and angiogenesis scores of all treatment groups showed a significant increase in comparison with vasaline base group ($P \leq 0.05$) but in collagen scores, the significant increase just found with β-sitosterol and the highest concentration of regadenoson 0.5% w/w in comparison with vasaline base group ($P \leq 0.05$).
Conclusion. According to the present findings we can conclude that regadenoson ointment is more efficient than petrolatum base and comparable in efficacy to β-sitosterol ointment in accelerating wound healing.
Keywords: regadenoson, adenosine A2A receptor agonist, wound assessment, histopathologic grading, vasaline base

■ INTRODUCTION

Wound healing is a mechanism that involves a series of coordinated and multi-phases including [1]: hemostasis, inflammation, proliferation and remodeling [2, 3]. In acute inflammation, tissue damage is followed by healing. Whereas in chronic inflammation, damage and repair continue for long duration [4]. Inflammatory cells such as neutrophils phagocyte invading pathogens, remove waste and debris, also increase angiogenesis which in turn may enhance the recruitment of inflammatory cells and the subsequent laying down extracellular matrix to repair tissue damage [5]. Long time inflammation may lead to tissue damage as well as aberrant or inadequate repair can lead to poorly ordered matrix deposition and fibrosis which affects normal tissue architecture [6].

Based on previous in vitro studies and in experimental animal models [7], we are proposing a new treatment for the promotion of impaired wound healing, is the use of adenosine receptor agonists.

Adenosine regulates cell functions by acting at specific receptors on the cell surface [8]. Generally, A1 and A3 receptors activate the Gi(inhibitory) family of G proteins, whereas A2A and A2B receptors activate the Gs(excitatory) family. Wound treatment with an adenosine receptor agonist accelerates the healing of the wound by acting on several stages involved in the wound healing process such as fibroblast migration, a rise in a matrix, and promotion of angiogenesis in the wound [9].

As soon as after the injury, the inflammatory response begins. The innate immune system is activated by evoking a local inflammatory response that recruits various types of inflammatory cells to the site of the wound [10]. Despite A2A agonists are inhibitors of the neutrophil oxidative flow, but they do not prevent the oxidative blast. Some of the oxidative blasts are resistant to inhibition by A2A agonist binding [11]. In addition to A2A receptors, neutrophils contain A1A receptors [12], and possibly A3 receptors that control neutrophil function [13].

Several findings suggest that adenosine A2A is important for angiogenesis by modifying the release of angiogenic factors from numerous cells and tissues [14]. collagen production is increased in wound healing by stimulation of the A2A receptors [15]. Collagen production also increased by activation of the A2A receptors [16].

■ PURPOSE OF THE STUDY

To evaluate the healing effect of regadenoson ointment in experimentally induced wounds in mice.

■ MATERIALS AND METHODS

Regadenoson powder: Royal pharm/China, B-sitosterol ointment: Philadelphia/Jordan, Formalin (MW=30.3): SOLVOCHEM/UK.

Preparation of regadenoson ointment

Since regadenoson is unavailable in a topical dosage form, the first step was to prepare a topical dosage form. The selection of a suitable base for the preparation is depending on the physical properties of regadenoson and its compatibility with chosen base. Two hundred fifty and five hundred milligrams of regadenoson powder was weighed separately and then, dispersed in castor oil (because regadenoson is water-insoluble

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compound) using spatula to obtain a smooth non-gritty dispersion. Afterwards, this dispersion was mixed well with vasaline base by incorporation method to complete the required weight of 100 gm. In incorporation method, a small amount of petrolatum base was mixed carefully with a small volume of regadenoson dispersion for about 45 minutes until a clear homogenized ointment was obtained. The prepared regadenoson ointment was then transferred into sterile plastic cups which were closed tightly and stored at 25c temperature..

Experimental animal groups

Sixty mice were involved in the experiment. Each group contained twelve mice which were selected randomly from the total mice involved in the experiment Fig. 1.

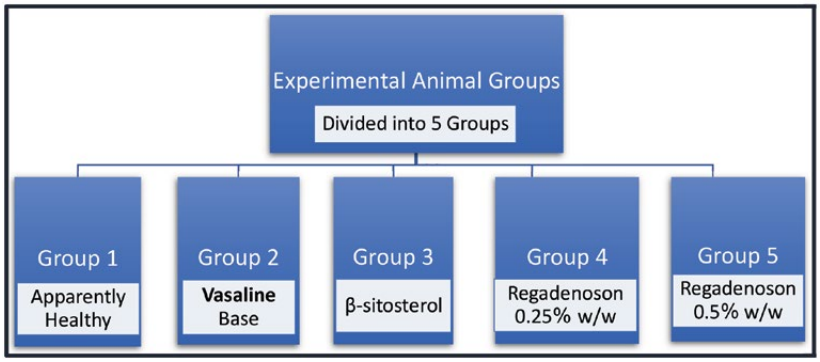

Fig. 1. Experimental Animal Group

Murine wound model

Mice were anesthetized by I.P. injection of "ketamine (100 mg/kg) / xylazine (10 mg/kg)". Afterwards, the back skin hair was shaved using shaving cream and 1-cm full-thickness excisional wound was then made on the back of each mice using 10-mm biopsy punch [17] (Fig. 2).

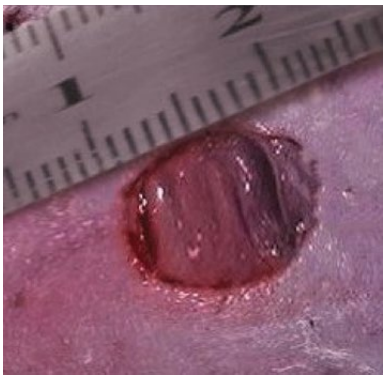

Fig. 2. Experimental Wound induction by punch biopsy

Wounds size measurement

Wounds size area for 6 mice from each group were measured by a ruler from edge to edge at 5th and 10th days of the experiment. The difference between wound size reduction between the experimental groups was compared using the following equation:

%Wound closure = (primary wound area – end wound area)/primary wound area × 100%,

primary wound area (in day 0) was defined as 1 cm [18].

Wounds area at tenth day were carefully dissected by a sharp, sterile surgical blade. Tissues were collected without folding by forceps which were stabilized in 10% buffered formalin solution and stocked into a sterile plastic container for storage ready for embedding in paraffin wax for histopathological and immunological study.

Assessment of the histopathological changes of tissue sections (H & E stains) were used for the staining of paraffin wax sections [19]. The skin histopathological changes for each mouse were scored as follows [20]. The amount of collagen, angiogenesis and re-epithelization scores were made and evaluated as: 0 = absent or a few, 1 = moderate presence, 2 = high [21].

■ RESULTS

Wound size reduction

The data obtained has revealed a highly significant reduction in the wound size of the treatment groups as compared with induced control group (petrolatum group) (Pa ≤0.001). However, there were no significant differences in comparisons between the treatment groups (Pb, Pc >0.05) as shown in Table 1 and Fig. 3.

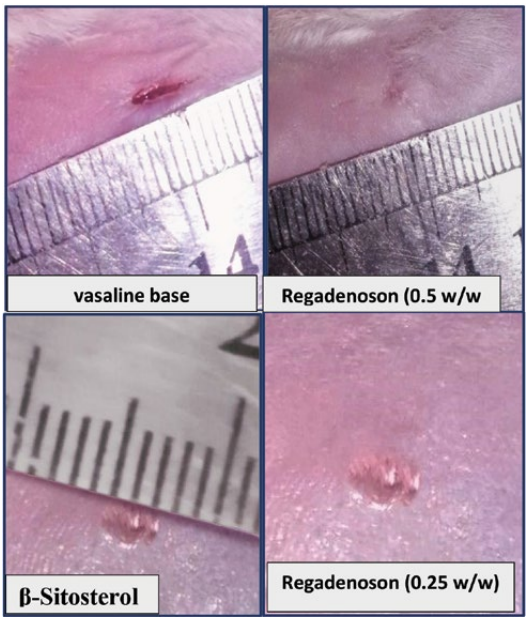

Fig. 3. Wound images on the tenth day showed completely wound closure in treatment groups, but incomplete wound closure in Vasaline base group

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Table 1
Comparison of wound size reduction between induced control and each other treatment groups and among other treatment groups by unpaired t-test

Day	Wound reduction %	Vasaline base, N=6	β-sitosterol, N=6	Regadenoson (0.25%), N=6	Regadenoson (0.5%), N=6
Day 5	Mean ± SE	28.33+1.67	48.33+1.67	49.67+1.86	47.67+0.92
	P-value a		<0.001	<0.001	<0.001
	P-value b			0.605	0.733
	P-value c				0.357
Day 10	Mean ± SE	67.5+1.12	98.0+1.0	95.0+1.83	97.67+1.23
	P-value a		<0.001	<0.001	<0.001
	P-value b			0.180	0.838
	P-value c				0.254

Notes: Data represented as Mean ± Standard error (SE); N = number of animals from each group tested per day; (a) Comparison between Vasaline base and each treatment group, (b) Comparison between β-Sitosterol and both regadenoson 0.25% and 0.5%, (c) Comparison between regadenoson 0.255 and regadenoson 0.5%.

Histopathological scores at tenth day re-epithelization score

The data has shown that there was a significant decrease in the petrolatum group in comparison to apparently healthy group (Pa ≤0.05). While, in all treatment groups, there were no significant differences with the apparently healthy group (Pa >0.05) respectively as illustrated in Table 2.

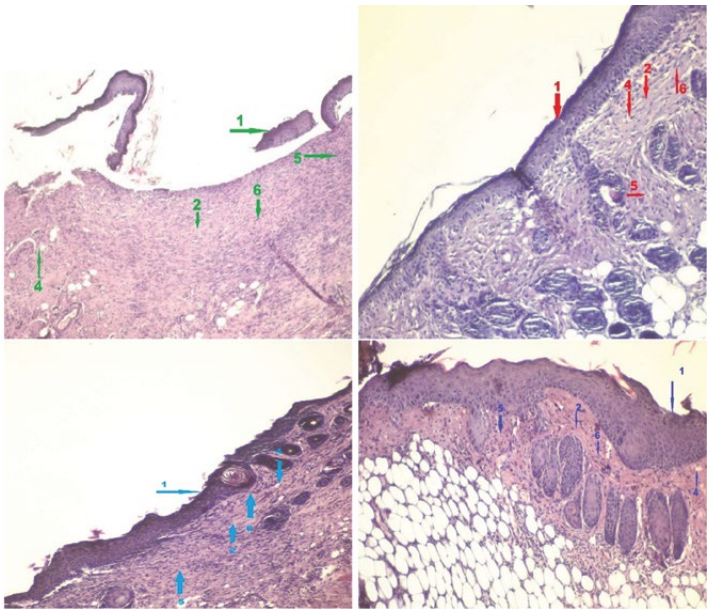

Fig. 4. Cross-section of wound tissue at tenth day for vasaline base (green arrow) showed high presence of inflammatory cells and fibroblasts but mild collagen, angiogenesis and re-epithelization, β-sitosterol (red arrow), regadenoson (0.25%) (bright blue arrow) and regadenoson (0.5%) (blue arrow) groups showed the presence of few inflammatory cells and fibroblasts, high angiogenesis, collagen and re-epithelization on microscopical examination (H&E staining) (10x). 1. Re-epithelialization. 2. Collagen. 3. Hair follicle. 4. Blood vessels. 5. Infllamatory cell. 6. Fibroblast

Table 2
Comparisons of histopathology at tenth day between healthy, induced control, and each other treatment groups and among each treatment group by Mann Whitney test

Histopath-ology		Apparent-ly healthy, N=6	Vasaline base, N=6	β-sitosterol, N=6	Regadeno-son (0.25%), N=6	Regade-noson (0.5%), N=6
Re-epitheliza-tion	Mean ± SE	2.0+0.0	1.0+0.0	2.0+0.0	2.0+0.0	2.0+0.0
	Median	2.0	1.0	2.0	2.0	2.0
	P-value a		*0.002*	**1.000**	**1.000**	**1.000**
	P-value b			*0.002*	*0.002*	*0.002*
	P-value c				**1.000**	**1.000**
	P-value d					**1.000**
Angioge-nesis	Mean ± SE	2.0+0.0	1.0+0.0	1.83+0.17	2.0+0.0	2.0+0.0
	Median	2.0	1.0	2.0	2.0	2.0
	P-value a		*0.002*	**0.699**	**1.000**	**1.000**
	P-value b			*0.015*	*0.002*	*0.002*
	P-value c				**0.699**	**0.699**
	P-value d					**1.000**
Collagen	Mean ± SE	2.0+0.0	1.17+0.17	2.0+0.0	1.67+0.21	2.0+0.0
	Median	2.0	1.0	2.0	2.00	2.0
	P-value a		*0.015*	**1.000**	**0.394**	**1.000**
	P-value b			*0.015*	**0.180**	*0.015*
	P-value c				**0.394**	**1.000**

Notes: Data represented as Mean ± Standard error (SE); N= number of animals from each group tested per day; (a) Comparison between healthy and all other groups, (b) Comparison between vasaline base and β-sitosterol, regadenoson 0.25% and 0.5%, (c) Comparison between β-sitosterol and both regadenoson 0.25% and 0.5%, (d) Comparison between regadenoson 0.25% and Regadenoson 0.5%.

Furthermore, all treatment groups had a significant increase in re-epithelization score as compared to the vasaline group (pb ≤0.05) respectively. Though, a nonsignificant difference has been demonstrated between the treatment groups as shown in Table 2 and Fig. 4.

Angiogenesis score

In a comparison of healthy group with other groups, vasaline group was significantly lowered (Pa ≤0.05). For other treatment groups, it was found that there are no significant differences with healthy group (Pa >0.05) respectively as shown in Table 2. A comparison of vasaline group with each treatment groups have shown that all treatment groups were significantly higher than vasaline group (pb ≤0.05) as demonstrated in Table 2 and Fig. 4. In addition, there were no significant differences in comparisons between the treatment groups (Pc and Pd >0.05) as shown in Table 2.

Collagen score

The results have shown that the C-Collagen score for petrolatum group is significantly lower than apparently healthy group (Pa ≤0.05) as illustrated in Table 2. In a comparison of vasaline group with each treatment groups, it was found that there is a significant increase in β-sitosterol and regadenoson (0.5% w/w) groups (Pb ≤0.05) respectively. While, there

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was no significant increase in the regadenoson (0.25% w/w) group (Pb >0.05) as shown in Table 2 and Fig. 4. Moreover, there were no significant differences in comparison between treatment groups (Pc and Pd >0.05) as demonstrated in Table 2.

■ DISCUSSION

The attractive properties of the ointment base (vasaline) including the ability to cover the site of application for a long time, and it will act as a barrier that prevents foreign substances and microorganisms from penetration into the tissue through the wound. In addition, the ointment base increases the hydration of the skin due to its oil nature that will prevent moisture evaporation leading to an increase in active ingredient penetration [22]. Furthermore, the prepared regadenoson ointment will be in a similar dosage form when compared to the positive control β-sitosterol ointment, since both of them have been used in an ointment dosage form. This will minimize any effect of the dosage form type on the wound healing process.

The present study showed complete wound closing in both regadenoson groups (0.25% and 0.5% w/w) in day ten in comparison to vasaline base group which presented incomplete wound healing. This proves the efficacy of regadenoson on the wound size reduction and acceleration of wound closure [23, 24]. Victor-Vega et al. [23] and Montesinos et al. [24] have found that a selective A2AAR agonist accelerates wound healing in the wild but not knockout mice which are in agreement with the findings of the present study in that an adenosine (A2A) agonist accelerates wound healing. The proposed mechanism for this wound reduction is by a contraction that reduces wound size with a central gravitational motion of dermis and epidermis [25].

The findings obtained from this study regarding re-epithelization showed that regadenoson significantly accelerates re-epithelization in comparison with vasaline base group [26] explained the expected mechanism that may be involved in accelerating re-epithelization by adenosine agonist is that adenosine stimulates macrophage differentiation into M2-type macrophages which increase wound healing by increasing growth factors such as VEGF that stimulates repair of tissue at sites of injury [25]. The histological examination to assess the re-epithelization of the wound and found a significant increase in the CGS-21680 (A2A adenosine agonist)-treated animals as compared to controls, this agreed with the present findings [24].

Both regadenoson concentrations of (0.25% and 0.5% w/w) found to significantly increase angiogenesis compared to vasaline base group. When compared to β-sitosterol ointment group found increase but statistically, it did not reach a significant level with the β-sitosterol group, showing that regadenoson has strong efficacy on angiogenesis. Authors proposed the mechanism of the angiogenic effects of adenosine acting on the subtype (A2A) receptor occurs directly via increased endothelial cell migration [27]. Additionally, other claimed that adenosine A2A receptor activation inhibits the generation of thrombospondin I, which is a powerful inhibitor of angiogenesis [28].

Although, there were no significant difference between both regadenoson and β-sitosterol treatment groups, regadenoson has shown a strong effect on collagen synthesis [28]. They found that adenosine A2A receptor activation motivates fibroblasts to generate type I and III collagen at a high level comparable to that stimulated by the transforming growth factor. In addition, Valls et al., [29] observed that A2AR activation can promote collagen and matrix production. Those results are in agreement with the present findings.

CONCLUSION

Topical application of A2A adenosine receptor agonist (regadenoson ointment) once daily for 10 days on induced wound seems to be more effective in accelerating wound healing in comparison to vasaline base and comparable in efficacy to β-sitosterol ointment in all parameters scores that have been measured.

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Zainab F. Salbookhh, Usama H. Ramadhan✉, Gibran K. Hassan
College of Pharmacy, University of Basrah, Basrah, Iraq

Evaluation of Serum Levels of Leptin, Nesfatin-1 and Electrolytes (Sodium, Potassium, Magnesium, Calcium and Chloride) in Patients with Hypertension

Conflict of interest: nothing to declare.
Authors' contribution: Zainab F. Salbookhh – conceptualization, methodology, investigation, resources, data curation, writing – original draft; Usama H. Ramadhan – conceptualization, methodology, investigation, resources, data curation, writing – original draft, soft wire; Gibran K. Hassan – investigation, resources, data curation, writing – original draft, soft wire.
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Contacts: usama_ramadhan@yahoo.com

Abstract

Introduction. Hypertension is a cardiovascular ailment that exhibits a high prevalence rate and poses significant risks of morbidity and mortality on a global scale. It is accountable for the onset of stroke, ischemic heart disease, and kidney failure. Numerous epidemiological studies have elucidated the correlation between hypertension and various biochemical markers.
Purpose. To determine the most significant changes in the parameters of serum leptin, nesfatin-1, and serum electrolytes such as sodium, potassium, calcium, magnesium, and chloride associated with patients who have only hypertension disease. Additionally, research the interactions of these adipokines with the electrolytes present in each group.
Materials and methods. This study was carried out at the Al-Basrah Teaching Hospital on patients with hypertension from December 2022 to March 2023. Fifty-three hypertensive patient samples, ranging in age from 30 to 60 years, were included in this study, and the various characteristics were compared to the same variables in fifty-three healthy subjects aged 30 to 40 years.
Results. While the electrolytes were within normal range for each group, the results indicate an increase in serum levels of leptin and nesfatin-1 in the hypertension group compared to the control group. The findings suggest no relationship between electrolytes and adipokines except for a positive correlation between leptin and chloride ions.
Conclusion. The current investigation determined that non-obese hypertension individuals had higher serum levels of leptin and nesfatin-1 than the healthy control group. At the same time, each group's electrolyte levels (sodium, potassium, calcium, magnesium, chloride) are within the normal range. Compared to the hypertension group, potassium, calcium, and chloride levels in the control group increased slightly.
Keywords: leptin, nesfatin-1, sodium, potassium, magnesium

■ INTRODUCTION

Essential hypertension is a chronic increase in blood pressure caused by various genetic and environmental factors. Its prevalence increases with age, regardless of the diagnostic criteria or measurement techniques. In both developed and developing nations, between 25 and 35 per cent of adults suffer from essential hypertension, and 60 and 70 per cent of people in their seventh decade are affected. Hypertension clusters with other cardiovascular risk factors like abdominal obesity, dyslipidemia, glucose intolerance, hyperinsulinemia, and hyperuricemia due to a shared underlying etiology [1]. Based on evidence from studies, older age, male sex and hyperlipidemia all increase the risk of developing hypertension [2].

According to the World Health Organization's estimate from 2006, an alarming 40% of people worldwide have hypertension, and elevated systolic blood pressure has been linked to 10.5 million deaths worldwide [3]. As measured by DALYs, the issue changed from the fourth Leading Global Burden of Disease Risk Factor in 1999 to the first risk factor in 2010 [4]. Various metabolic processes, including blood pressure, glucose homeostasis, inflammation, and angiogenesis, are influenced by the numerous adipokines secreted by adipose tissue. Leptin, one of the main adipokines produced by adipocytes, is encoded by the LEP gene [5] [6].

Leptin promotes blood pressure in the endocrine system. Leptin's endocrine system-mediated effects on blood pressure regulation include: (i) The LR is expressed in the adrenocortical zona glomerulosa's aldosterone-secreting cells, which may boost leptin's potential to trigger adrenal aldosterone release, which raises blood pressure directly. (ii) Leptin regulates anterior pituitary hormones and thyroid-stimulating hormones. TSH and ACTH regulate blood pressure now and indirectly. (iii) When stressed or fasting, leptin suppresses the hypothalamus-pituitary thyroid and adrenal axes, which may contribute to stress-induced blood pressure increase [7]. Nesfatin-l is an N-terminal 82 amino acid peptide nucleobindin-2-derived adipokine. Nesfatin was initially identified as an anorexigenic molecule secreted by the hypothalamus, testes, pancreatic cells, subcutaneous adipose tissue and gastric mucosa. Its exact mechanism of action is still unclear [8].

The regulation of cardiovascular function is aided by nesfatin-l. Nesfatin-l distribution at the central level may be crucial in controlling cardiovascular mechanisms and processes which support cardiovascular homeostasis. Nesfatin-l injection into the cerebrospinal fluid, for instance, raises arterial blood pressure. By depolarizing the paraventricular nucleus, nesfatin-l stimulates the release of oxytocin. It is also understood that oxytocin, in conjunction with nesfatin-l, activates the melanocortin pathway. Therefore, it is assumed that the central oxytocin or melanocortin pathways are responsible for the hypertensive effect. Nesfatin-l was given intravenously to animals in a study, and it was discovered that this resulted in vasoconstriction, high blood pressure, and inhibition of nitric oxide production [9].

Electrolytes are electrically charged molecules crucial for maintaining a healthy acid-base balance, blood clotting and muscle contractions. This balance is vital for hydration, nerve impulses, muscle function and pH level. The body's primary electrolytes are potassium, sodium, calcium, magnesium, and chloride. The main factors determining the myocardial membrane's electrophysiological characteristics are sodium, potassium, and chloride [10]. The goal of figuring out how hypertensive patients' trace element variations

vary contributes to addressing and lowering the risk of CVD (cardiovascular disease) and the associated morbidity and mortality globally. Currently, numerous researchers have pinpointed the essential variations in each of these trace elements separately.

It is essential to comprehend the significance of these ions. First, $Na^{\pm}$ regulates the extracellular fluid volume between the blood and the surrounding cells, an osmolality component that plays a significant role in the development of hypertension. The extra fluid in the blood causes the blood pressure to rise and stresses the kidneys' ability to filter out water. When consumed in large amounts, $Na^{\pm}$ and Cl^{-} have been highly influential in developing hypertension [11]. On the other hand, potassium dietary supplements can lower blood pressure in both standard and hypertensive patients. Potassium channels are vital in regulating the resting membrane potential and cell volume, along with $Na^{\pm}$-$K^{\pm}$-ATPase (also called the $Na^{\pm}$-$K^{\pm}$ pump).

The activation and subsequent opening of potassium channels cause the plasma membrane to become hyperpolarized because the potassium concentration in the intracellular, as opposed to the extracellular medium, is significantly higher. This alters the electrogenic driving force for $Na^{\pm}$ reabsorption in the distal nephron [12].

Even though magnesium is the second necessary mineral in the body and crucial for many vital activities, hospitalized patients' serum magnesium concentrations are rarely routinely examined. Therefore, most magnesium problems go undiagnosed [13]. Calcium affects blood coagulation, neurotransmitter release, blood pressure regulation, cardiac electrophysiology and contraction. Calcium and magnesium help maintain blood pressure by providing calcium channels for smooth muscle contraction and relaxation at each site [14].

Thus, excessive salt and chloride concentrations and $Mg^{\pm 2}$ deficits contribute to global blood pressure homeostasis disorders such as hypertension [11]. Clinical investigations have linked low serum chloride levels to hypertension and cardiovascular disease mortality. The present study investigated the serum concentrations of leptin and nesfatin-1 and the electrolytes sodium, potassium, calcium, magnesium, and chloride. Furthermore, the study aimed to explore the potential relationships between these variables. We specifically investigated hypertension patients and contrasted them with a control group of healthy individuals due to the significant effect that these electrolytes and adipokines have on hypertension.

■ PURPOSE OF THE STUDY

To determine the most significant changes in the parameters of serum leptin, nesfatin-1, and serum electrolytes such as sodium, potassium, calcium, magnesium, and chloride associated with patients who have only hypertension disease. Additionally, research the interactions of these adipokines with the electrolytes present in each group.

■ MATERIALS AND METHODS

Place and time of data collection

A study was carried out at the Internal Medicine Division of Al-Basrah Teaching Hospital between December 2022 and March 2023. As the primary referral hub for private, direct, and secondary healthcare providers, the hospital is a significant healthcare facility in this region. Twenty contributors were not included in the investigation, which involved

126 participants. Two groups were created out of the remaining participants. Regarding characteristic data, the 53 hypertensive patients who made up the first group were compared to the 53 healthy adults who made up the second group.

Inclusion and exclusion samples

The study included everyone who had been given a diagnosis of hypertension and was receiving care from internal medicine specialists. People with hypertension who also had diabetes, kidney disease, or thyroid disorders were not eligible for the study and were excluded.

Measurements

Babyly company's device evaluated blood pressure for all participants. After 5 minutes of rest, the individual sat with their right arm on the desk. Also, body mass index has been calculated following weight and height measurements for all individuals. A 5ml blood sample was taken from all participants and then collected in a sterile disposable syringe. Then, blood was transferred to a gel tube that did not have an anticoagulant. To assess the fasting serum electrolyte levels, including sodium, potassium, calcium, magnesium, and chloride, as well as the levels of adipokines (leptin and nesfatin-1) and the ability for clot retraction, the tubes were allowed to stand at room temperature for 30 minutes. The serum was extracted from the blood samples using a Kokusan centrifuge H-F and centrifuged at 3000 rpm for 15 minutes. The serum was then transferred to polyethene Eppendorf tubes and stored at –20 °C for electrolytes and adipokines analysis. The electrolytes were analyzed using an ARCHITECTplus c4000. Enzyme-linked immunosorbent assay (ELISA) kits from the Wuhan, China-based company Elabscience were used to measure the serum levels of leptin and nesfatin-1.

Statistical analysis

The data were initially entered into Microsoft Excel files. The IBM Corporation in Armonk, New York, the United States, provided SPSS version 22, which was used for all statistical studies. The Shapiro-Wilk test was used to determine whether each variable's distribution was normal. The independent Student's t-test was used for the analysis of the data. The analyzed data were represented by mean ± standard deviation. P-value equal to 0.05 or less, which represents Statistical significance. A Pearson correlation analysis was also used to determine the correlation between the serum leptin level, nesfatin-1, and serum electrolyte levels.

■ RESULTS

The characteristics of the individuals, like their biochemistry and anthropometrics, are delineated in Table 1. A significant difference was found in the mean age of both groups ($p=0.00$). A significant difference was shown in BMI between the control group (25.108 ± 2.711) and the hypertensive group (27.947 ± 2.781). All participants in both groups were considered non-obese individuals due to a BMI under 30. The control group and hypertensive patients had significant differences in systolic BP ($p=0.000$, $p<0.001$) and diastolic BP ($p=0.000$, $p<0.001$), respectively. The hypertensive patients had significantly higher levels of leptin ($p=0.032$) than the control group. Also, the serum nesfatin-1 level of the hypertensive patients (814.907 ± 239.107) was considerably more than that of the

control group (544.923±374.475). A descriptive analysis of the electrolyte study variables is represented in Table 1. There is no significant difference in serum electrolyte levels between the control group and hypertensive groups for Na (p=0.581), Mg (p=0.145), and K (p=0.196). At the same time, there were statistically significant differences between patients and controls for Ca (p=0.003) and Cl (p=0.020).

The correlation between the serum level of leptin for both the control and hypertensive groups with serum electrolytes is represented in Table (2). There was no significant relationship observed between leptin level and Na (p=0.425), K (p=0.385), Mg (p=0.070), Ca (p=0.085) and Cl (p=0.167) in the control group. Moreover, the hypertensive group did not show a significant relationship between leptin with Na (p=0.227), K (p=0.079), Mg (p=0.738) and Ca (p=0.858) except Cl (p=0.015) which exhibits a positive relationship. Leptin has no significant association with systolic BP, diastolic BP, and BMI. Leptin relationship with nesfatin-1 shows significance in the control group (p=0.000), while in a hypertensive group no significant relationship (p=0.158).

The correlation between serum level of nesfatin-1 for both the control and hypertensive groups with serum electrolytes was represented in Table 3. There was no

Table 1
Anthropometric and clinical characteristics of the study groups

Group	No.	%No.	M/F	%M/F
Control	53	50	24/29	45.28/54.72
Patients	53	50	24/29	45.28/54.72
	BMI (Kg/m²)	**Age (years)**	**Systolic (mm Hg)**	**Diastolic (mm Hg)**
Control	25.108±2.711**	35.962±4.612**	72.377±5.368**	111.584±6.428**
Patients	27.947±2.781**	41.056±6.726**	89.886±10.053**	141.47±16.82**
	Leptin (pg/ml)	**Nesfatine-1(pg/ml)**	**Na (mmol/L)**	**K (mmol/L)**
Control	454.91±386.07	544.92±374.47	139.26±3.97	4.08±0.41
Patients	598.69±323.61*	814.90±239.10**	138.86±3.37	3.97±0.38
	Mg (mg/dL)	**Ca (mg/dL)**	**Cl (mmol/L)**	
Control	1.933±0.173**	9.147±0.9214**	106.188±3.138**	
Patients	1.98±0.18	9.611±0.607**	104.698±3.343**	

Notes: values are expressed as mean ± standard deviation in each group; * significant at p<0.05; ** significant at p<0.01.

Table 2
The relationship of leptin with other parameters of patients and control in terms of probability and correlation

Leptin		Na	K	Mg	Ca	Cl
Control	R	–0.112	0.122	–0.251	–0.239	0.193
	P	0.425	0.385	0.070	0.085	0.167
Patients	R	0.169	0.243	0.047	0.025	0.332*
	P	0.227	0.079	0.738	0.858	0.015
		Nesfatine-1	**BMI**	**Systolic**	**Diastolic**	
Control	R	0.720**	0.161	–0.116	0.157	
	P	0.000	0.251	0.408	0.261	
Patient	R	0.197	0.097	–0.146	–0.188	
	P	0.158	0.491	0.298	0.178	

Table 3
The relationship of nesfatin-1 with other parameters of patients and control in terms of probability and correlation

Nesfatine-1		Na	K	Mg	Ca	Cl
Control	R	–0.069	0.052	–0.248	–0.024	0.176
	P	0.622	0.712	0.073	0.864	0.207
Patients	R	0.090	–0.076	–0.014	–0.007	0.147
	P	0.520	0.590	0.919	0.962	0.293
		Leptin	**BMI**	**Systolic**	**Diastolic**	
Control	R	0.720**	0.002	–0.204	0.160	
	P	0.000	0.991	0.142	0.252	
Patient	R	0.197	0.423**	–0.275*	–0.137	
	P	0.158	0.002	0.046	0.326	

significant relationship detected between leptin level and Na (p=0.622), K (p=0.712), Mg (p=0.073), Ca (p=0.864) and Cl (p=0.207) in the control group. Additionally, the hypertensive group did not display a significant relationship between leptin with Na (p=0.520), K (p=0.590), Mg (p=0.919), Ca (p=0.962) and Cl (p=0.293). There is no significant relationship between nesfatin-1 and diastolic BP for each group, while BMI and systolic BP represent a significant relationship with nisfatin-1 only in the hypertensive group (p=0.046) and (p=0.002), respectively. The nesfatin-1 relationship with leptin displays significance in the control group (p=0.000), while in the hypertensive group no significant relationship (p=0.158).

■ DISCUSSION

The hypertensive group's serum leptin level has increased compared to the control group. The present result agrees with the study of Al-garawi et al., which shows the levels of leptin hormone, low-density lipoprotein cholesterol, and triglycerides are significantly higher in many persons with hypertension [15]. The idea is that the downstream effects of hypothalamic leptin signalling mediate the effects of leptin on blood pressure (BP) and ultimately lead to the activation of particular melanocortin receptors found on sympathetic neurons in the spinal cord. The renin-angiotensin system is activated due to this sympathetic activity of the heart and kidney, which also causes sodium retention and circulatory expansion, then blood pressure is also increased [7]. Compared to patients with normotension, those with hypertension exhibited significantly higher leptin and lower adiponectin levels. Leptin, through its probable association with arterial stiffness (AS), causes salt retention and volume expansion by activating the renin-angiotensin-aldosterone pathway. Leptin has been demonstrated to affect men's systolic and diastolic blood pressure without regard to BMI] 16[. For instance, some research has linked high leptin levels to renal failure, natriuresis, hyperglycemia, and increased blood pressure] 6[.

The study's results indicate that the serum level of nesfatin-1 in hypertensive patients is higher than in the control group. The findings of Zhao et al. indicated that the hypertension group's fasting plasma nesfatin-l level was substantially greater than that of the control group. The overweight/obese hypertension patients had significantly greater fasting plasma nesfatin-l levels than BMI-matched controls but not those with normal BMI, suggesting nesfatin-l may be crucial in obesity hypertension. In addition to its effects

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on controlling energy homeostasis, nesfatin-l delivery to the central nervous system has cardiovascular consequences [17].

For instance, it has been demonstrated that intravenously administering nesfatin-l elevates mean arterial pressure, and an increase in heart rate accompanies this effect. Also, central nervous nesfatin-l enhances renal sympathetic nerve activity, which is known to be involved in blood pressure regulation through the renin-angiotensin system. At the same time, it is still unclear if it stimulates cardiac sympathetic innervation] 18[. In the previous study, nesfatin-l positively correlated with apelin and lipid profile. On the other hand, there are no noticeable effects on blood pressure since control values were nearly identical to those of the patients [19].

The electrolyte values could differ depending on the geographical position and people's lifestyle factors like diet and exercise] 11[. In this study, serum sodium level was within normal levels for each group. Wu et al. also found a non-linear connection between serum sodium and potassium and hypertension [20]. The results contradict the hypothesis that the hypertensive patients had increased $Na^{\pm}$ levels [11]. In contrast, the result agreed with Abdul-Razak et al. that sodium levels in the hypertensive and control groups did not differ significantly [21]. In many (but not all) patients with excessive blood pressure, dietary salt restriction has been demonstrated to reduce blood pressure in clinical investigations [22]. An extensive meta-analysis revealed that, regardless of sex or ethnicity, a minor reduction in salt intake for four or more weeks significantly lowers blood pressure in hypertensive and normotensive people. Additionally, more considerable salt intake reductions are associated with larger drops in systolic blood pressure]23[.

A study involving hypertension and a healthy group showed that total body potassium was linked inversely with blood pressure] 24[. According to study data, the control and hypertension groups' potassium levels were within the normal range. However, the potassium levels in the hypertensive group were lower than in the control group. Additionally, a study in a Dutch population indicated that after controlling for confounding variables, there was a slight negative connection between serum potassium and blood pressure [24]. Although the mechanisms by which potassium disruptions affect patient life are understood, little is known about the ideal range of serum potassium in disease and the levels associated with elevated risk [25]. Renal excretion and the movement of serum potassium between intracellular and extracellular fluid compartments keep it between 3.5 and 5.3 mmol/l. Despite a negative concentration gradient, the sodium potassium-ATPase pump maintains a high intracellular potassium concentration. Aldosterone, catecholamines, insulin, hyperkalemia and other factors all act as stimulants] 26[.

The serum calcium concentration in the hypertension group had higher levels than the control group. It is crucial to remember that the calcium levels in both groups were normal. In specific epidemiological research, more elevated serum Ca levels were linked to a higher risk of hypertension. In contrast, lower serum Ca levels were linked to a higher risk of hypertension]27[. The main regulators of plasma calcium levels are parathyroid hormone (PTH) and 1, $25(OH)_2$ D. Although it has no well-defined physiological function in humans, calcitonin can also be considered a calcitropic hormone [28]. Serum calcium was considerably lower and adversely linked with systolic and diastolic blood pressure in hypertensive individuals] 29[.

The magnesium level is assessed in control people and patients with hypertension. The results were determined to be indistinguishable, and the magnesium concentration

was within the normal range. The result disagreed with a study that showed lower magnesium levels in hypertensive patients compared to the control group [11]. Three organs work in dynamic equilibrium to control the amount of magnesium in the blood: the kidneys (renal transport and excretion), the intestine (facilitating magnesium uptake) and the bone (the magnesium storage system: availability of magnesium to maintain constant serum levels) [30].

The magnesium level directly influences the ability of vascular smooth muscle cells to relax and control other cations crucial to blood pressure, including intracellular calcium and the ratio of sodium to potassium in cells. Nutritional magnesium affects blood pressure control and, consequently, the prevalence of hypertension directly and indirectly. In 2021, magnesium deficit may affect blood pressure and result in hypertension [31]. Except for a tiny increase of 0.06mmol/L at menopause, plasma magnesium concentrations did not significantly differ with age or between the sexes. Like calcium, plasma magnesium levels with estrogen replacement decrease to premenopausal levels [28]. However, numerous research on serum magnesium revealed no correlation with hypertension or blood pressure] 20[. Serum chloride levels and systolic blood pressure are correlated directly, and diastolic blood pressure has an inverse association that is not statistically significant. It was agreed with Nakajima et al. [32].

Leptin and electrolytes (sodium, potassium, calcium, and magnesium) have no link, as seen in Table 2. Even though Cl and leptin have a strong association. One of the many factors contributing to hypertension, sodium retention, is thought to increase leptin levels in the blood [7]. One study's findings indicate that although leptin levels were not related to lithium clearance, leptin accounted for 18% of the variation in lithium clearance, suggesting that leptin may be able to raise blood pressure through increasing renal sodium reabsorption [33]. The observed alterations in aldosterone and CYPI 1 B2 levels are due to leptin's induction of an elevated cytosolic calcium concentration. In addition, leptin infusion causes aldosterone synthase protein expression and blood aldosterone levels to rise in a dose-dependent manner in wild-type or ob/ob female mice lacking leptin in vivo without changing levels of serum sodium, potassium, Angll (angiotensin II), or ACTH (adrenocorticotropic hormone) [34].

Nesfatin-1 was not related to electrolytes. In the kidney, nesfatin-1 and epithelial sodium channels (ENaC) are co-expressed in the renal collecting duct. By increasing sodium retention in the kidney, activation of the renal sympathetic nervous system contributes to the development of salt-induced hypertension [35]. The levels of Nesfatin-l are positively correlated with BMI, hs-CRP (high-sensitivity C-reactive protein), HOMA-IR (homeostasis model assessment as an index of insulin resistance), luteinizing hormone, heart rate, systolic and diastolic blood pressure all of which were measures that exacerbated the syndrome [36]. In contrast to the present findings, the hypertension group shows a link with BMI and systolic blood pressure, while the control group shows no significant correlation with any of these variables [37].

■ CONCLUSION

The current investigation determined that non-obese hypertension individuals had higher serum levels of leptin and nesfatin-1 than the healthy control group. At the same time, each group's electrolyte levels (sodium, potassium, calcium, magnesium, chloride) are within the normal range. Compared to the hypertension group, potassium, calcium,

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and chloride levels in the control group increased slightly. There was no connection between blood leptin and nesfatin-1 and (sodium, potassium, magnesium, calcium, and chloride). Serum chloride revealed a positive correlation with serum leptin, while there was no correlation between serum nesfatin-1 and serum chloride.

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Arwa H. AL-Saeed[1], Alyaa S. Jasam[2], Ali B. Aldeewan[2], Muna H. AL-Saeed[2], Basil A. Abbas[2]✉
[1] College of Science, University of Basrah, Basrah, Iraq
[2] College of Veterinary Medicine, University of Basrah, Basrah, Iraq

Assessment of Antioxidant, Anticancer and Antimicrobial Activities of Silver Nanoparticles N-Acetylcysteine in Sarcoma Male Rat Induction by 7,12 Dimethylbenz[a] Anthracene

Conflict of interest: nothing to declare.
Authors' contribution: Arwa H. AL-Saeed – conceptualization, investigation, resources, data curation, writing – original draft, soft wire; Alyaa S. Jasam – conceptualization, resources, data curation, writing – original draft, soft wire; Ali B. Aldeewan – conceptualization, resources, writing – original draft, soft wire; Muna H. AL-Saeed – conceptualization, data curation, writing – original draft, soft wire; Basil A. Abbas – conceptualization, investigation, resources, data curation, writing – original draft, soft wire, supervisor.
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Contacts: basil.abbas@uobasrah.edu.iq

Abstract

Introduction. Nanoparticles of silver are used in a variety of applications, including catalysts, pharmaceutical constituents and chemical and biological sensing. Animal experiments have shown that carcinogenicity of chemicals is higher in human and animals.
Purpose. To investigate the biosynthesis of silver (Ag) nanoparticles using N-acetylcysteine (NAC) and effects of NAC-NPs on oxidative stress and sarcoma induced by 7,12 dimethylbenz [a] anthracene (DMBA) and antimicrobial.
Materials and methods. We investigated sensitivities to a carcinogen in rats with a model of sarcomas-induction by a subcutaneous injection of chemo-carcinogen that has rarely done in rats. Male rats were injected with 150μg of 7,12-DMBA at 2 months, which resulted in sarcomas-induction in 80% of rats. In grossly observations for the site of injection, sarcomas develop.
Results. The result of NAC-AgNPs lead to significant ($p \leq 0.05$) increased body weight and body weight gain, CAT,SOD, GPx, MOP and TOS compared with DMBA group. The results showed significant decrease the MDA, ALP, AST, Calprotectin, IL-6, TNF-α ,CRP, and TAS diameter and volume of tumors and significant ($p<0.05$) reduced the serum levels of serum p53 CA15.3 in therapeutic group.
Conclusion. Silver nanoparticles were biologically manufactured through N-acetylcysteine (NAC) antioxidant, anticancer, anti-inflammatory, and their antibacterial activity against pathogenic microorganisms We consider these findings may contribute a little to elucidation of process of sarcomas-induction in rats. NAC-AgNPs as antimicrobial agents are among the top uses of silver nanoparticles.
Keywords: antioxidant, anticancer, antimicrobial, n-acetylcysteine, nanoparticles

INTRODUCTION

Cancers allocate numerous features, such as their origins, their explosion, defeat of segregation, and attack of nearby tissues [1]. Cancer therapies include chemotherapy, surgery, hormonal therapy, and radiotherapy, which possess several side effects including toxicity to normal cells and drug resistance problems [2]. To overcome these problems; natural products were used as an alternative approach for cancer therapy [3]. Humans are daily exposed to polycyclic aromatic hydrocarbons (PAHs) which are a ubiquitous class of highly lipophilic environmental organic pollutants [4]. PAHs are released into the environment in large quantities mainly due to human activities [5]. PAHs have detrimental biological effects, toxicity, mutagenicity, and carcinogenicity effects [6]. Exposure to 7,12-dimethylbenz[a]anthracene (DMBA) which is a carcinogenic synthetic PAHs [7] underlie the development of many tumor types including; mammary gland, skin, liver, lung, hematopoietic system, and pancreatic tumors [8]. DMBA requires metabolic activation in liver and extra-hepatic tissues to become an ultimate carcinogen [9].

In recent years, noble metal nanoparticles (NPs) have been intensively utilized for biomedical applications, such as diagnostics, drug delivery, and tissue engineering, due to their unique physicochemical and optoelectronic properties [10–13]. Among various noble metal nanoparticles, silver nanoparticles (AgNPs) have received great attention in a variety of applications, including nanoelectronic devices, sensors, imaging contrast agents, filters, and antimicrobial agents due to their good electrical conductivity, stability, optical property, and antimicrobial activity [14, 15]. AgNPs have also extended their applications to cancer therapy. Several in vitro studies using AgNPs have demonstrated their potential as effective anticancer agents [16–19]. They have exhibited apoptosis-mediated, strong anticancer efficacies in a variety of cancer cells, including human cervical cancer [17], lung cancer [18], and breast cancer cells [19].

The acetylated precursor of L-cysteine, N-acetylcysteine (NAC) has been used for a long time as a mucolytic agent, and in conditions such as acetaminophen intoxication, doxorubicin-induced cardiotoxicity, stable angina pectoris, acute respiratory distress syndrome, and psychiatric disorders. Moreover, it has a very low toxicity level [8]. Being oxidized by various radicals and also acting as a nucleophile, NAC, thanks to these characteristics, can reduce the disulfide bridges in proteins, act as a free-radical capturer, and produce metal chelation [20]. Furthermore, NAC is also known for its anti-inflammatory and antiapoptotic properties [21]. In addition to these direct effects, NAC also exerts its effect by increasing GSH levels [19, 20].

PURPOSE OF THE STUDY

To investigate the biosynthesis of silver (Ag) nanoparticles using N-acetylcysteine (NAC) and effects of NAC-NPs on oxidative stress and sarcoma induced by 7,12 dimethylbenz [a] anthracene (DMBA) and antimicrobial.

MATERIALS AND METHODS

Animals

All experimental measures were accepted by the health check explore principled team at University of Basrah and according to the Guide for the care and use of animals house. Wistar rats (total 32 rats) weighing 200–220 g were used for these studies. All rats were

housed at temperatures of 23±1 °C and a 12 h light: 12 h dark cycle. Rats had free access to tap water and fed standard foodstuff during the adaptation period.

Experimental design

Purpose: to examine the incidence of sarcomas in male rats injected with DMBA. After a one week adaptation period, rats were randomly assigned into five groups (n=8; each) and were distributed in their corresponding cages and classified as follows: group1(Control): non treated rats that were injected S/C with vehicle for 3 months; group 2: the incidence of sarcomas in rats injected with 150 µg/kg doses of DMBA dissolved in 0.05 ml sesame oil was injected into subcutis below panniculus carnosus at the site of tumor of male rats for 2 months and leave 1 month without treated. Group 3: The rats were treated with150 µg/kg doses DMBA dissolved in 0.05 ml sesame oil was injected into subcutis for incidence of sarcomas in rats for 2 months and 4 mg/kg of NAC-AgNPs dissolved in 1ml of normal saline for 1 month. Group 4: The rats were treated with 4 mg/kg of NAC-AgNPs dissolved in 1ml of normal saline for 1 month and incidence of sarcomas in rats injected with 150 µg/kg doses of DMBA dissolved in 0.05 ml sesame oil was injected into subcutis 2 months. Group 5: The rats were treated with 4 mg/kg of NAC-AgNPs dissolved in 1ml of normal saline for3months.

The tumor was observed weekly until necropsy. The latent period was defined as the interval between the day of injection and the day when a nodular lesion became recognizable by palpation at first. When the lesion reached more than 20 mm in diameter, the rat was necropsied. All of the rats were euthanized at the necropsy.

Blood samples were collected by cardiac puncture under anaesthesia (chloroform at 40 mg/kg body weight), and animals were then culled and tissues were harvested. Blood samples were collected without anticoagulant, left for 10 minutes, then centrifuged for 10 minutes at 4000 r/min to obtain serum, which was stored at −20 °C until further biochemical analysis.

Measurement of body weight, body weight gain and weight of pancreas. The animals were weighed before starting the experiment and at the end of the experiment [14].

Determination of blood levels of Calprotecine, TNF-α, and IL-6. MDA, SOD, GPx and Catalase. At day 60, animals were sacrificed and serum levels of, TNF-α (Abcam, Cambridge, UK), and IL-6 (RayBio, GA, USA) were determined using ELISA kits according to the manufacturer's instructions.

Antimicrobial activity assay

The testing microbes used in this study were previously characterized and obtained from central lab in veterinary college that were two Gram-positive bacteria (Staphylococcus aureus, Bacillus cereus, Escherichia coli , Proteus and four fungal isolates Candida albicans, Aspergillus fumigatus, Aspergillus flavus and Aspergillus terreus.

For microbial culture preparation, the testing bacteria were cultivated in nutrient broth at 37 °C for 18 h, whereas fungal isolates was cultivated in potato dextrose broth at 30 °C for 18 h. Then, the cell suspensions were adjusted to approximately 10^8 CFU/mL (equal to 0.5 McFarland standard).

Application of discs

The sterile paper discs (6 mm in diameter) were then soaked with 10 mL of the chemical solutions with different concentrations previously dissolved in DMSO (100 µg/mL, 50 µg/ml, 25 µg/ml, 12.5 µg/ml [22].

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Inoculum preparation

A sterile cotton swab was dipped into the microbial suspension, excess fluid inoculum from the swab was removed by pressing the swab firmly on the side wall of the tube above the fluid level then was inoculated by streaking over the entire sterile agar surface. This procedure was repeated by streaking 2 more times, rotating the plate approximately 60 °C each time to ensure an even distribution of inoculum as a final step, the rim of the agar was swabbed.

The plates were left at room temperature for 15 min, to allow for any excess surface moisture to be absorbed before applying the drug impregnated discs. The discs were air-dried thoroughly, and placed on the surface of the inoculated medium. These inoculated plates were then incubated for 24 h at 37 °C for bacteria, and 30 °C for yeasts and Aspergillus species. Each experiment was carried out in two replicates. The data, recorded by measuring the zone of growth inhibition around the discs by ruler in millimetres [23].

Inoculation of test plates

A sterile cotton swab was dipped into the suspension, excess fluid inoculum from the swab was removed by pressing the swab firmly on the side wall of the tube above the fluid level then was inoculated by streaking over the entire sterile agar surface. This procedure was repeated by streaking 2 more times, rotating the plate approximately 60 °C each time to ensure an even distribution of inoculum as a final step, the rim of the agar was swabbed. The plates were left at room temperature for 15 min, to allow for any excess surface moisture to be absorbed before applying the drug impregnated discs.

Statistical analysis

The data were tabulated as a means and standard deviations (SD) and compared using analysis of variance (ANOVA) followed by post-hoc analysis (Tukey test). A significant difference was considered when P-value ≤0.05. Calculations were made on SPSS software (version 23).

■ RESULTS

Induction of sarcoma by7,12 dimethylbenz[a] anthracene

The latent period was defined as the interval between the day of injection and the day when a nodular lesion became recognizable by palpation at first. When the lesion reached

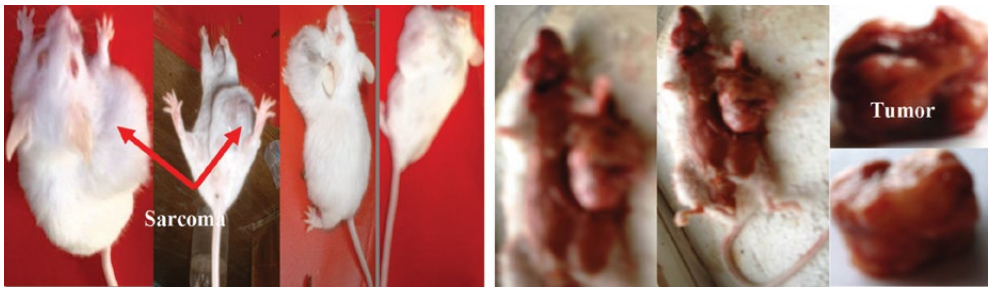

Fig. 1. Show tummour in rat induction sarcoma by 7,12DMBA

more than 20 mm in diameter, the rat was necropsied. All of the rats were euthanized at the necropsy. The hair on the site presence of tumor was shaved and the tissues were widely excised including skin, subcutaneous tissue, muscles and adjacent organs in case of tumor invasion. The abdomen and the thorax including their cavities were also inspected (Fig. 1).

Therapeutic and protective effect of NAC-AgNPs on body weight and body weight gain in sarcoma male rats induced by 7,12DMBA

The study showed a significant (P≤0.05) decrease in final body weight and body weight gain in cancer male rats group(+ve) compared to (-ve) control group. While the result of final body weight and body weight gain in sarcoma treated NAC-AgNPs (4 mg/kg dose) and treated NAC-AgNPs (4mg/kg alone significant (P≤0.05) increase compared with control positive.

The results in Table 1 revealed a significant increase (P≤0.05) in weight of cancer male rats (+ve) control compared with control(-ve) and treated with NAC-AgNPs (4 mg/kg dose).

Table 1
Therapeutic and protective effect of NAC-AgNPs on body weight and body weight gain in sarcoma male rats induced by 7,12DMBA (Mean±SD) (n=8)

Parameters / Treatments	Initial Body Weight (g)	Final Body Weight (g)	Body Weight Gain (g)
Control (-ve) Normal Saline (0.9% NaCl)	209.11± 0.47 NS	229.47±2.75 a	20.6±0.33 a
Control (+ve) Induction sarcoma (7,12DMBA)	211.58±1.01 NS	170.67±2.36 c	–40.94±0.11 d
Induction sarcoma (7,12DMBA) + NAC- AgNPs (Therapeutic Group)	210.63±3.01 NS	218.67±8.01 b	8.04±0.26 c
NAC- AgNP+ Induction sarcoma (7,12DMBA) (Protective Group)	213.43±6.21 NS	226.17±5.11 a	13.74±0.22 b
NAC- AgNP	207.73±2.23 NS	232.23±6.29 a	24.50±0.01 a

Notes: N – number of animals, Small letters denote differences between groups, P≤0.05 vs. control, NS – non-significant.

Therapeutic and protective effect of NAC-AgNPs on antioxidant stress in serum sarcoma male rats induced by 7,12DMBA

The results in Table 2 observed that the effect of NAC-AgNPs on biochemical analysis in sarcoma male rats. The results were showed significant (P<0.05) increase of ALP, ACP, while the results of GPx and SOD levels revealed significant (P≤0.05) decrease in serum sarcoma male rats. Also the results were showed significant (P≤0.05) decrease of concentration, ALP, ACP.

MDAn is the most representative product of lipid peroxidation, and its concentration can indicate the rate and intensity of lipid peroxidation within the body. The current study revealed a significant elevation in MDA level (mg/dl) and marked reduction in GST and CAT activity (P<0.05) in NAC-AgNPs treated groups compared to control group in all examined tissues. pretreatment with Ag-NPs NAC showed no significant difference (P>0.05) in all oxidative parameters in control, while a significant decrease (P<0.05) in the MDA level and a significant increase (P<0.05) in the activity of both CAT and GST were recorded in serum of all treated groups, and that indicated the protective effect of NAC-AgNPs against NAC-AgNPs oxidative stress without affecting NAC-AgNPs antitumor activity.

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Table 2
Therapeutic and protective effect of NAC-AgNPs on antioxidative stress in serum of sarcoma male rats induced by 7,12DMBA (Mean±SD) (n=8)

Parameters / Treatment	GPx (mmol/L)	SOD U/dL	MDA mg/dl	CAT mg/dl	ALP U/L	ACP U/L
Control (-ve) Normal Saline (0.9% NaCl)	16.25±0.86 a	91.3±1.29 a	103.67±14.70 c	569.45±3.38 a	26.7±2.5 b	29.3±7.11 b
Control (+ve) Induction sarcoma (7,12DMBA)	7.68±0.15 b	56. 36±0.13 b	215.63±29.37 a	337.43±6.49 b	57.83±5.78 a	68.31±2.89 a
Induction sarcoma (7,12DMBA) + NAC-AgNPs (Therapeutic Group)	15.46±0.37 a	89.45±0.28 a	149.93±5.51 b	506.67±9.27 a	27.66±3.69 b	31.47±3.86 b
NAC- AgNP+ Induction sarcoma (7,12DMBA) (Protective Group)	16.46±0.11 a	90.05±0.33 a	123.13±5.91 b	546.17±9.27 a	26.55±3.69 b	28.26±3.74 b
NAC- AgNP	17.05±0.19 a	90.29±0.15 a	110.67±8.58 c	565.37±7.6 a	25.91±1.2 b	27.4±3.18 b

Notes: n – number of animals, a, b, c – differences between groups, P≤0.05 vs. control.

Therapeutic and protective effect of NAC-AgNPs on calprotectin, IL-6, TNF-α, CRP, MOP, TOS and TAS in males rats induced by 7,12DMBA

The results in Table 3 observed that the effect of NAC-AgNPs on inflammatory parameters in sarcoma male rats. The results were showed significant (P<0.05) increase of Capl, IL-6, TNF-α CRP and TAS compared with control group while the results of MPO and TOS levels revealed significant (P≤0.05) decrease in serum sarcoma male rats compared with control group.

Table 3
Effect therapeutic and protective of NAC-AgNPs on calprotectin, IL-6, TNF-α, CRP, MPO, TOS and TAS in sarcoma males rats induced by 7,12DMBA (Mean±SD) (n=8)

Parameters / Treatment	Calprotectin ng/ml	TNF-α ng/L	IL-6 Pg/ml	CRP Pg/ml	MPO ng/mg	TOS µmol/g	TAS Mmol/g
Control (-ve) Normal Saline (0.9% NaCl)	350.92±10.01 b	120.12±36.43 c	1.85±0.37 d	0.67±0.011 c	1.12 ±0.29 b	0.27±0.01 a	0.11±0.02 c
Control (+ve) Induction sarcoma (7,2DMBA)	3670.34±39.09 a	200.60±45.23 a	5.56±0.09 a	15.28 ±0.89 a	0.62 ±0.20 d	0.15±0.03 c	0.19±0.06 a
Induction sarcoma (7,12DMBA) + NAC-AgNPs (Therapeutic Group)	765.41±19.51 b	160.17±14.21 b	2.26±0.27 c	7.77±0.25 b	1.04±0.18 c	0.19±0.003 b	0.15±0.03 b
NAC- AgNP+ Induction sarcoma (7,12DMBA) (Protective Group)	695.21± 14.71 b	150.18±19.21 b	2.20±0.12 c	4.37± 0.44 b	1.01±0.16 c	0.16±0.001 b	0.13±0.01 b
NAC- AgNP	310.52±14.36 b	140.11±30.25 b	4.05±0.35 b	0.63±0.012 c	1.64 ±0.32 a	0.30±0.02 a	0.12±0.05 c

Notes: n – number of animals, small letters denote differences between groups, P≤0.05 vs. control.

Therapeutic and protective effect of NAC-AgNPs on CA15.3 and tumor p53 in serum sarcoma male rats induced by 7,12DMBA

The results in Table 4 observed that the effect of on CA15.3 and tumor p53 in sarcoma male rats. The results were showed significant (P<0.05) increase of CA15.3 and tumor p53 in serum sarcoma male rats compared with control group and other groups.

Table 4
Therapeutic and protective effect of NAC-AgNPs on CA15.3 and tumor p53 in serum sarcoma male rats induced by 7,12DMBA (Mean ± SD) (n=8)

Parameters / Treatment	CA15.3 (U/ml)	Tumor p53 (pg/ml)
Control (-ve) Normal Saline (0.9% NaCl)	5000.70±28.01 b	80.60±24.32 b
Control (+ve) Induction sarcoma (7,2DMBA)	7000.96±70.8 a	140.32±32.19 a
Induction sarcoma (7,12DMBA) + NAC- AgNPs (Therapeutic Group)	5500.37±52.90 b	120±17. 32 b
NAC- AgNP+ Induction sarcoma (7,12DMBA) (Protective Group)	5100.37±52.90 b	94.23±10. 12 b
NAC- AgNP	4500.19±27.86 c	75.20±15.84 c

Notes: N – number of animals, a, b, c – differences between groups, P≤0.05 vs. control.

Therapeutic and protective effect of Ag-NPsNAC on final tumor diameters and tumor volume in male rats induced by 7,12DMBA

The results in Table 5 observed that the effect of Ag-NPsNAC on final tumor diameters and tumor volume in sarcoma male rats. The results were showed significant (P<0.05) increase of final tumor diameters and tumor volume in sarcoma male rats compared with control group and other groups. While final tumor diameters and tumor volume in sarcoma male rats were showed significant (P<0.05) decrease in group treated with Ag-NPsNAC**.**

Table 5
Therapeutic and protective effect of Ag-NPsNAC on diameter and tumors volume in male rats induced by DMBA (Mean ± SD) (n=8)

Parameters / Treatment	Tumor Diameter (mm)	Tumor Volume (cm3) rat-1
Control (-ve) Normal Saline (0.9% NaCl)	0.00±0.00 c	0.00±0.0 c
Control (+ve) Induction sarcoma (7,2DMBA)	40.61±1.705 a	25.35±2.01 a
Induction sarcoma (7,12DMBA) + NAC- AgNPs (Therapeutic Group)	27.30±2.10 b	11.56±3.52 b
NAC- AgNP+ Induction sarcoma (7,12DMBA) (Protective Group)	0.00±0.00 c	0.09±0.00 b
NAC- AgNPs	0.00±0.0 c	0.00±0.00 c

Notes: N – number of animals, a, b, c – differences between groups, P≤0.05 vs. control.

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Interpretation of results

After 24–72 hr of incubation, the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. The plates were incubated at 37 °C for 24 hr and the inhibitory zone diameters were measured and interpreted according to the manufacturer's instructions (DHN PAN Kraków, Poland).

Effect of the NAC-AgNPs against bacterial growth

The results observed in Table 6 that the NAC-AgNPs exhibited activity against the gram-positive bacteria such as Staph. aureus and gram-negative bacteria such as E. coli. All the concentrations studies showed a zone of inhibition against bacteria. The maximum antibacterial activity against Bacillus cereus, Staph. aureus, E. coli and Proteus spp. with a zone of inhibition was 61±1.41, 59±1.41, 42.50±0.70 and 36.50±0.70 mm at 100 µg/ mL of silver nanoparticles NAC-AgNPs respectively; furthermore, there was increase in the zone of inhibition with an increase in the concentration of silver nanoparticles gainst all studied bacteria and the zone inhibition with NAC-AgNPs greater than standard antibiotics (Fig. 2).

Table 6
Inhibition zones in diameter (mm) of NAC-AgNPs against bacterial isolates

No.	Microbial isolates	Concentrations (µg/ml)			
		100	50	25	12.5
1	Staphylococcus aureus	59±1.41 aA	45.50±0.30 bA	42.50±0.70 bA	40.50± 0.20 bA
2	Bacillus cereus	61±1.41 aA	32±1.41 bC	31.50± 0.70 bB	0±0 cC
3	Echerichia coli	42.50±0.70 aC	36.50±0.70 bB	34±1.41 bB	20.50±0.70 cB
4	Proteus spp.	36.50±0.70 aD	0±0 bD	0±0 bC	0±0 bC

Notes: captal letter denote differences between groups, small letters denote differences concentrations, P≤0.05 vs. control, NS – non-significant.

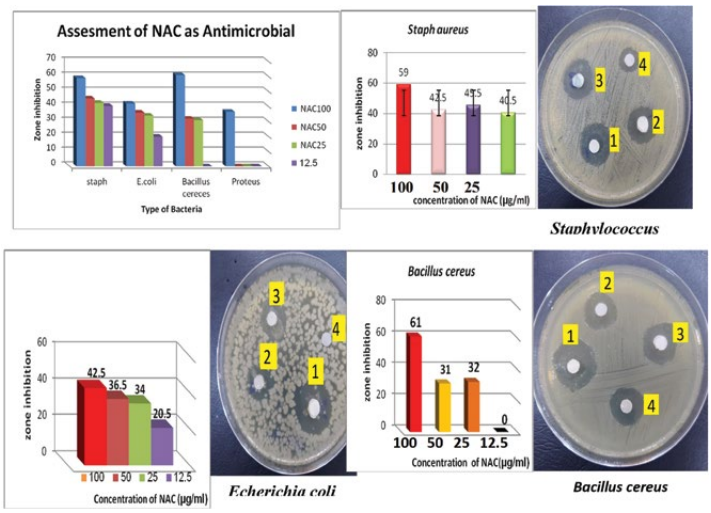

Fig. 2. Antibacterial activity of NAC aginst bacterial isolates

Effect of some antibiotics against bacterial isolates in this study

The results in the Table 7 and Figure 3 showed the standard antibiotics such as amoxicillin, erythromycin and Oxacillin were exhibited zero zone inhibition against the gram-positive bacteria Staph. aureus and gram-negative bacteria E. coli. But the standard antibiotics such as tetracycline and gentamicin were exhibited zone inhibition 35.27±0.32 and 38.12±0.66, 25.19±0.11 and 27±0.16 against the gram-negative bacteria E. coli and the gram-positive bacteria Staph. aureus respectively.

Table 7
Effect of some antibiotics against bacterial isolates in this study

Bacterial species	AM	TE	E	CN	OX
Escherichia coli	0	35.27±0.32	0	25.19±0.11	0
Staphylococcus aureus	0	38.12±0.66	0	±270.16	0

Notes: AM – Amoxicillin; TE – Tetracycline; E – Erthromycin; CN – Gentamicin, OX – Oxacillin.

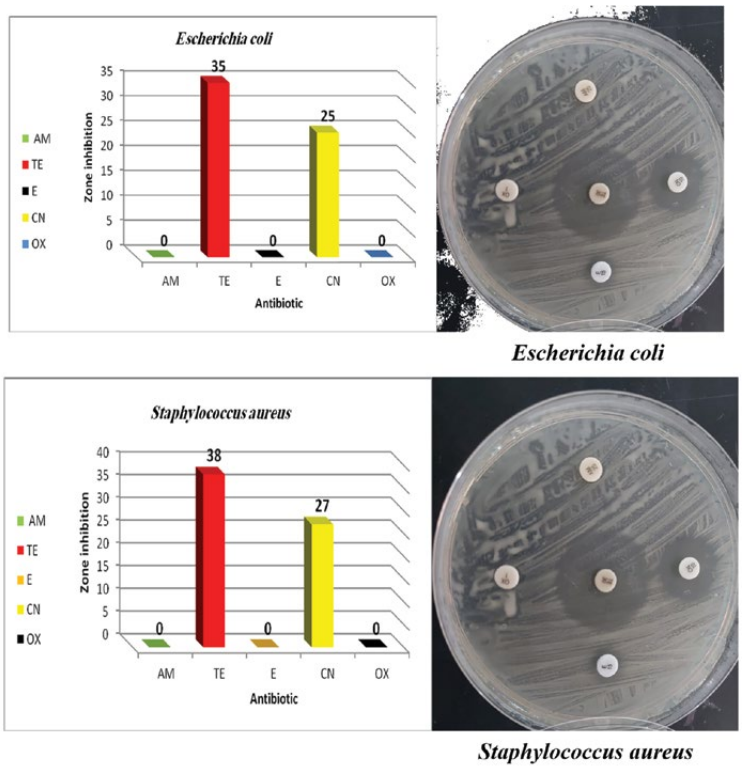

Fig. 3. Effect of some antibiotics against bacterial isolates

Effect of NAC-AgNPs against fungal isolates

The results observed in the Table 8 the NAC-AgNPs exhibited activity against the fungi. The concentrations studies showed a zone of inhibition. The zone of inhibition was concentration dependent. The maximum antifungal activity against Candida

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Table 8
Inhibition zones in diameter (mm) of NAC-AgNPs against fungal isolates

No.	Microbial isolates	Concentrations (µg/ml)			
		100	50	25	12.5
1	Aspergillus fumigatus	45.50±0.70 aC	38.50±0.70 bB	0±0 cC	0±0 cC
2	Candida albicans	50.50±0.70 aB	30.50±0.70 bC	31±1.41 bB	0±0 cC
3	Aspergillus terrus	31.50±0.70 aE	33.50±0.70 aC	0±0 bC	0±0 bC
4	Aspergillus flavus	0±0 F	0±0 D	0±0 C	0±0 C

Notes: captal letter denote differences between groups, small letters denote differences concentrations, P≤0.05 vs. control, NS – non-significant.

albicans, Aspergillus fumigates and Aspergillus terreus with a zones of inhibition were 50.50±0.70, 45.50±0.70 and 31.50±0.70mm at 100 µg/mL of silver nanoparticles NAC-AgNPs respectively; furthermore, there was increase in the zone of inhibition with an increase in the fungi except Aspergillus flavus showed zero zone of inhibition in all studied concentration of silver nanoparticles NAC-AgNPs against Aspergillus flavus (Fig. 4).

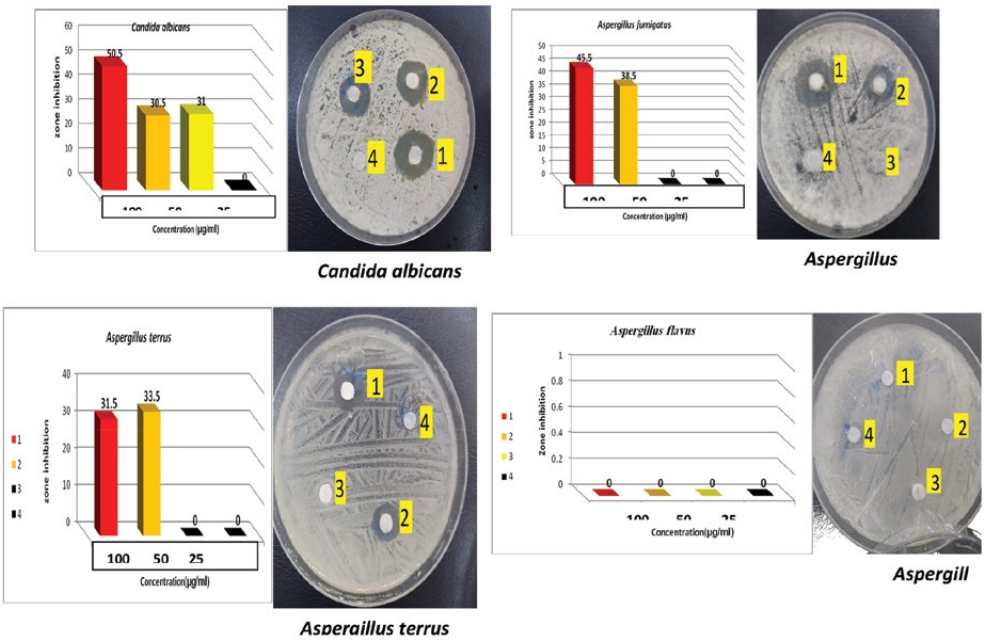

Fig. 4. Antifungal activity of NAC-AgNPs aginst fungal isolates

Effect of some antifungal against fungal isolates

The results in the Table 9 of showed the standard antifungal such as posaconazole and econazole were exhibited zone inhibition20±1.06 and 22±0.16, 21±2.33 and 25±4.92, 18±2.28 and 20±2.81 against the Candida albicans, Aspergillus flavus and Aspergillus terreus respectively (Fig. 5).

Table 9
Effect of some antifungal against fungal isolates

Fungal species	EON	POS
Candida albicans	20±1.06	22±0.16
Aspergillus flavus	21±2.33	25±4.92
Aspergillus terreus	18±2.28	20±2.81

Notes: POS – Posaconazole; EON – Econazole.

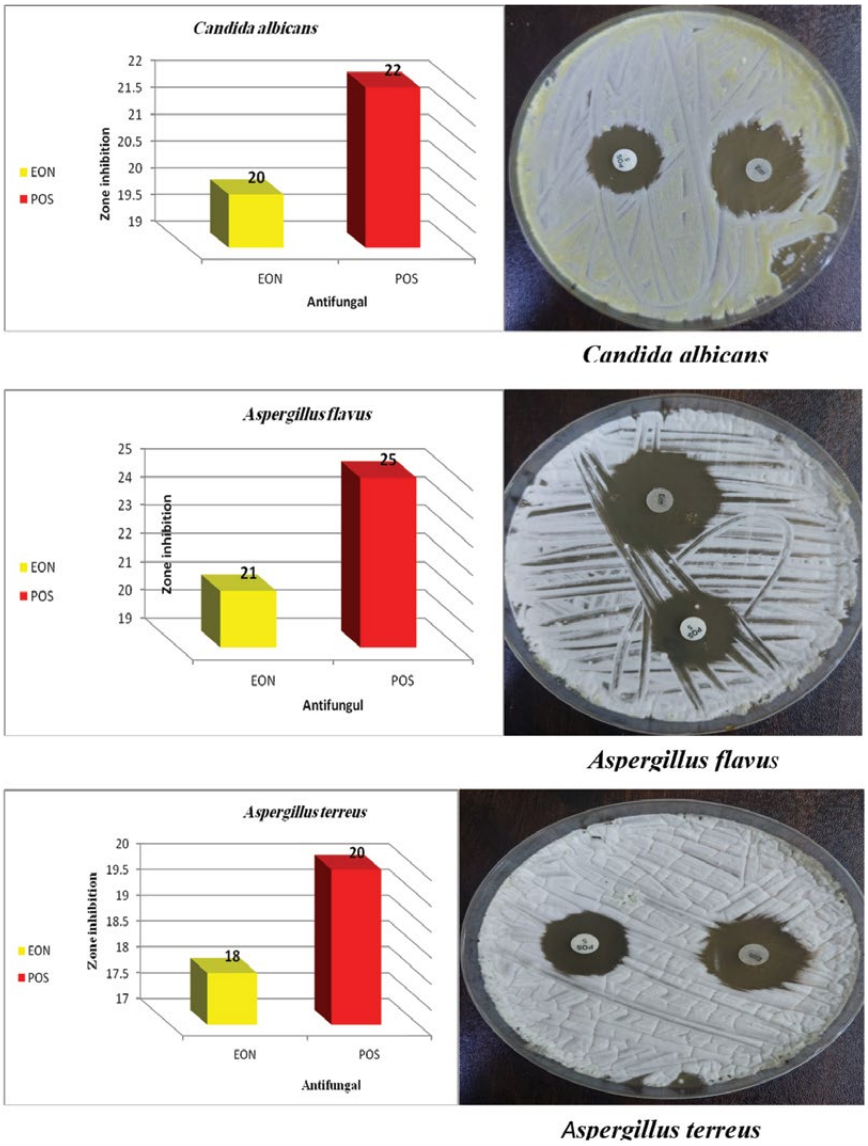

Fig. 5. Effect of some antifungal against fungal isolates

DISCUSSION

7,12DMBA-induced tumor in rats is a well-known model which has been widely used for evaluation of different compounds as chemopreventive drugs for breast cancer in humans [24]. DMBA carcinogenicity is associated with its oxidative metabolism leading to the formation of reactive metabolites, which bind covalently to nucleophilic sites on cellular macromolecules eliciting cancerous responses [25].

The present data showed that effectively Ag-NPsNAC reduced diameter and volume of the tumor in tumor-bearing rats. In order to reveal some possible mechanisms involved in anticancer effect of Ag-NPsNAC, we first investigated some events that lead to cell cancer development. Cell proliferation is regulated by multiple mechanisms. PCNA, a nuclear protein in proliferating cells, is essential for replication and serves as a cell proliferation marker [26].

PCNA overexpression was reported in a variety of human tumors and carcinoma induced by DMBA. Serum marker CA15.3, which is used widely for breast cancer diagnosis, is recommended for evaluation of metastatic breast cancer response to treatment and monitoring [27, 28]. It was shown that high levels of CA15.3 in serum suggest poor response to immunotherapy [29]. As shown, oral treatment of rats with Ag-NPsNAC [30].

Numerous studies demonstrated that tumor CA15.3 and p53 status is an important determinant of the response of tumors to anti-neoplastic agent [28, 31]. Mutations in p53 were reported to occur in 40% of all human tumors. Mutant p53 acts as an oncogene, loses its ability to act as a tumor suppressor, and enhances cell proliferation. Overexpression of mutant p53 may increase genetic instability by facilitating cell proliferation [31].

Our results showed that serum levels of mutant p53 in DMBA-treated group are significantly increased in comparison to the control group. Injection Ag-NPsNAC significantly decreased the serum levels of mutant P53 compared to the DMBA group [32].

The results of the present study show that the Ag-NPsNAC had an antibacterial effect against all the tested microorganisms (Gram positive and Gram negative bacteria), candida and filamentous fungi at all concentrations that were used. The inhibition zones against the tested microorganisms were increased with increasing the concentrations of Ag-NPsNAC as shown in table 5. Except the Aspergillus flavus is more resistance to NA Ag-NPsNAC C in all concentration compared with another fungi. The mechanism of antimicrobial activity of Ag-NPsNAC action against microorganisms is not completely understood but, it is supposed that the plasma membrane of the cell is the target, as the NAC possess an amphipathic nature that allows it's to interact with phospholipids of plasma membrane [33].

Another suggestion about the mechanism action of the NAC was increasing the membrane permeability of microbial cells with consequent alteration of this membrane causing cell damage [34]. During this present study we investigation the Ag-NPsNAC is considered as excellent antimicrobial.

The carcinogenic and mutagenic effect of DMBA requires its metabolic activation by mixed function oxidases [35]. The hydroxylation of DMBA at 7-methyl group is a crucial step towards its carcinogenesis [19].

The trans-3,4-dihydrodiol-1,2-epoxide is the carcinogenic product of DMBA [36]. When present inside body, hampers ROS-antioxidant balance by overproduction of free radicals and the body in turn reacts by modulating activities of antioxidant enzymes to curb the damaging effects of an increased ROS [37].

Hematological and biochemical parameters may be affected by a variety of factors such as race, age, gender, pregnancy, lactation, muscular activity, region, season, environmental heat, maintenance, and nutrition [38].

Oxidative products derived from mutagen metabolism, such as DMBA, might impair vital cellular function by damaging proteins and lipid membranes. Oxidative state plays an important role in cell integrity and function, including bacteria [39, 40].

We showed here that the NAC application to staphylococcus aureus biofilm in our in vitro biofilm system leads to a reduced ratio of NAD^+/NADH inside the bacteria, indicating high levels of oxidative stress [27, 28].

After 24 h of treatment with NAC, the NAD^+/NADH ratio significantly declines as the NAC concentration increases, which can potentially be due to the buildup of NADH or reduced levels of NAD^+ leading to the increase of the intracellular oxidative status [41]. Low concentration of reactive oxygen species (ROS) is critical for inhibition growth bacteria [42].

There is a dramatic increase in multi-resistant bacteria to commonly used antimicrobial agents that emerge as a serious medical problem worldwide. Therefore, there is a crucial need to manage and control this challenge through progressive alternative approaches that led to discover novel source of antibacterial compounds [37, 40].

■ CONCLUSION

Finally, we are currently undertaking the studies to examine the effects of Ag-NPsNAC and its mechanism of action on biofilm-forming bacteria such as Staphylococcus. These studies are designed to determine whether Ag-NPsNAC affects other biofilm-forming bacteria in the same manner as it affects P. aeruginosa. In the end, when we understand better how NAC affects the various biofilm forming bacteria individually, we will be able to create complex biofilms in vitro to determine how to best use Ag-NPsNAC to kill bacteria and dismantle biofilm and how to use it in combination with other drugs using high-throughput screening.

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Ihsan Hameed Khudhair
College of Science, University of Thi-Qar, Thi-Qar, Iraq

Biological Effects of Some Conocarpus Lancifolius Extracts and Silver Nanoparticles on Almond Moth Ephestia Cautella (Walker)

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Contacts: ehsan1981hameed@sci.utq.edu.iq

Abstract

Introduction. The almond moth, Ephestia cautella is one of the most important warehouse insects which is classically controlled by insecticides that have many disadvantages related to human health and environment as well as resistance.
Purpose. To achieve control this pest by ecofriendly ways, through the use of silver nanoparticles and some plant extracts, as well as some secondary metabolite compounds isolated from these extracts.
Materials and methods. A pure insect culture was taken from the Integrated Control Center and was bred on medium consisting of 60 g molasses, 810 g semolina, and 10 g of dough yeast, 120 g of glycerin, placed at the laboratory temperature and renewed between and over the duration of the research. Three concentrations 100, 500, 1000 ppm were prepared by dissolving 10, 50, and 100 mg, respectively, of silver nanoparticles in 2 ml of solvent then complete the volume to 100 ml of deionized distilled water. Thin layer chromatography (TLC) was used and the solvent (Hexane 3: Ethyl acetate7) was determined as a separation solvent.
Results. The alkane compound Decane was the most efficient in causing the act of killing in the larvae of this insect, as its LC50 was the lowest among the other coefficients (1307.88 ppm), and the toxicity index of this compound was the highest (100%) with a significant difference ($p \leq 0.05$) from the rest of the compounds. As for the Feeding Deterrence Index (FDI), silver nanoparticles were the most efficient (57.71%) with a significant difference ($p \leq 0.05$) from the rest of the compounds.
Conclusion. Conocarpus lancifolius leaves contain effective compounds such as the alkane compound Decane, which can be used to control insects, including the almond moth cautella Ephestia. Silver nanoparticles can be used to control insect and limit its impact and spread.
Keywords: biological effects, conocarpus lancifolius extracts, silver nanoparticles, almond moth, Ephestia cautella

■ INTRODUCTION

The almond moth (Walker) Ephestia cautella belongs to the Lepidoptera order of the moth family Pyralidae. The larvae of this moth produce webs on top of their food, therefore they are called web worms [1]. This moth reproduces inside warehouses at a temperature ranging from 24–28 °C and a relative humidity of 15–75%, and the length of its generation varies according to the temperature [2]. The female often lays eggs in the food or on its surface. Upon hatching, the larvae pierce the flesh of the fruit or the surface of the grain. The transformation in this insect is of the complete metamorphosis, as its life cycle goes through the following stages: egg and larva and Pupa as well as Adult, pupae are obtect and enclosed in a cocoon [3]. This insect has many names, as it is called in some African countries the Tropical warehouse moth, and in North Africa it is called the almond moth, It is also called the date moth [4], and in most countries it is called the fig moth [5]. Authors mentioned that this insect is widespread in different countries of the world as it was recorded in Saudi Arabia, Iraq and North African countries such as Egypt and others [2], and it was recorded as a pest in many European countries such as Georgia [6] and Greece [7] and in several Asian countries such as Malaysia [8], India [9] and in some Latin American countries such as Brazil [10]. This insect is considered the most important among the insect pests that afflict date fruits [11], as it infects fallen dates in the farms and continues to infect dates after harvesting in presses and warehouses. It was also observed that this insect infects dates before harvesting while it is on the palm tree [12]. Authors considered it one of the most important factors affecting the economics of the date industry [11]. He explained that it infects dates during storage and during the packing and export stages and cause damage. The presence of this insect on the stored crops and products leads to significant economic losses due to the large contamination left by these insects on the stored materials through feces, uric acid, remnants of moulting skins, pupae, and dead insect parts, which makes these foodstuffs and their products unacceptable [13], and the larval role is considered to be the harmful role primarily through feces and feeding on stored materials and the silky tissues that it weaves in order to live inside it [14].

Among the other damages caused by storehouse insects in general to crops and their products is the dissemination of many fungi and their spores that are already present on crops in the largest possible quantity, as well as adding fungi on their bodies to stored crops. This study came to combat this important insect pest in eco- friendly ways and not harmful to health, such as the use of nanoparticles and plant extracts within the following items: Preparation of two types of extracts from the leaves of Conocarpus lancifolius, which are hot aqueous and ethanolic alcoholic extract; Separation and identification of two secondary metabolite compounds from the ethanolic alcoholic extract; and Some biological effects (LC50, Toxicity Index, Relative Efficiency, and Feeding Deterrence Index) of silver nanoparticles and hot aqueous and ethanolic alcoholic extracts of Conocarpus lancifolius leaves, as well as ether compound (Cyclohexyldimethoxymethyl-Silane and alkane compound (Decane), which were extracted from the leaves of the same plant.

■ PURPOSE OF THE STUDY

To achieve control this pest by ecofriendly ways, through the use of silver nanoparticles and some plant extracts, as well as some secondary metabolite compounds isolated from these extracts.

■ MATERIALS AND METHODS

Collection and breeding of almond moth (Ephestia cautella)

A pure insect culture was taken from the Integrated control Center / Ministry of Sciences and technology / Baghdad / Iraq , and was bred on medium consisting of 60 g molasses, 810 g semolina, and 10 g of dough yeast, 120 g of glycerin, placed at the laboratory temperature and renewed between and over the duration of the research.

Silver nanoparticles

It was obtained ready from one of the competent offices.

Concentrations used in the study

Three concentrations (100, 500, 1000) ppm were prepared by dissolving (10, 50, and 100) mg, respectively, of silver nanoparticles in 2 ml of solvent ((Hexane 3: Ethyl acetate7), then complete the volume to 100 ml of deionized distilled water.

Preparation of hot aqueous and alcoholic ethanolic extract of C. lancifolius leaves

A group of fresh leaves of the plant was taken and the hot aqueous and alcoholic extracts were prepared according to [15].

Concentrations used in the study

The concentrations were prepared as in the preparation of concentrations of silver nanoparticles, with the placement of dry powder of the ethanolic and hot water extract instead of silver nanoparticles, and distilled water was used instead of deionized water.

Isolation and characterization of some secondary metabolite compounds of the alcoholic ethanolic extract of leaves of C. lancifolius

Several chemical solvents were tried to find out the most efficient one for use in separating some active compounds from the ethanolic alcoholic extract. Thin layer chromatography (TLC) was used, according to the method [16] and the solvent (Hexane 3: Ethyl acetate7) was determined as a separation solvent. Where two compounds were separated and characterized by a device.

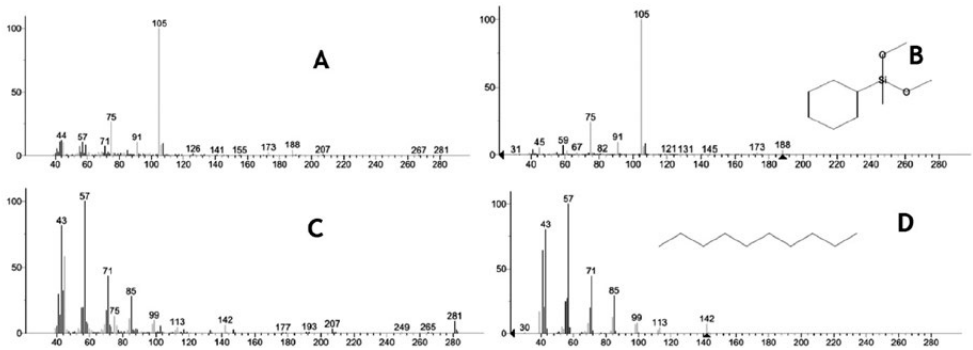

Fig. 1. Two separated compounds etheric compound Cyclohexyldimethoxymethyl-Silane [B]) and alkane compound (Decane [D]) as report of the GC-MS / MS device, [A and C] no record

GC – MS / MS (MS Model= 5973 Network Mass Selective Detector, Electron Impact (EI) 70ev, Analyzer = Quadrupole, = Ion source; Ion source Temperature = 230 °C, Temperature = 230 °C)

(and the two separated compounds are etheric compound Cyclohexyldimethoxymethyl-Silane) and alkane compound (Decane) as shown in the report of the GC-MS / MS device (Fig. 1).

Concentrations used in the study

The same method was used to prepare concentrations of silver nanoparticles with the use of two compounds Cyclohexyldimethoxymethyl-Silane and Decane instead of silver nanoparticles and the use of distilled water instead of deionized water.

Biological effects of silver nanoparticles, hot aqueous extract, ethanolic alcohol, cyclohexyldimethoxymethyl-silane and decane on almond moth E. cautella

Ten newly hatched larvae, one or two days old, were taken and placed in plastic cans containing artificial food consisting of date molasses, semolina and yeast dough, mixed with 1 ml of each of the concentrations of the previously mentioned treatments. As for the control treatment, they were treated with distilled water with addition 2 ml of solvent (Hexane 3: Ethyl acetate 7) /100 ml, with three replicates for each of the treatments and control. All the cans were incubated under a temperature of 28±2 C and a relative humidity of 5±70%, and were examined after seven days. The following biological parameters were calculated Lethal Concentration 50 (LC50), according to [17] as equations follow:

$$TI = \frac{LC50\ (toxic)}{LC50\ (any\ compound)} \times 100,$$

TI – toxicity index;
LC50 – Lethal Concentration 50 value for toxic compound and any other compound.

$$FDI = \frac{C - T}{C + T} \times 100,$$

FDI – Feeding Deterrence Index;
C – food consumed in the control;
T – food consumed in the treatment.

Statistical analysis

The statistical design (C.R.D.) Complete Randomized Design was adopted in the implementation of the experiments, and the percentages were converted into angle numbers, then they were analyzed statistically by variance analysis, using the SPSS program, and the Least Significant Difference (L.S.D.) was used to compare the statistical differences under the probability level (0.05).

■ RESULTS

Table 1 shows that the LC50 values were as follows (2339.62, 2529.28, 1889.31, 1307.88, 1889.31 ppm) for the ethanolic alcoholic extract, the hot aqueous extract, the ether compound Cyclohexyldimethoxymethyl-Silane, the alkane Decane, and silver nanoparticles, respectively. Through these results, it is clear that the alkane compound Decane is the lowest material for the LC50 (1307.88 ppm), which means that it is the most effective compound in causing killing in the larvae of the almond moth E. cautella.

Table 1
LC50 and Toxicity Index values of ethanolic alcoholic and hot aqueous extract of Conocarpus lancifolius leaves, ether compound Cyclohexyldimethoxymethyl-silane, alkane compound Decane and silver nanoparticles in almond moth Ephestia cautella

Compound	LC50 (ppm)	Toxicity Index %
Ethanolic alcoholic extract	2339.62	55.90
Hot aqueous extract	2529.28	51.70
Cyclohexyldimethoxymethyl-Silane	1889.31	69.22
Decane	1307.88	100
Silver nanoparticles	1889.31	69.22

In table 2, and with regard to the effect of the type of compound, it can be seen that the highest rate of feeding deterrence index (FDI) was in the treatment of silver nanoparticles, as it reached (57.71%), and it differed significantly ($p \leq 0.05$) from each of the extracts; Ethanolic alcohol, hot aqueous extract, cyclohexyldimethoxymethyl-silane ether, and the alkane compound Decane, which had an feeding deterrence index for each of them (32.58%, 26.22%, 41.51%, and 50.73%), respectively.

Table 2
Feeding deterrence index (FDI) for almond moth larvae cautella Ephestia treated with (alcoholic ethanolic and hot aqueous extract of Conocarpus lancifolius leaves, the etheric compound cyclohexyldimethoxymethyl-silane, alkane compound decane, and silver nanoparticles

Compound type / Concentration	Ethanolic alcoholic extract	Hot aqueous extract	Cyclohexyl dimethoxymethyl-Silane	Decane	Silver nanoparticles	Mean
500	31.95*	22.79	31.95	36.27	52.54	35.10
1000	32.58	18.43	32.58	37.46	49.02	34.01
1500	33.21	37.46	60.00	78.46	71.57	56.14
Mean of compound	32.58	26.22	41.51	50.73	57.71	41.75

Fig. 2 showed that the concentration (1500 ppm) within the treatment with the alkane compound decane is the highest feeding deterrence index (78.46%) and it differed significantly ($p \leq 0.05$) from the other concentrations within the different treatments, followed by the same concentration within the treatment of silver particles nanoparticles (71.57%).

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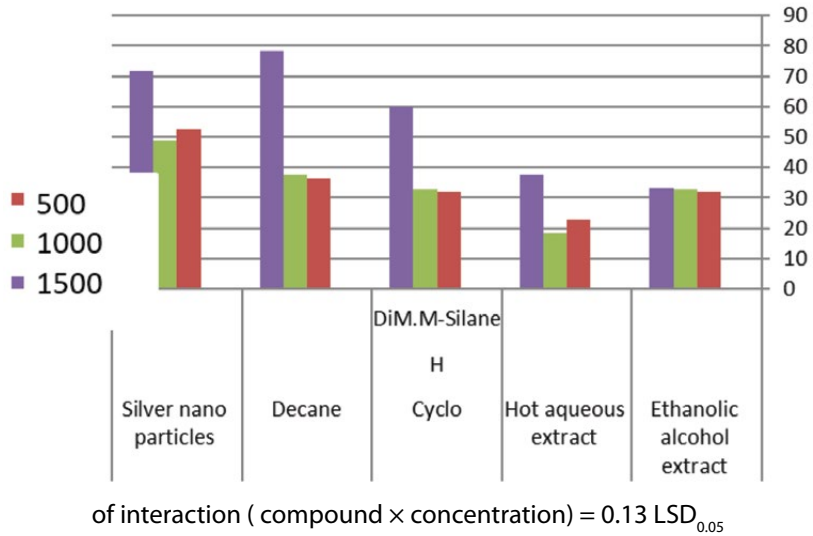

of interaction (compound × concentration) = 0.13 $LSD_{0.05}$

Fig. 2. Interaction between the compound and the concentrations in feeding deterrence index (FDI) of the larvae of the almond moth Ephestia Cautella treated with (ethanolic and hot aqueous extract of Conocarpus lancifolius leaves, the ether compound cyclohexyldimethoxymethyl-silane, the alkane compound Decane and silver nanoparticles)

■ DISCUSSION

The results in In table 1 are consistent with the findings of [18] that the alkanes isolated from the leaf extract of Sphagneticola trilobata had a significant lethal effect on many lepidopteran larvae such as the tobacco worm Spodoptera litura (LD50 = 15.32) mg/ larva) and the beet worm (S. exigua) (LD50 = 7.98 mg/larva) and Plutella xylostella moth (LD50 = 5.68 mg/larva), as may agree with [19] who indicated the efficiency of alkanes in killing Mosquitoes (LD50 = 47 mg/insect) and houseflies (LD50= 189 mg/larva), The results of the statistical analysis of the Toxicity Index showed that there was a significant difference between the alkane compound Decane and other compounds , where the Toxicity Index of this compound was (100%). The effectiveness of alkanes in killing insects can be attributed to their association with the ATPase enzyme present in the cell membranes of insects, which It leads to the deprivation of those cells of the energy provided by this enzyme, and thus the death of cells and insects in the end [20]. The current result in table 2 that silver nanoparticles achieved the highest feeding deterrence index is consistent with what was concluded by [21] that these particles have a significant feeding deterrence index effect against the larvae of the cotton worm Helicoverpa armigera, as these particles can negatively affect the digestive enzymes in the stomach of insects, [22] found that these particles, when mixed with food, affected the enzymes (Lipase, Amylase, and Protease) in the stomach of the tobacco worm insect Spodoptera litura, as well as [23] and [24] showed that after the arrival of silver nanoparticles to the epithelial membrane of the stomach of insects, they work to inhibit enzymes and the production of peroxide, which leads to the death of stomach cells, as for the effect of concentration, the concentration (1500 ppm) was the most efficient, as it produced the highest feeding deterrence index (56.14%),

and it differed significantly ($p \leq 0.05$) from both concentrations of 500 ppm (35.10%) and 1000 ppm (34.01%).

The result in fig. 1 is consistent with what was noted by [25] that the alkanes isolated from the Bauhinia scandens plant have an effect of preventing feeding against the larvae of the Plutella xylostella moth, and the lowest feeding deterrence index was in the concentration of 1000ppm within the treatment with hot aqueous extract (18.43%).

■ CONCLUSION

Conocarpus lancifolius leaves contain effective compounds such as the alkane compound Decane, which can be used to control insects, including the almond moth cautella Ephestia, as it kills the insect's larvae. Silver nanoparticles can also be used to control this insect and limit its impact and spread. Through the effect of these particles by significantly reducing the ability of insect larvae to feed.

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Ibrahim H. Muslim, Nadheerah F. Neamah✉, Falah H. Shari
College of Pharmacy, University of Basrah, Basrah, Iraq

Sodium Copper Chlorophyllin Assist Healing of Gastric Ulcers Induced by Indomethacin in Male Rat

Conflict of interest: nothing to declare.
Authors' contribution: Ibrahim H. Muslim – conceptualization, data curation, investigation, methodology, project administration, resources, software, validation, visualization, writing – original draft and writing – review & editing; Nadheerah F. Neamah – conceptualization, data curation, methodology, project administration, resources, supervision, validation, visualization, writing – original draft and writing – review & editing; Falah H. Shari – conceptualization, data curation, investigation, methodology, project administration, resources, supervision, visualization, writing – original draft and writing – review & editing.
Ethics statement: the study was approved by the College of Pharmacy, University of Basrah (No. #4 of 3/5/293 on 21/10/2021). The article is published in the author's edition.

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Contacts: nadheerah.neamah@uobasrah.edu.iq

Abstract

Introduction. Gastric ulcer is a firm gastrointestinal erosion disease that penetrates the muscle mucosa and affects the full thickness of the mucosa. The main treatment option for GU at present is drug therapy, however, actually the side effects of medications limit its usage.
Purpose. To determine the effect of Sodium copper chlorophyllin on indomethacin-induced stomach ulcers in rats.
Materials and methods. The study included fifty adult albino male rats, which were randomly divided into five groups. Each group consists of ten rats and is housed in separate cages. The control group served as a negative control, while the other four groups were given 50 mg/kg of indomethacin in a single dose. The third group was given Rabeprazole 20 mg/kg. The fourth group received Sodium copper chlorophyllin 35 mg/kg. Finally, the fifth group received Sodium copper chlorophyllin 70 mg/kg.
Results. The findings indicated a significant decrease in gastric acidity in the second group compared to all other groups, including the treated and control groups. However, the groups treated with Sodium copper chlorophyllin showed an increase in pH values, reaching normal levels. Indomethacin-induced gastric ulcers are diagnosed based on observable indications such as edema, bleeding, necrosis, erosion, and ulcers, which can be identified through macroscopic and microscopic examination. The groups treated with Rabeprazole, Sodium copper chlorophyllin (35 mg/kg) and Sodium Copper chlorophyllin (70 mg/kg) exhibited a significant decrease in the occurrence of macroscopic and microscopic symptoms such as hemorrhage, necrosis, and ulcers, both in terms of count and length, compared to the indomethacin group. Additionally, all treated groups showed a higher percentage of recovery.
Conclusion. The available information suggests that chlorophyll may be an effective natural treatment for stomach ulcers. The fact that chlorophyll reduced the gastric adverse effects of indomethacin in the GU rat model suggests that it has exceptional stomach-protective properties. Research on the anti-GU properties of chlorophyll and the potential synergistic effects of these compounds in GU prevention is ongoing.

Keywords: Sodium Copper Chlorophyllin, gastric ulceration, indomethacin, Rabeprazole, ulcer

■ INTRODUCTION

Gastric ulcer (GU) is a firm gastrointestinal erosion disease that penetrates the muscle mucosa and affects the full thickness of the mucosa. For many years, it was thought that excessive stomach acid secretion was the primary cause of gastrointestinal ulcerations; however, many patients who presented with such illnesses had normal rates of acid secretion. Subsequently, scientists discovered that peptic ulcers resulted from an imbalance between several established defense mechanisms and hostile stimuli. Hydrochloric acid, pepsin, pancreatic enzymes, bile, and other exogenous aggressive factors minimized the gastrointestinal mucosa's defense mechanisms, such as intercellular junctions, local blood flow, mucus/bicarbonate secretion, and cellular growth. These external aggressive factors also caused tissue necrosis through mucosal ischemia, free radical generation, and cessation of nutrient delivery [1]. Even though the precise etiology of gastric ulcers remains unclear, it is widely recognized that the ratio of aggressive to protective agents determines the degree of damage to the gastric mucosa [2].

The main treatment option for GU at present is drug therapy, however, the considerable side effects of most medications limit its practical use. To avoid GU bleeding, suppression of stomach acid is commonly employed. Proton pump inhibitors (PPIs) and somatostatin work together to effectively suppress the secretion of stomach acid. In contrast, somatostatin preserves a high intragastric pH more effectively than PPIs. Conversely, long-term PPI use may result in thrombocytopenia. Somatostatin therapy frequently causes hypoglycemia unexpectedly and abruptly. A drug called sucralfate is used to treat GU. In an animal model, sucralfate is used to treat GU. It decreases inflammation, TNF-α levels, and leukocyte adherence in post-capillary venules, as well as promoting GU repair [3].

Carotenoids and chlorophyll are involved in photon absorption, light harvesting, and the transfer of excitation energy to the photosynthetic reaction center. Nevertheless, inside the reaction center, only chlorophyll carries out charge separation across the cell membrane. Chlorophyll initiates a series of electron transfer processes that convert carbon dioxide (CO2) to carbohydrates [4].

Chlorophyll is primarily made up of a porphyrin ring that chelates a Mg atom. Because of its 20-carbon phytol tail at carbon 17, chlorophyll is extremely hydrophobic and can integrate into cellular lipid membranes. The a and b forms of chlorophyll in naturally occurring chlorophylls are defined, respectively, by the functional group of C7, a -CH3 or a -CHO group. Pheophytin, chlorophyllide, and pheophorbide are naturally occurring a and b derivatives of chlorophyll that are found in plants as breakdown products and in animals as byproducts of digestion of chlorophyll [5].

Sodium copper chlorophyllin (Chlo) is a natural chlorophyll derivative that is being used more and more as a food supplement and colorant. Without any noticeable undesirable effects, Chlo has been linked to a wide range of health advantages, including anti-inflammatory, deodorizing, and erythropoietic properties. Additionally, it was demonstrated that phase II enzymes such quinone reductase might be induced by Chlo derivatives. Because of such studies, there is a lot of interest in Chlo and its potential

applications in both the treatment and prevention of chronic illness. More research has shown that LvWei Capsule, whose primary ingredient is Chlo, is a potentially effective medicinal medication for the treatment of stomach ulcers and boosting immunity [2].

The leaves contain a variety of pigments. This comprises the blue-green pigment chlorophyll a, the yellow-green pigment chlorophyll b, the yellow-green pigment xanthophylls, and the yellow-orange carotenoids. Additionally, the primary pigment that promotes optimal photosynthesis is chlorophyll a. Adjunct pigments are the other pigments. Chlorophyll a receives the light energy absorbed by these auxiliary pigments. One derivative of chlorophyll that has been shown to boost peripheral leukocyte counts and alleviate leukopenia patients' complaints of weariness and dizziness is chlorophyllin copper. Chlorophyll has been used in the past to treat cancer, anemia, and gastrointestinal issues. Chlorophyll has been shown by Fahey et al. to enhance the performance of vital detoxification processes [6].

The non-steroidal anti-inflammatory medication indomethacin is derived from indomethacim and has anti-inflammatory, analgesic, and antipyretic properties. Because of its efficient reduction of pain, temperature, color, and edema, it is used in the treatment of ankylosing spondilitis, osteoarthritis, rheumatoid arthritis, gout arthritis, bursitis, tendonitis, synovitis, and other inflammatory illnesses [7]. Unfortunately prolong use of NSAIDs, such aspirin or indomethacin (IND), can result in gastric ulcers through a number of different processes, such as damage via suppression of prostaglandin (PG) synthesis, decreased local blood flow, irritation in specific areas, and inhibition of tissue regeneration. As there are currently a variety of synthetic anti-ulcerative medications in the market, they may result in minor to severe side effects [8].

As an instance, the proton-pump inhibitor (PPI), Rabeprazole (Rabe) as a reference drug act by inhibiting gastric acid secretion. Headaches, upset stomach, vomiting, or diarrhea are among the most likely common adverse effects. Serious side effects are extremely rare however they can include allergic reactions, liver issues, and joint discomfort from subacute cutaneous lupus erythematosus brought on by prolonged treatment. Another indication of prolonged usage could be a drop in blood magnesium levels following more than three months of omeprazole treatment. Although the exact mechanisms by which PPIs increase the risk of fracture remain unknown, several theories have been put forth, such as decreased calcium absorption and decreased gastric acid secretion, as well as decreased absorption of other nutrients (like B vitamins) that may be crucial for bone health and hypergastrinemia [9].

■ PURPOSE OF THE STUDY

To determine the effect of Sodium copper chlorophyllin on indomethacin-induced stomach ulcers in rats.

■ MATERIALS AND METHODS

Study of animals and ulcers induction

This study included fifty adult albino male rats, weighing between 180 and 200 grams, that were provided by the University of Zakho College of Veterinary Medicine. The animals were separated randomly into five groups (n=10) and kept in distinct cages of polypropylene with sawdust lining for a week prior to onset the study for acclimatization. The standard rat pellet diet and tap water were provided to the four or five rats in each

cage. The animals were housed in a room with well controlled parameters, such as 21±2 °C temperature, 30±11% humidity, and a 12-hour light/dark cycle. For the duration of the experiment, they had full access to their regular diet and water.

The preferred reference anti-ulcer medication for this investigation was Rabeprazole. The medication was prepared and taken orally at a dosage of 20 mg/kg Bw of body weight. The article was authorized by the Basrah University Animal Ethics Committee (No.EC 30 updated to 1/9/2023) for all animal handling protocols.

Experimental design

The rats were randomly assigned to five groups, each with ten rats. Prior to the experiment, The rats were allowed unlimited access to water but were denied a diet for twenty-four hours. Each group received the following exact treatment at a given dose. Rats were given once a day for a maximum of seven days:

- Control Group (C): as a negative control received 2 mL of Distilled water –DW- orally by mouth gavage for seven days.
- Indomethacin treated group (Indol): as Ulcers –induced group received oraly, 50 mg/kg BW of pure indomethacin dissolved in DW as suspension at 21°C using magnetic hot plate. One dose at the first day, six days later the rats received 2 ml of DW.
- Rabeprazole treated group(indol-Rabe): as positive group, at the first day indomethacin, then after two hours Raberazole administer orally at the dose 20 mg/kg BW. Rabeprazole were administered daily at 9:00 AM in the 6 days that followed.
- Sodium-Cupper chlorophyll treated group (indol-Chlo): first group of Chlo at dose 35mg/kg, at the first day indomethacin, then after two hours Chlo was administered orally. Only Chlo was administered in the 6 days
- Sodium-Cupper chlorophyll treated group (indol-Chlo D): Chlo was given at dose 70 mg/kg BW after two hours after Indomethacin administration at first day. The 6 days followed only Chlo was administered.

Preparation of the stomach

Following anesthesia with chloroform, every rat was sacrificed, then the stomach was ligated at both ends after the abdomen was opened. At that point, the stomach opened along its greater curvature, separating it from other viscera. Using a regular syringe, the stomach's contents were removed, examined, and the volume measured. After that, the stomach had been immersed in usual saline solution to remove out food particles, and the length of the damaged regions was measured (Ulcers and lesions were examined and measured using a magnifying glass). Ultimately, the stomach was preserved in 10% formalin for a whole day in order to perform a histological analysis.

Measurement of gastric lesions

The total length (in millimeters) of ulcers measured using a regular ruler is the ulcer index, the number of gastric ulceration in each stomach, also estimated, finally the percentage of ulcers inhibition were calculated. The measurements for proliferations and hemorrhagic lesions were same. The percentage of ulcer healing was determined as:

Ulcer recovery (%) = ((UI control-UI test group)/UI control group) × 100%.

IU means ulcer index [10].

Measurement of pH

A pH of the gastric juice was measured using pH meter S2K712 (Isfetcom Co., Ltd., Shimokayama, Japan) after the stomach wall was cut [11].

Histological examination

Gastric tissue was removed from the body while under the influence of chloroform and then cleaned with regular saline. Following sample fixation in 10% formalin, histological sections and staining were carried out in a Al Fayhaa private laboratory in Al Basrah city by a licensed pathologist. Samples were embedded in paraffin and stained with hematoxylin and eosin (H&E; Coolaber, Beijing, China) for histopathological examination. The slides were examined, and histopathological photographs of the stomach sections using BS-2026BD1 Binocular Digital Microscope (genex-china).

Statistical analysis

The One-Way ANOVA test and then LSD multiple comparison test with a 95% significance level is used to statistically analyze the results, which are shown as mean values±SEM.

■ RESULTS

Macroscopic evaluation of gastric mucosa

The control group's stomach mucous lining was normal, and there was no evidence of macroscopic appearance of lesions such as erosions, ulcers or hemorrhage. In the present study, intragastric indomethacin to the rats of Indol group experienced a typical macroscopically symptoms including edema, congestion, hemorrhage, necrosis, erosions and ulcers with elevated border, and perforation. Indol+Rabe group revealed decreased in ulcer index include length of ulcers and recovery percentage than in Indol group. Some of gastric mucosa of (indol+Chlo), (indol+ChloD) with yellowish color which may indicate

Fig. 1. Macroscopic investigation of indomethacin induced ulcers. (A) mild edematous, necrosis, ulcers(black arrows) with elevated borders. (B) sever edema, ulcers with elevated irregular inflammatory border(blue arrow), (C) hemorrhage (red arrow) ulcers and necrosis (black arrows) sever loss of muscularis mucosae. (D) perfuse necrosis and ulcers

hemosiderin precipitation, with minor erosions, also a marked decline in the number of gastric ulcers. Gastric mucosa exhibited normal mucosa in almost stomach regions, with markedly reduced in edema, necrosis, hemorrhage and other macroscopic signs than in Indol group.

The treated groups (Indol+Rabe, Indol+Chlo and Indol+ChloD) showed minor injuries, with a marked decline significantly in the number of Indol+Rabe than in Indol group. Gastric mucosa showed normal mucosa in almost stomach regions, and macroscopic signs than in Indol group. Fig. 1 illustrated the macroscopic signs.

Gastric ulceration measurements

The ulcers index both number and length of Indol group significantly differ in length of ulcers (8.7±2.311805 mm) and number of ulcers (5.2±2.097618) compare to Control group (0±0). The third group, Indol+Rabe group exhibited significant differences in comparison in number and ulcer length (1.1±1.197219, 2.1±2.330951 respectively) with control and Indol groups. Rats given Chlo(Indol+Chlo) showed significant decline in number 0.5±0.849837 in comparison with indomethacin treated rats(indol), but there was no important differences with control group. however; ulcer length (1.5±1.433721) significantly differed from both Indol and Control groups. The fifth group (Indol+ChloD) showed important differences only with Indol group in both number and length (0.5±0.849837, 0.9±1.523884 respectively). The ulcers recovery percentage measured according to ulcer index inIndol group. In Indol+Rabe group the ulcer index decline to 75.86207%, while ulcer index at both Indol+Chlo and Indol+ChloD ulcer index decreased to 82.75862% and 89.65517%. The data illustrated by table 1.

Table 1
Effects of Indomethacin, Chlorophyll and Rabepirazole on Gastric ulcers rate, lenghth and PH

Groups	PH	GU No/rat	GU length mm	Ulcers recovery%
Control (C)	3.23 ±0.411096	0±0	0±0	
Indol (50 mg/kg)	1.82 ±0.248551 a	5.2±2.097618 a	8.7 ±2.311805 a	
Indol+Rabe (20 mg/kg)	2.82±0.364539 a b	1.1±1.197219 ab	2.1±2.330951 ab	75.86207
Indol+chloro (35 mg/kg)	3.11±0.406749 bc	0.8±0.788811 b	1.5±1.433721 ab	82.75862
Indol+chloro. D (70 mg/kg)	3.52±0.297396 bcd	0.5±0.849837 b	0.9±1.523884 b	89.65517
LSD	0.316555	1.079212	1.568186	

Notes: The letters (a), (b) and (c) stand for significant differences with the control, indol and Rabe treated groups, respectively.

PH measurement

Following sacrifice, the pH of the stomach fluid significantly decreased in the group of gastric ulcer model animals exposed to indomethacin compared to the control group. The pH value of the Indol group (1.82±0.248551) was significantly lower than that of the Control group (3.23±0.411096); on the other hand, the pH value of the Indol+Rabe group was significantly higher than that of the Indol and control groups. The control group's PH value was not substantially different from the Indol+Chlo and Indol+ChloD groups', whereas the PH of the Indol group dramatically increased (Fig. 2).

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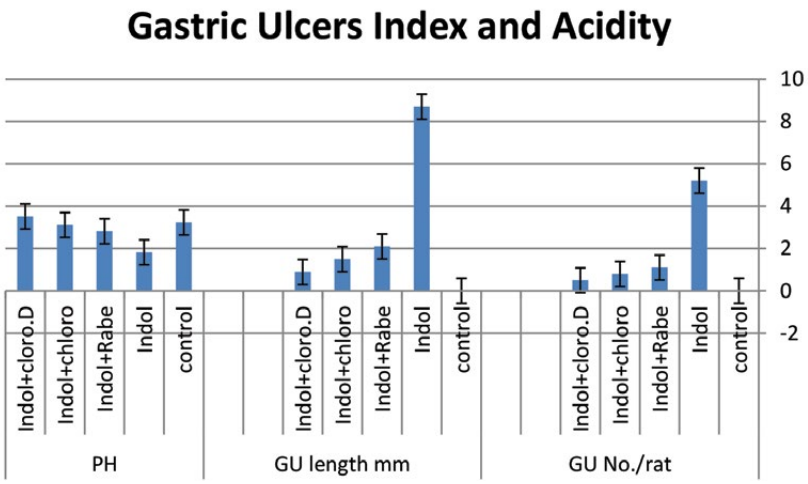

Fig. 2. Ulcer index including ulcers length, number of ulcers each rat and gastric acidity

Histopathological evaluation

Hematoxylin and eosin-stained (H&E) stomach slices from the control rats performed histopathological analysis, which showed normal histological structure (Fig. 3A). Analyzing the portions taken from the indomethacin group revealed excessive infiltrate of cells

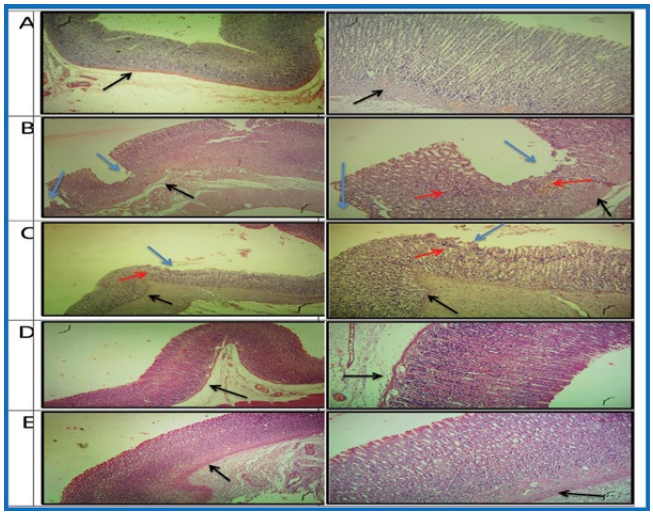

Fig. 3. Analysis of the histology. (A) Stomach from control group shows normal gastric mucosa, normal lamina muscularis (black arrows). (B) The ulcerative mucosal surface on the stomach of the Indo-treated group is related to the pigment hemosiderin deposition (blue arrow), inflammatory infiltrate and hemorrhage (red arrows) and thin lamina muscularis and signs of mucosal perforation(black arrows). (C) Determined mucosal surface erosion on the stomach with Indo+Rabe (mg/kg) (blue arrow), with mild accumulation of inflammatory cells(red arrow), the lamina muscularis to less extend was affected than in indo-group. (D) and (E) The mucosa on the stomach from Indo+Chlo (35 mg/kg) and Indo+Chlo (70 mg/kg) appeared to be normal mucosa and submucosa and lamina muscularis

associated with inflammation in the mucosa and submucosal layers was observed in many sections. The submucosa layer demonstrated dispersion of the connective tissue with extensive edema, congested blood vessels, and inflammatory cells infiltration. significant mucosal erosion that extends to the lamina muscularis (Muscularis mucosae). Moreover, significant hyperemia of the arteries, edema in the submucosa, and hemorrhagic infiltration were noted (Fig. 3B). Rabeprazole (20 mg/kg) pretreatment showed a notable protective impact on the tissue architecture. Thus, in the sections selected from the Rabe-group, mild hyperemia in the arteries, slight degeneration and necrosis of the mucosal epithelium, and minor erosion of the mucosal layer were observed (Fig. 3C). Histological examination verified that chlorophyll has a gastroprotective effect. Actually, Indol+chlo (35 mg/mL)and Indol+Chlo Importantly reduced the effects of Indomethacin while exerting protective benefits identical to those of Rabeprazole. In particular, there was slight hyperemia in the arteries, mild degeneration and some necrosis in the mucosal epithelium observed in few rats of both groups (Fig. 3D and E).

■ DISCUSSION

Initially pilot screening has been carried out to determine the optimum dose and time interval to reach the optimum ulcerogenic state, three doses were tested 35, 50, and 70 mg/kg BW. The results showed at dose 35, small ulcers had been identified, most of them were recovered spontaneously at the day 8th indomethacin administration, normal mucus layer with no signs of hemorrhage or edema. while at the dose 70 mg/kg, the ulcers characterized by increase in depth and number with edema in the gastric mucosa than produced by 35 mg with sever signs of hemorrhage, necrosis, which may need long term treatment and high doses of test medicines. The dose 50 was preferred for its acceptable ulcer index which agree with some previous study [12].

While stomach ulcers are generally frequent diseases caused by a variety of factors, such as free oxygen radicals, Since indomethacin possesses a higher potential for ulcer formation than other NSAIDs, it was the drug of choice when it was first used producing an experimental ulcer model. The stomach's lumen receives gastric acid secreted from the stomach parietal cells, which have a vast secretory network of their own. These cells are found in the gastric mucosa's epithelial fundic glands. The rat stomach lumen's pH of gastric acid is roughly 3–4. This pH is controlled by the proton pumps H+ and K+ ATPase [13].

It is impossible to link the gastrointestinal system side effects of indomethacin and other NSAIDs to the inhibition of only the COX-1 enzyme because the medication, which is less selective for COX-2, can inhibit NSAID-induced gastric damage, while celecoxib and rofecoxib, which are more selective for COX-2 (350 to 800 times as selective), cannot inhibit these ulcers. According to some theories, indomethacin causes damage to the digestive tract by reducing the release of protective factors like mucus, prostaglandin E2 (PGE2), cyclooxygenase-1 (COX-1), and bicarbonate; enhancing aggressive factors like acid; and promoting oxidant parameters while reducing antioxidant parameters. By acting against indomethacin (raising PGE2, bicarbonate production, and mucus; interfering with acid secretion; minimizing oxidant parameters; or increasing antioxidants), typical antiulcer medications are known to induce antiulcer effects. The review by Suleyman et al. [7] proposed a connection between α 2 adrenergic receptors and ulcers caused by indomethacin to explain the discrepancies in the data described above. The elevation in aggressive factors induced by indomethacin may be caused by blocking α2 adrenergic

receptors, while the increase in protective factors induced by antiulcer medications may be caused by stimulating α 2 adrenergic receptors.

Currently, traditional medicine seems to be increasingly important. Medical plants provide effective, safe, and generally accessible alternative treatments for gastric ulcers. One example of this type of medicine is herbal medicine, on which the WHO has published several articles. One of them discusses the experimental and clinical data that is currently available supporting the efficacy of several medicinal plants that have been utilized for peptic ulcer treatment in the past. One of the most prevalent naturally occurring pigments in plants are carotenoids, especially lutein, which give plants their attractive hues in their fruits, leaves, and flowers. According to epidemiological research, there is an associations between consuming a diet that contains more carotenoids and a reduced risk of degenerative conditions like cardiovascular diseases, ocular illnesses, cancer, and ulcers. Carotenoids have been believed to have protective effects against oxidative damage to cells and tissues via their antioxidant activity. It has also been demonstrated that carotenoids are potent anti-oxidants that optimize cytoprotection in peptic ulcers and effectively promote gastric ulcer healing [14].

There is evidence that the pH in the stomach varies in certain upper gastrointestinal disorders. Contrary to common belief, gastric ulcers are not caused by excessive HCl or gastric acid output. However, non-steroidal anti-inflammatory medications (NSAIDs) or the Helicobacter pylori (H. pylori) bacteria are typically attributed for stomach ulcers. An further possible cause of anemia is H. pylori colonization of the human stomach [15].

To the best of our knowledge the only study done by Lv et al. [2] described the gastroprotactive effect of Sodium Copper Chlorophyllin, and the mechanisms that involved. This study illustrated that, the pH of gastric juice decreased significantly in the indomethacin- induced gastric ulcers. While; in Chlorophyll pretreatment groups in combination with indomethacin significantly increased pH compared with gastric ulcers induced group, this result agree with results of pH values in the current study. The current research indicate that by blocking the NF-κB pathway, the protective effects of SCC may be advantageous as a possible treatment and preventive drug for stomach ulcers [2]. When combined, SCC treatment showed a strong anti-inflammatory effect as seen by a decrease in proinflammatory mediator secretions such IL-6 and TNF-α in the serum of mice exposed to ethanol. It also dramatically lowered the levels of MPO, NO, and MDA in gastric tissue [15].

■ CONCLUSION

The evidence currently available supports the use of chlorophyll as a natural remedy for stomach ulcers and shows promise in this regard. In general, the reduction of indomethacin's gastric affronts through its treatment indicates chlorophyll's remarkable stomach-protecting qualities in the model of GU rats. Studies on chlorophyll's anti-GU characteristics and the possibility of their combined efficacy in GU prevention are still currently under way.

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Safaa Jassim Mohammed✉, Nahlla Amer Sabir
Nineveh Health Directorate, Ministry of Health, Nineveh, Iraq

Changes in Hematological Parameter in Patients with Type II Diabetes Mellitus

Conflict of interest: nothing to declare.
Authors' contribution: Safaa Jassim Mohammed – conceptualization, data curation, investigation, methodology, project administration, software, supervision, validation, visualization, writing – original draft and writing – review & editing; Nahlla Amer Sabir – conceptualization, project administration, resources, validation, visualization, writing – original draft and writing – review & editing.
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Contacts: Safaa.jasim.mohammed@gmail.com

Abstract

Introduction. Diabetes mellitus is a chronic metabolic syndrome characterized by persistent hyperglycemia and increased risk of microvascular and macrovascular complications. Most of patients with diabetes mellitus have signs of metabolic syndrome which is a cluster of phenotypes associated with increased risk of cardiovascular disease.
Purpose. To determine haematological indices and their correlation with blood glucose level in type II diabetic patients in comparison with healthy controls.
Materials and methods. 192 subjects were studied. 75 were non diabetic healthy subjects (Group1). 117 were known diabetic patients, divided into two groups: HbA1c <7, Group 2 and HbA1c >7 Group 3. Sample for glucose estimation and CBC were collected in vacutainersgel tube and K 2EDTA respectively. Glucose, HbA1c and CBC estimation was carried out by auto analyzers.
Results. Mean age of group 1 58.3±14 where the mean age of group 2 60.6±9.3 and mean age of group 3 61.114±10. There is significant different between group 1 and group 2 in platelet count and platelet distribution width where p-value 0.00. There is significant different between group 1 and group 3 in mean corpuscular hemoglobin content (MCH) and mean cell hemoglobin concentration (MCHC) where p-value 0.00. There is significant different between group 2 and group 3 in mean corpuscular hemoglobin content (MCH), mean corpuscular volume (MCV) platelet count and platelet distribution width where p-value 0.00
Conclusion. Haematological parameters should be routinely determined among diabetes mellitus type II patients for proper management and reduction of further complications.
Keywords: diabetes mellitus, metabolic diseases, sedentary life style, mean corpuscular hemoglobin, platelet count

■ INTRODUCTION

Diabetes mellitus is a global metabolic disorder. Incidence of diabetes mellitus continues to rise in elderly and also in young age group due to adverse life style changes like excess calorie intake and sedentary life style [1].

Diabetes is a complex, chronic condition requiring continuous medical care with multifactorial risk-reduction strategies beyond glycemic management [2].

The number of people with diabetes rose from 108 million in 1980 to 422 million in 2014. Prevalence has been rising more rapidly in low- and middle-income countries than in high-income countries. Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation. Between 2000 and 2019, there was a 3% increase in diabetes mortality rates by age. In 2019, diabetes and kidney disease due to diabetes caused an estimated 2 million deaths. A healthy diet, regular physical activity, maintaining a normal body weight and avoiding tobacco use are ways to prevent or delay the onset of type 2 diabetes. Diabetes can be treated and its consequences avoided or delayed with diet, physical activity, medication and regular screening and treatment for complications [3].

Diabetes is diagnosed based on the following stress-free hyperglycaemia levels:

- fasting plasma glucose (FPG) of ≥7.0 mmol/L (126 mg/dL);
- plasma glucose of ≥11.1 mmol/L (200 mg/dL) post-oral glucose tolerance test (OGTT), with 1.75 g/kg (max 75 g) of anhydrous glucose;
- polyuria, polydipsia, nocturia, unexplained weight loss, or a random plasma glucose of ≥11.1 mmol/L (200 mg/dL);
- glycated haemaglobin (HbA1c) of >6.5% [4, 5].

Type of diabetes: majority of cases of diabetes can be broadly classified into 2 categories: type 1 diabetes and type 2 diabetes, although some cases are difficult to classify. Gestational diabetes (GDM) refers to glucose intolerance with onset or first recognition during pregnancy [6].

■ PURPOSE OF THE STUDY

To determine haematological indices and their correlation with blood glucose level in type II diabetic patients in comparison with healthy controls.

■ MATERIALS AND METHODS

The study was a randomized cross-sectional study aimed at comparison the CBC parameters between three groups (first group non diabetic healthy subjects and second group patient with D.M II and HbA1c less than 7 and third group patient with D.M II and HbA1c more than 7).

Study population

192 subjects were studied. 75 were non diabetic healthy subjects (Group1). 117 were known diabetic patients, divided into two groups: HbA1c <7, Group 2 and HbA1c >7 Group 3.

The exclusion criteria from the study were endocrinological diseases affecting the metabolism of glucose, Haematological diseases, systemic diseases, pregnancy, acute diseases and malignancy. And patients with type I diabetes

Blood collection

Blood samples were collected and Testing of samples was carried at the Department of Laboratory of Ibn sena Teaching Hospital: start 15/may/2023 to End: 1/September/2023. The material used to determine CBC 5-part difference and HbA1c was about 2 ml of

venous blood collected in vacuum tubes with an anticoagulant EDTA (Ethylene Diamine Tetracetic Acid).

Complete blood count determination included: total white blood cells – (WBC), differential of WBC which included neutrophils (N), lymphocytes (L), monocytes (M), eosinophils (E), basophils (B). Red blood cell parameter which included :The red blood cells – count (RBC), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCH), mean cell hemoglobin concentration (MCHC) and red blood cell distribution width (RDW). Total platelets count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), mean platelet component (MPC).

The HbA1c value was analyzed by A high-performance liquid chromatographic (HPLC) method in hemolized full blood.

Statistical analysis was carried out with SPSS 24 software package and p values less than 0.05 were considered as statistically significant.

■ RESULTS

Total number of diabetic patient 117 where divided into group HbA1c less than 7 (n=82, 70%) and HbA1c more than 7 (n=35, 30%).

Mean age of group 1 – 58.3±14 where the mean age of group 2 – 60.6±9.3 and mean age of group 3 – 61.114±10.

Table 1
Parameters of groups studied

Parameters	Group 1 (n=75)			Group 2 (n=82)			Group 3 (n=35)		
	mean	±	SD	mean	±	SD	mean	±	SD
WBC	8.408	±	2.067	9.090	±	2.426	8.756	±	2.289
Lymphocyte	2.483	±	0.682	2.787	±	1.002	2.574	±	0.860
RBC	4.763	±	0.412	4.887	±	0.509	4.691	±	0.813
Hb	13.933	±	1.076	13.638	±	1.430	13.374	±	1.732
HCT	39.892	±	3.028	40.359	±	4.150	38.817	±	5.356
MCV	83.258	±	5.543	81.846	±	4.512	83.669	±	4.115
MCH	29.075	±	1.639	27.676	±	1.921	28.849	±	1.736
MCHC	34.983	±	1.398	34.050	±	1.709	34.431	±	1.208
RDW	12.725	±	0.867	12.378	±	0.873	12.446	±	0.755
PLT	238.25	±	59.463	246.622	±	67.363	247.286	±	74.295
PDW	15.950	±	1.562	16.588	±	1.066	16.271	±	1.188
Age	58.333	±	14.035	60.659	±	9.351	61.114	±	10.067
Neutrophil	5.517	±	1.879	6.004	±	1.987	5.866	±	1.932

There is a significant different between group 1 and group 2 in platelet count and platelet distribution width where (p=0.001).

There is a significant different between group 1 and group 3 in mean corpuscular hemoglobin content (MCH) and mean cell hemoglobin concentration (MCHC) where (p=0.001).

Table 2
Hematological parameter in groups 1 and 2

Hematological parameter	Group 1	Group 2	P-value	
	mean	mean		
WBC	8.408	9.090	0.65	NS
Lymphocyte	2.483	2.787	0.74	NS
RBC	4.763	4.887	0.77	NS
Hb	13.933	13.638	0.30	NS
Hct	39.892	40.359	0.51	NS
MCV	83.258	81.846	0.79	NS
MCH	29.075	27.676	0.69	NS
MCHC	34.983	34.050	0.20	NS
RDW	12.725	12.378	0.29	NS
PLT	238.25	246.622	0.001	<0.05
PDW	15.950	16.588	0.001	<0.05
Neutrophil	5.517	6.004	0.59	NS

Table 3
Hematological parameter between group 1 and group 3

Hematological parameter	Group 1	Group 3	P-value	
	mean	mean		
WBC	8.408	8.756	0.36	NS
Lymphocyte	2.483	2.574	0.31	NS
RBC	4.763	4.691	0.42	NS
Hb	13.933	13.374	0.49	NS
Hct	39.892	38.817	0.71	NS
MCV	83.258	83.669	0.33	NS
MCH	29.075	28.849	0.02	<0.05
MCHC	34.983	34.431	0.04	<0.05
RDW	12.725	12.446	0.20	NS
PLT	238.25	247.286	0.68	NS
PDW	15.950	16.271	0.04	<0.05
Neutrophil	5.517	5.866	0.43	NS

There is a significant different between group 2 and group 3 in mean corpuscular hemoglobin content (MCH), mean corpuscular volume (MCV) platelet count and platelet distribution width where (p=0.001).

Table 4
Hematological parameter between group 2 and group 3

Hematological parameter	Group 2	Group 3	P-value	
	mean	mean		
WBC	9.090	8.756	0.49	NS
Lymphocyte	2.787	2.574	0.28	NS
RBC	4.887	4.691	0.12	NS
Hb	13.638	13.374	0.39	NS
Hct	40.359	38.817	0.10	NS
MCV	81.846	83.669	0.04	<0.05
MCH	27.676	28.849	0.001	<0.05
MCHC	34.050	34.431	0.23	NS
RDW	12.378	12.446	0.69	NS
PLT	246.622	247.286	0.001	<0.05
PDW	16.588	16.271	0.001	<0.05
Neutrophil	6.004	5.866	0.73	NS

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DISCUSSION

Hematological parameters give information on the physiological states of blood cells, and these parameters may suffer distortions in type 2 DM [7].

In this study, show that patients diabetics with HbA1c <7 (group 2) and with HbA1c >7 (group 3) had significant high mean PLT and PDW($p<0.05$) values when compared to non-diabetic healthy subjects (Group1) The results in the study were in agreement with study Charles LE et al, [8] and Kodiatte TA [9] also observed significant

There might be small vascular bleeds due the rupture of a throthrombotic plagues leading to bone marrow stimulation to recruit larger hyperactive platelets [8, 9]. Moreover, osmotic swelling of platelets, as a result of hyperglycemia and the platelet granule secretions, may contribute to platelet size variation and MPV elevation in T2DM patients [8].

While RBC parameter (Hb, HCT, MCV, MCH and RDW) is not significant different between group 1 and group 2. The results in the study were in agreement with study Abbas et al. [10], Adam et al. [11] and Biado et al. [12] also observed no significant.

While WBC parameter (total WBC and differential WBC) is not significant different between group 1 and group 2, 3. The results in the study were in agreement with Ebrahim et al, [13] but results in the study were in dis agreement with study Biado B et al also observed no significant [12]. The variation might be attributed to dissimilarities in size of sample.

In this study, show that patients diabetics with HbA1c >7 (group 3) had singingly lower mean MCV, MCH ($p<0.05$) values and significant high mean PDW ($p<0.05$) values when compared to non-diabetic healthy subjects (Group1) and the results in the study were in agreement with Ebrahim H et al.multiple risk factors such as hyperglycemia, hyperosmolarity, oxidative stress, inflammation, and lipid metabolic disorder may affect RBCs metabolism as they may increase aggregation, reduce cell deformability, and reduce membrane fluidity [13].

In this study, show that patients diabetics with HbA1c >7 (group 3) had significant lower mean MCV and MCH ($p<0.05$) values when compared to diabetics with HbA1c <7 (group 2) suggesting that good diabetic control is essential to prevent development of anemia in diabetic individuals. The results in the study were in agreement with study by Afsar N. et al. and Ebrahim Het al. also observed significant low MCV and MCH [14].

In this study, show that patients diabetics with with HbA1c >7 (group 3) had significant high mean PLT and PDW ($p<0.05$) values when compared to HbA1c <7 (group 2). No such comparison was made in any of the previous studies.

CONCLUSION

Haematological parameters should be routinely determined among diabetes mellitus type II patients for proper management and reduction of further complications.

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