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STUDY OF THE EFFECT OF LEAD ACETATE ON THE HEART, LUNGS, SPLEEN AND SOME BLOOD VARIABLES OF SPRAGUE–DAWLEY RATS

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Abstract

This study was conducted on thirty-six (Sprague-Dawley) male rats to determine the effect of LD50 (0.046) of lead acetate on some internal organs and whether it also affected some blood variables were divided to six groups, a control group (T1) which rats were fed on regular food and distilled water. The second group was the lead acetate group (T2) which was fed on regular food and distilled water containing lead acetate. The groups (T3 and T6) was fed on regular food mixed with mint powder with a concentration of (2-4%) respectively and lead acetate in distilled water. (T5 and T4) which were differ with (T3 and T6) in parsley powder instead of mint powder. The results showed that lead acetate had significant negative effects on the organs and blood variables (indicated in the study) and that (T6) gave a good resistance to lead acetate, followed by (T5) in terms of preference. The groups (T4, T3) were equal in terms of blood content from hemoglobin. When comparison between groups (T4 and T3), we find that (T4) gave better results than (T3) with regard to lung weight and spleen, as well as blood variables (Cholesterol, T.G, HDL, W.B.C, RBC, Hematocrit and M.C.V) while T3 gave better results compared to (T4) heart weight variables and blood variables (lead acetate concentration, LDL). conclude that the best types of therapeutic nutrition within this study variables are the nutrition on mint powder in the concentration (2%), that is, it is treatment (T6).

Key words: Lead (Pb) acetate toxicity; Mint; Parsley; Rat heart; Rat blood variables.

Introduction

Lead is one of the heavy metals that have no nutritional value while it has a lot of negative effects on the organism, as well as it is a dangerous environmental pollutant, the sources of heavy metals in general and lead in particular (mining, agriculture, coal production and burning) (Kim *et al.*, 2020). One of the bad characteristics of heavy metals is their easy access into the food as well as accumulation in the body of the organism (Kim *et al.*, 2020). As for the lead access to the human body, it is either through our inhalation of air or dust, food and water contaminated with this element. the mechanism of its effect on the cells and causing their damage, due to the oxidative stress of the accumulated lead in the cells, especially the types of oxygen (excessive reactivity) as well as the content of cells from Low antioxidants which

help to speed up the damage of the cells concerned. Lead have affect on many biological molecules, through the effect on its functions of regulatory proteins and enzymes by change the signals for these molecules (Mohamed *et al.*, 2020). Lead also affects the internal organs of the human body. The kidneys, liver, brain and other organs are vulnerable to the toxic effects of lead. The effect of lead on the level of anti-inflammatory cytokines production and other changes in the body in general extends from the smallest cells to the largest organ of the body (Mohamed *et al.*, 2020).

One of the most important internal organs of the human body is the heart, as it is responsible for pumping blood, which in turn is the means of life and transporter for all the needs of the cells, as well as its waste. Heart get start its work after only 21 days of pregnancy. All

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four chambers of the heart (atria and ventricles) are formed from the heart tube after the bulging, that is, after 30 days of pregnancy (Asp *et al.*, 2020). In general, the heart delivers the oxygen-carrying blood to the body's different cells through the capillaries through the arteries, as well as the transfer of carbon dioxide through the blood also from the cells to the capillaries through the veins and then into the lungs in two main cycles (the pulmonary circuit and the systemic circuit) (Lewis *et al.*, 2016). If we look carefully, we will find that deaths around the world in cardiovascular disease (CVD) constitute a very high rate, we also find that there is a clear relationship between these diseases (CVD), oxidative stress and infections (Roshan *et al.*, 2011). Has been found that the widespread prevalence of lead and its apparent role in many chemical and physiological imbalances as well as behavioral had a clear impact on the increase in the number of deaths from diseases (CVD) and this is indicated by (Roshan *et al.*, 2011) because the heart is one of the internal organs which characterized by its low antioxidants defense. We mentioned that the lead has a close relationship with the formation of free radicals, especially the free radicals of oxygen which confirmed by (Roshan *et al.*, 2011), adding that many blood variables

are affected by lead toxicity, including blood sugar and lipid peroxide and other variables, as well as the toxic effects of lead on the enzymatic system and qualitative and quantitative changes of the heart muscle. Lead acetate has a direct effect on the blood content of red blood cells and the rise of white blood cells, as it affects the size and shape of these cells. In most cases, white blood cells do not perform due to the deformation of their shape despite the high total number. Lead acetate affects the percentage of hemoglobin in the blood as it leads to a decrease Its ratio and many other blood variables (Ekanem *et al.*, 2015). Lead acetate has a major effect on the respiratory system in general and the lung and its tissues in particular through the oxidative stress caused by these acetates. The decrease in antioxidants resulting from lead acetate leads to a high incidence of asthma, especially in children if we compare with adults, there is also a relationship between Lead acetate and (concentrations of tocopherols and ascorbate) in the lining of the respiratory tract for the asthma patients, where lead acetate leads to a decrease in these concentrations in addition to its negative role in the imbalance of the bronchial washing fluid content of antioxidants. Therefore, we find that the oxidative stress caused by lead acetate

is associated with many respiratory diseases such as pulmonary fibrosis and ischemia. In addition to the other diseases and the negative effects the above (Samarghandian *et al.*, 2015). In addition to the foregoing, we can say for sure that the majority of people in the world suffer from the accumulation of lead in their bodies and with the different amounts of lead accumulated according to each person (depending on the location and type of work, as well as the type of food he consumes). In spite of this, we can point out that the majority of lead accumulated in our bodies accumulates in the bones by up to 75% of the total accumulated lead in the body, followed by the rates of lead accumulation of blood and soft tissues at rates (5-20%) (Ekanem *et al.*, 2015). Medicinal plants have played an important role as successful alternatives to chemical and industrial treatments, especially in the last two decades, as they have broad biological activities because of the various receptors and various secondary chemical compounds. Therefore, we

Table 1: Shows the concentration of lead acetate in blood, as well as the effect of lead acetate on the weight of the rats' internal organs (lung, spleen, heart) and the efficiency of therapeutic feeding is in Fighting the negative impact of lead acetate.

Treatment	Parameters				
	Blood Lead concentration $\mu\text{g/dl}$	W lungs *2 g	W Spleen g	W Heart g	
T1	4.8 ^a ±	1.536 ^a ±	0.8 ^a ±	0.8 ^a ±	
	0.1	0.0016	0.0002	0.00034	
T6	65 ^b ±	1.4 ^b ±	0.9 ^b ±	0.76 ^b ±	
	0.1	0.0003	0.0003	0.00016	
T5	96.2 ^d ±	1.11 ^c ±	0.95 ^c ±	0.653 ^b ±	
	0.4	0.0002	0.0002	0.0002	
T3	85.1 ^c ±	0.939 ^c ±	1.25 ^c ±	0.6 ^d ±	
	0.8	0.0005	0.0003	0.00024	
T4	103.2 ^c ±	1.05 ^d ±	1.202 ^d ±	0.582 ^c ±	
	0.5	0.0005	0.0044	0.00016	
T2	314.9 ^f ±	0.855 ^f ±	1.5 ^f ±	0.399 ^f ±	
	0.7	0.0002	0.0003	0.00026	
P-Value	Treatment	<.0001	<.0001	<.0001	<.0001
	Time	<.0001	<.0001	<.0001	<.0001
	T × Ti	<.0001	<.0001	<.0001	<.0001

T1= Control group (regular fodd), T2=Lead Acetate, T3= Mint at 4% concentration, T6= Mint at 2% concentration, T4= Parsley at 4% concentration, T5= Parsley at 2% concentration. In each Colum: (a, b, c, d, e, f). Means indented with different letters are significantly at the specified confident level with significantly by Duncan's multiple range test $p > 0.05$.

find that approximately 50% of the newly used treatments contain compounds extracted from medicinal plants or the like (Hanafy *et al.*, 2017). Peppermint is one of the medicinal plants that contain many compounds beneficial to human health, In general, it was and still is to this day used in preparing many foods, Also, it is used in many food industries as a main ingredient or as a flavoring additives, in addition to its use in the production of many types of nuts, as well, It can be eaten fresh as in many countries of the world (Hanafy *et al.*, 2017). Despite the lack of studies on mint in general or its content of phenolic compounds in particular, mint contains many plant phenols including (hesperitin, naringenin, eriodictyol, luteolin, apigenin,) and its cyclosides as well as acids (caffeic, salvianolic, rosmarinic) And other important compounds (Hanafy *et al.*, 2017). Parsley is distinguished by its attractive leaves with distinctive aromatic odors. Parsley uses different uses in the food industry as flavoring materials or as decoration materials for various dishes. Parsley is used either dry or fresh (Zhang *et al.*, 2006). Parsley is important in fighting cancer, some studies

mentioned that parsley has an anti-oxidant effect, its role in fighting cancer may be due to its (b-phellandrene, p-menthatriene, myrcene, myristicin) as basic ingredients (Zhang *et al.*, 2006). The current study aimed to study the effect of lead acetate on the heart, lung, spleen, as well as some blood variables (Cholesterol, T.G, HDL, W.B.C,RBC, Hematocrit and M.C.V, lead acetate concentration, LDL) and the resistance of both (parsley and mint) the oxidative stress to lead acetate.

Materials and Methods

Preparation of medicinal plants

Fresh mint leaves and parsley has been collected. Thoroughly washed and dried at room temperature (22-25°C). After these leaves dried up, they were ground and added to the rat bush, according to the type (mint or parsley) and the concentration according to the type of treatments.

Lead source

The lead acetate (CH₃COO)₂Pb.3H₂O Molar. - Gew.379.34 E. Merck, Darmstadt, Germany) at 0.46%

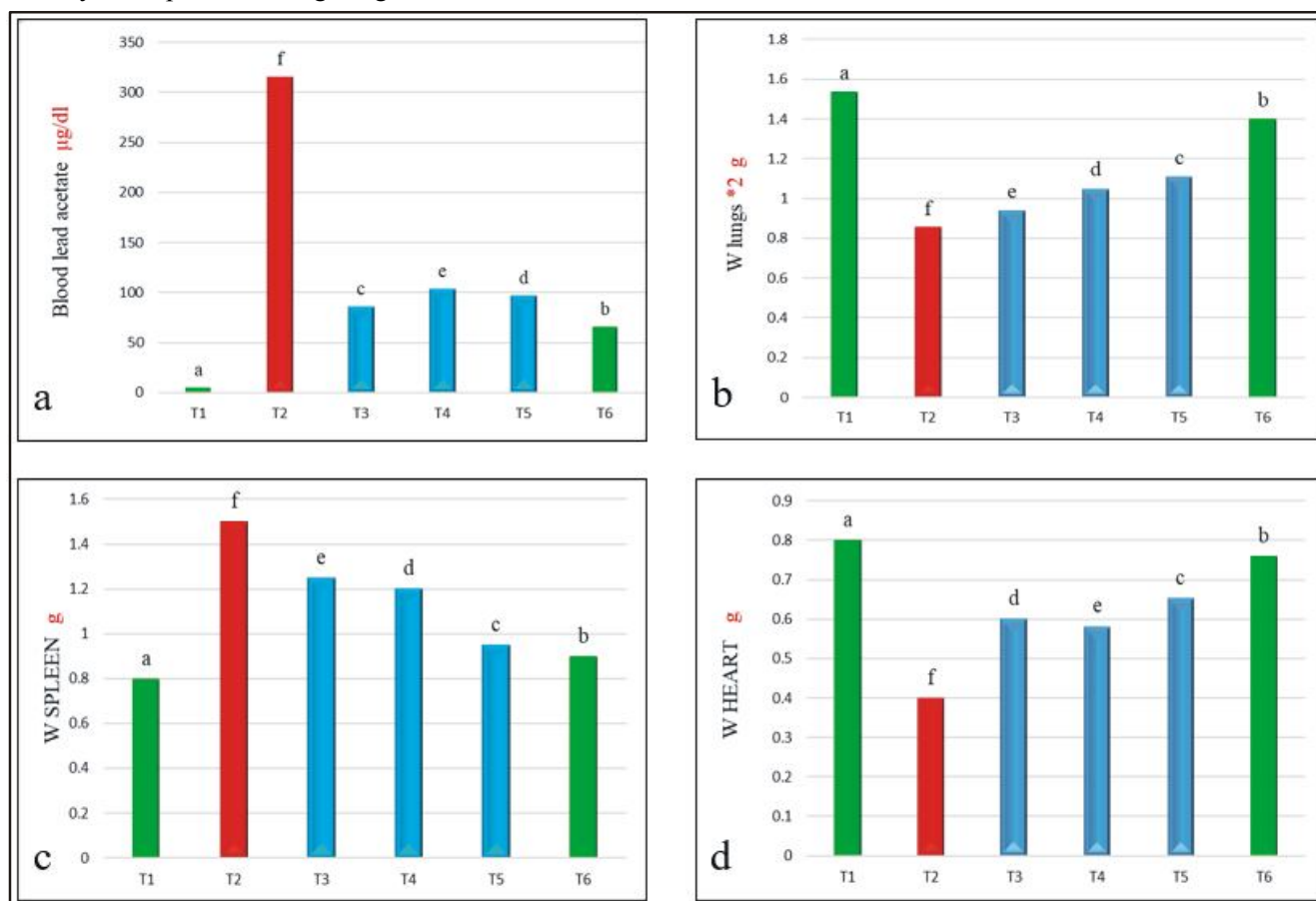


Fig. 1: Shows the concentration of lead acetate in blood (a), as well as the effect of lead acetate on the weight of the rats' internal organs (lung (b), spleen (c), heart (d)) and the efficiency of therapeutic feeding is in Fighting the negative impact of lead acetate. T1= Control group (regular food), T2=Lead Acetate, T3= Mint at 4% concentration, T6= Mint at 2% concentration, T4= Parsley at 4% concentration, T5= Parsley at 2% concentration. In each Column: (a, b, c, d, e, f). Means indented with different letters are significantly at the specified confident level with significantly by Duncan's multiple range test $p > 0.05$.

concentrate mixed with Distilled Water Provided to the rats.

Ethics statement

The experiment was carried out according to standards and specifications similar to the experiments and research of experimental animals, especially working to reduce the number of used animals within one experiment throughout the trial period (Tomaszewski *et al.*, 2015).

Experimental design and data analysis

This experiment was completely randomly designed and lasted for a period of 60 days during which the rats underwent similar breeding conditions with (Tomaszewski *et al.*, 2015) in terms of temperature, humidity, lighting periods, ventilation and breeding boxes. The experiment was done using thirty-six (Sprague-Dawley) male rats (Amos *et al.*, 2016) that were randomly divided, as we mentioned to several groups (T1, T2, T3, T4, T5, T6). The control group was called T1 which were fed to the organized feed adding to distilled water free of any additives (the water bottles are filled daily), all other groups are supplied with distilled water containing lead acetate with a concentration (0.046%). Also, it is ensured

Table 2: Shows the effect of lead acetate on the Blood parameters as blood (cholesterol, Triglycerides, High-density lipoprotein, Low-density lipoprotein) and the efficiency of therapeutic feeding is in Fighting the negative impact of lead acetate.

Treatment	Parameters			
	Cholesterol mg/dl	Triglycerides (TG) mg/dl	High-density lipoprotein (HDL) mg/dl	Low-density lipoprotein (LDL) mg/dl
T1	75.98 ^a ±0.1	44 ^a ±0.16	48 ^a ±0.16	11 ^a ±0.16
T6	76.02 ^a ±0.16	70 ^b ±0.16	46 ^b ±0.16	14 ^b ±0.16
T5	80.3 ^b ±0.4	81 ^c ±0.16	41.96 ^c ±0.11	16.07 ^c ±0.12
T3	87.37 ^c ±0.3	110.72 ^d ±0.41	38 ^c ±0.16	20 ^d ±0.16
T4	89.04 ^c ±0.2	116 ^e ±0.16	40.98 ^d ±0.16	21.07 ^c ±0.12
T2	97.98 ^e ±0.1	142.02 ^f ±0.19	32.92 ^f ±0.13	37 ^f ±0.16
P-Value	Treatment	<.0001	<.0001	<.0001
	Time	<.0001	<.0001	<.0001
	T × Ti	<.0001	<.0001	<.0001

T1= Control group (regular fodd), T2=Lead Acetate, T3= Mint at 4% concentration, T6= Mint at 2% concentration, T4= Parsley at 4% concentration, T5= Parsley at 2% concentration. In each Colum: (a, b, c, d, e, f). Means indented with different letters are significantly at the specified confident level with significantly by Duncan's multiple range test $p > 0.05$.

that the bottles of water are filled daily, group T2 is fed on regular fodd in addition to the lead acetate solution. the groups (T6 and T3) were fed on the fodd after its Remixed with mint powder with a concentration of (2-4%) respectively, as well as the groups (T5 and T4) but the parsley powder instead of mint replaced in the fodd with concentrations (2-4%) respectively.

Blood tests

The rats were anesthetized by diethyl ether (inhalation), after it was confirmed that the rats lost consciousness. the blood samples were drawn for each rat by approximately 5 ml and one sample of blood was distributed over two (EDTA) test tubes and a (z serum Sep clot Activator) tube (Lee *et al.*, 2017).

The internal organs (liver, kidneys, testes)

The rat's internal organs have been extracted and weighed after we used a surgical blade to open the abdominal cavity down to the rib cage. The rat's internal organs samples it has been saved with a formalin solution with (10%) concentration.

Statistical analysis

Statistically significant differences between the averages were compared by testing the least significant difference (LSD) and the use of general statistics, (2012) in the statistical analysis of the data studied.

Results and Discussion

Blood Lead Acetate level and rats' internal organs: Through the results which related to the blood, we find a clear rise in the blood lead acetate concentration of the group (T2)(314.9 µg/dl) compared to the control group (T1)(4.8 µg/dl) (Table 1), whilst the therapeutic feeding groups, it showed resistance to the negative effects of lead acetate (Fig. 1a), this is clear by containing lower levels than Lead acetate in blood compared to group (T2). The closest of these groups in terms of the concentration of lead acetate in blood to the control group (T1) is group (T6) (65 µg/dl) is the lowest in terms of levels of lead acetate in blood, followed by therapeutic feeding groups (T3) (85.1 µg/dl), (T5) (96.2 µg/dl), (T4) (103.2 µg/dl) (Table 1). The results also showed the clear

effect of lead acetate on the internal organs (T2) (lungs, spleen, heart) when compared to the control group (T1). Through the results (Table 1), (Fig. 1b) we note the negative effect of lead acetate that led to a decrease in heart (0.399 g) and lung (0.4275 g for each one) weight and a noticeable rise in the weight of the spleen (1.5g). In regards to the therapeutic feeding of the treatments (T3, T6, T4, T5), the best results in reducing the negative harm of lead acetate were the therapeutic feeding of the group (T6) in terms of the internal organs weight (Table 1), it was the closest to the control group (T1) followed by the group (T5). The group (T3) gave better results compared to (T4) in terms of heart weight, while the group (T4) outperformed (T3) in resisting the negative effects of lead acetate on the weight of the lung and spleen (Fig. 1b, 1c, 1d). The results of the study came close to the role of mint as an antioxidant (Hanafy *et al.*, 2017) about the variables in this study, it is having been believed to be the first in this field.

The Blood parameters as blood (cholesterol, T.G, HDL, LDL): Among the important blood variables of this study (LDL, HDL, T.G, Cholesterol) it was noticed through the results that these variables were affected by lead acetate, according to the results we find that lead acetate helped to raise (cholesterol) in blood (Fig. 2a), especially with (T2) group (97.98 mg/dl) compared with (T1)(75.98 mg/dl), while therapeutic feeding led to resistance to this height in particular, the group (T6) (76.02 mg/dl) (Table 2) which was the closest to (T1) at the level of (cholesterol) followed by the groups (T5), (T3), (T4) (Table 2). The lead acetate also led to a high blood content of (T.G) (Fig. 2b). It reached its highest levels in (T2) group (142.02 mg/dl) and its lowest levels in (T1) (44 mg/dl). As for the therapeutic feeding groups, the lowest content was (T.G) is (T6) which have (70 mg/dl) also there is no significant difference between (T1a) and (T6a) (Table 2). As for the rest of the groups (T5), (T3), (T4), respectively, in terms of blood content of (T.G) (Fig. 2b). The results of the study indicated a clear decrease

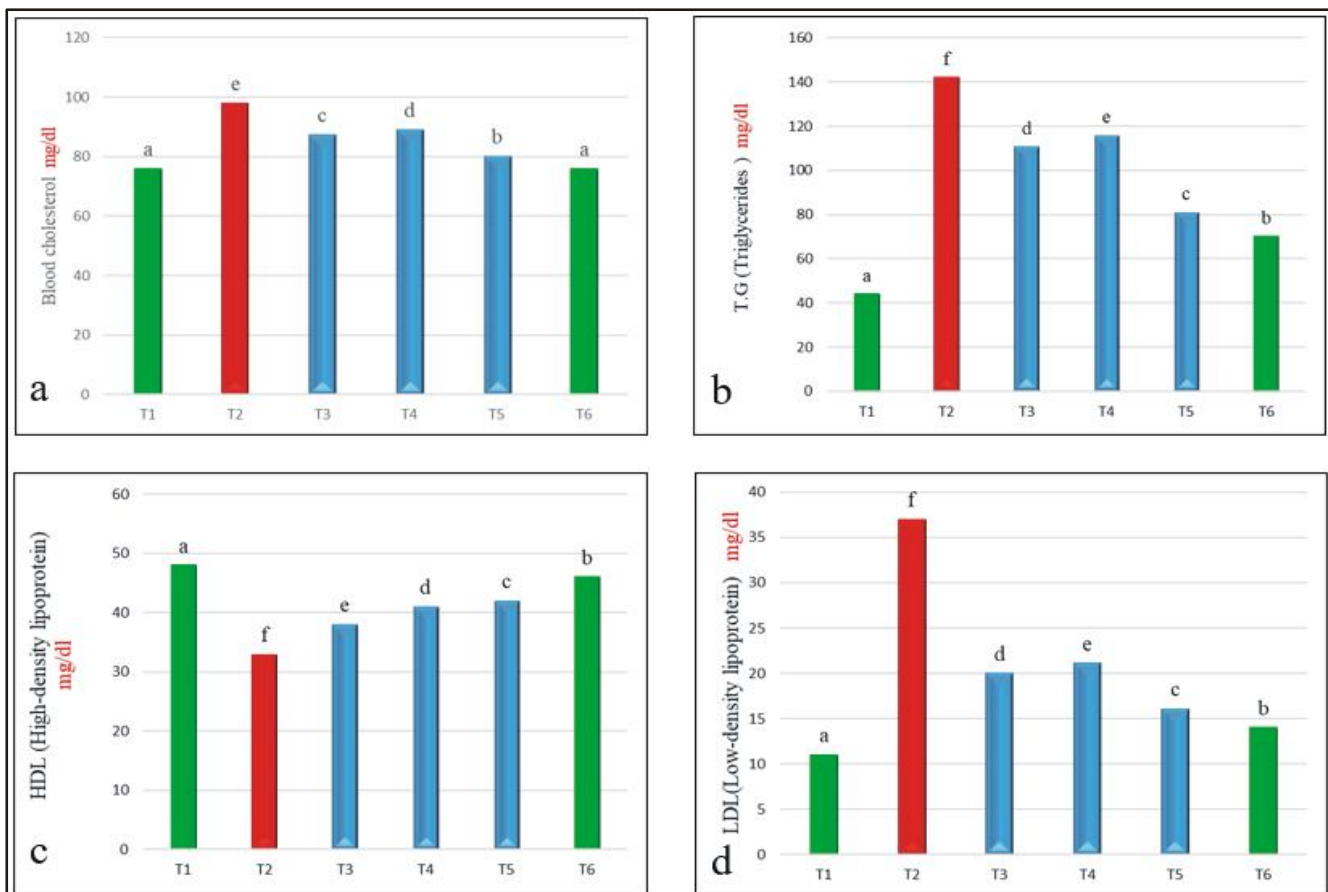


Fig. 2: Shows the effect of lead acetate on the Blood parameters as blood (cholesterol (a), Triglycerides (b), High-density lipoprotein (c), Low-density lipoprotein (d) and the efficiency of therapeutic feeding is in Fighting the negative impact of lead acetate. T1= Control group (regular fodd) , T2=Lead Acetate, T3= Mint at 4% concentration, T4= Parsley at 4% concentration, T5= Parsley at 2% concentration, T6= Mint at 2% concentration . In each Colum: (a, b, c, d, e, f). Means indented with different letters are significantly at the specified confident level with significantly by Duncan's multiple range test $p > 0.05$.

in HDL (T2) group (32.92 mg/dl) and a noticeable rise in blood (LDL) (T2) group (37 mg/dl) due to the effect of lead acetate and these changes were explained by the blood of the group (T2). The best resistance to the effect of the negative lead acetate was through the therapeutic feeding of group (T6) group (46 mg/dl H.D.L and L.D.L 14mg/dl) followed by (T5) with respect to each of (L.D.L,H.D.L) (Table 2). As for the comparison between (T3) and (T4), we find that (T4) is the best blood content of HDL and (T3) in terms of blood content of (LDL) (Fig. 2c, 2d).

The Blood parameters as blood (W.B.C, R.B.C, Hemoglobin, Hematocrit, M.C.V C): Among the most important variables studied in this research is the blood content of white blood cells (WBC) as an indicator of the effect of lead acetate on the immune system, we find that the results of (WBC) showed a marked increase in the content of (WBC) within the blood of group (T2) (4417 mm³) Compared to the control group (T1) (1703 mm³) (Table 3), has been find a good resistance to this increase by the therapeutic feeding groups, especially (T6) (4402 mm³) (Table 3) which were the lowest in

terms of the amount of (WBC) in the blood followed by the therapeutic feeding groups (T5) (10106 mm³), (T4) (13802 mm³), (T3) (16451 mm³) (Fig. 3a). When we look at the results of each of (RBC, Hemoglobin, Hematocrit), we find that there is a clear decrease in the number of (RBC) due to the negative effect of lead acetate (T2) ($4.417 \times 10^6/\text{ul}$). When compared to group (T1) ($8.83 \times 10^6/\text{ul}$) in terms of blood (RBC) content followed by in terms of resistance and positive effect groups of therapeutic feeding (T6) ($8.494 \times 10^6/\text{ul}$), (T5) ($7.401 \times 10^6/\text{ul}$), (T4) ($7.109 \times 10^6/\text{ul}$), (T3) ($6.65 \times 10^6/\text{ul}$) (Table 3) (Fig. 3b). As for the negative effects of lead acetate at the level of (Hemoglobin) in the blood, it appeared clear in the group (T2) (8.494 g/dl), in terms of low blood content of (Hemoglobin), while we find that the therapeutic feeding led to the desired results, it showed clear resistance to the negative effects of lead acetate, either better The results were for the group (T6) (14.78 g/dl), followed by the group (T5) (14.09 g/dl), while the groups (T4) (13.31 g/dl) and (T3) (13.06 g/dl) (Table 3), were equally resistant to the effects of lead acetate on (Hemoglobin) (Fig. 3c). It can be observed from the study

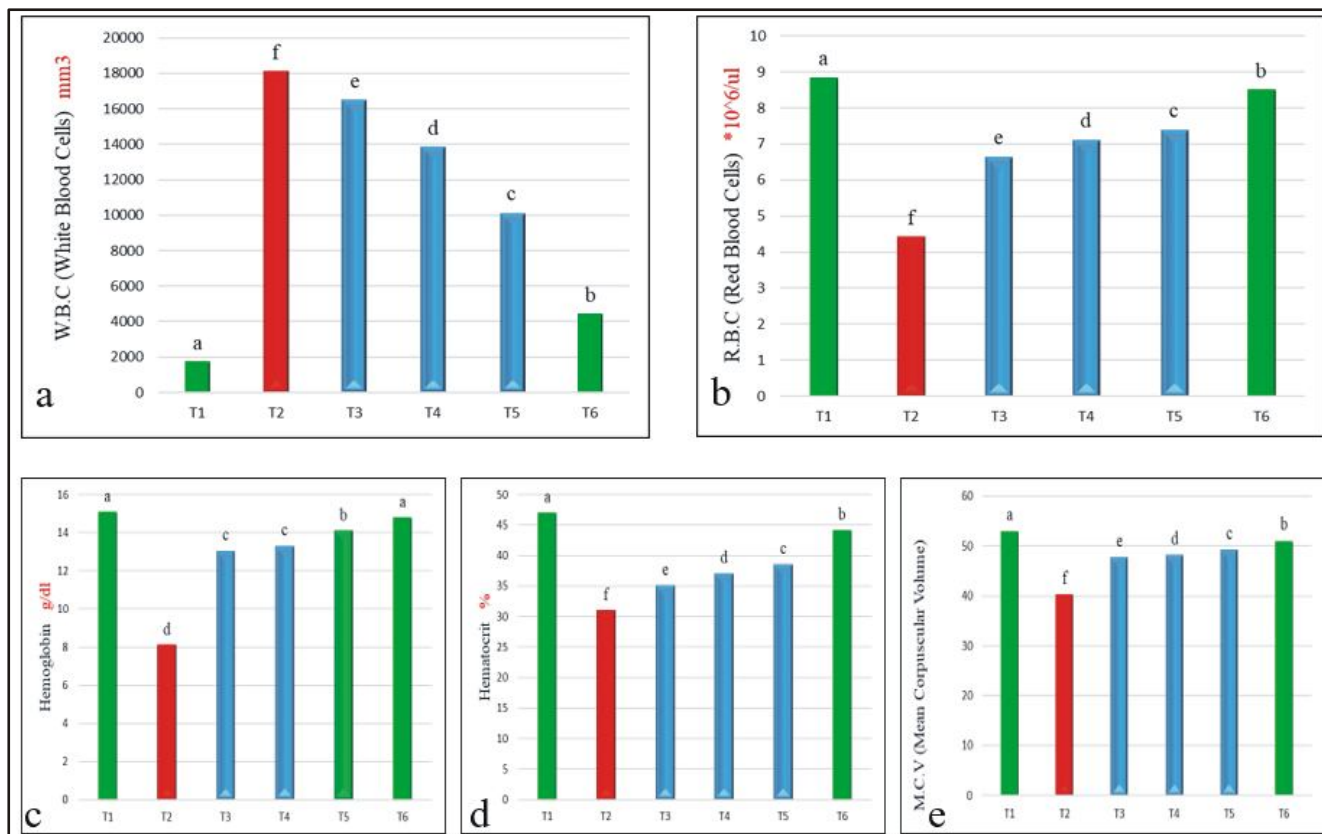


Fig. 3: Shows the effect of lead acetate on the Blood parameters (White Blood Cells (a), Red Blood Cells (b), Hemoglobin (c), Hematocrit (d), Mean Corpuscular Volume (e)) and the efficiency of therapeutic feeding against the negative effect of lead acetate. T1= Control group (regular fodd), T2=Lead Acetate, T3= Mint at 4% concentration, T4= Parsley at 4% concentration, T5= Parsley at 2% concentration, T6= Mint at 2% concentration. In each Colum: (a, b, c, d, e, f). Means indented with different letters are significantly at the specified confident level with significantly by Duncan's multiple range test $p > 0.05$.

Table 3: Shows the effect of lead acetate on the Blood parameters (White Blood Cells, Red Blood Cells, Hemoglobin, Hematocrit, Mean Corpuscular Volume) and the efficiency of therapeutic feeding against the negative effect of lead acetate.

Treatment	Parameters					
	White Blood Cells (W.B.C) mm3	Red Blood Cells (R.B.C) *10 ⁶ /ul	Hemoglobin g/dl	Hematocrit %	Mean Corpuscular Volume (M.C.V) %	
T1	1703 ^a ±3.5	8.83 ^a ±0.0	15.08 ^a ±0.606	47.01 ^a ±0.011	52.8 ^a ±0.007	
T6	4402 ^b ±5.4	8.494 ^b ±0.005	14.78 ^b ±0.017	44.15 ^b ±0.013	50.9 ^b ±0.16	
T5	10106 ^c ±4.4	7.401 ^c ±0.001	14.09 ^b ±0.003	38.56 ^c ±0.011	49.1 ^c ±0.009	
T3	16451 ^c ±0.002	6.65 ^c ±0.002	13.06 ^c ±0.034	35.06 ^c ±0.08	47.6 ^c ±0.016	
T4	13802 ^d ±5.3	7.109 ^d ±0.01	13.31 ^c ±0.00	37.05 ^d ±0.034	48.08 ^d ±0.037	
T2	4417 ^f ±0.022	4.417 ^f ±0.022	8.1 ^d ±0.01	30.94 ^f ±0.04	40.1 ^f ±0.01	
P-Value	Treatment	<0001	<0001	<0001	<0001	<0001
	Time	<0001	<0001	<0001	<0001	<0001
	T×Ti	<0001	<0001	<0001	<0001	<0001
T1= Control group (regular fodd), T2=Lead Acetate, T3= Mint at 4% concentration, T6= Mint at 2% concentration, T4= Parsley at 4% concentration, T5= Parsley at 2% concentration. In each Colum: (a, b, c, d, e, f). Means indented with different letters are significantly at the specified confident level with significantly by Duncan's multiple range test p > 0.05.						

results that lead acetate has a clear negative effect in reducing the blood content of (Hematocrit and M.C.V) by comparing the group (T2) with the control group (T1) groups (Table 3), therapeutic feeding (T3) (T4) (T5) (T6) When we note the results of (Hematocrit), we find that the percentage of blood in it decreased for the group (T2)(30.94%), but it did not decrease significantly with respect to (T6)(44.15%). This is evidence of the positive effect of feeding this group in reducing the effects of lead acetate followed by the groups (T5) (38.56 %) (T4) (37.05%) (T3) (35.06 %) (Fig. 3d). The results of (M.C.V) also showed the clear positive effect of the therapeutic feeding of mint (2%) (T6) (50.9%) on the resistance of low blood content of (M.C.V) compared to the group (T2) (38.56%), it is the closest in terms of (M.C.V) content of the control group (T1) (52.8 %). (T5) (49.1%) followed and also followed by (T4) (48.08%) and the last in terms of lead acetate resistance is the group (T3) (47.6%) (Fig. 3e) (Table 3).

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