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Antioxidant Activity of Schiff Base Derived from Methionine.

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ABSTRACT

The aim of study is to investigate the role of new Schiff base derived from methionine in preventing the oxidation of hemoglobin in vitro and preventing the disturbances in lipid metabolism, and liver enzymes induce by sodium nitrite toxicity in rats (*Ratus norvegicus*). Certain parameters were measured as inhibit sodium nitrite induce met hemoglobin formation, serum TC,TG, LDL,VLDL, HDL, serum alanine transaminase and aspartate transaminase (ALT & AST) activity. The new Schiff base causes inhibit sodium nitrite induce met hemoglobin formation, moreover the new Schiff base causes significant decrease of TG and VLDL while HDL significant increase, and causes significant decrease in ALT and AST in the $NaNO_2$ with synthesized compound group as compared with $NaNO_2$ group.

Keywords: Schiff base, Sodium nitrite, Biochemical tests, Antioxidant.

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INTRODUCTION

Sodium nitrite widespread use in the food industry contributes to the potential health risk if not handled cautiously and causes decreased whole blood viscosity. A growing body of evidence indicates the beneficial effect of sodium nitrite. In medicine is used as antidote in the treatment of cyanide poisoning [1], and as a potential new treatment for peripheral vascular disease [2]. However, toxicity to humans and animals is well documented in nitrite over exposure [3].

Acute intoxication is manifested primarily by met hemoglobin formation and resultant anoxia [4]. A major concern considering the toxicology of sodium nitrite is the induction of met hemoglobinemia, a condition in which there is reduction in oxygen transport ability of hemoglobin [5].

Antioxidants are defined as substances that are capable of deactivating or stabilizing free radicals before they attack living cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being functions. In case of exposure to free radicals, from a variety of sources, has led organisms to develop a series of defense mechanisms [6]. Antioxidants like, vitamin C, vitamin E, Moreover, selenium, are intimately involved in the prevention of cellular damage, the common pathway for cancer, and a variety of diseases [7]. The aim of study is to investigate the role of new Schiff base derived from methionine in preventing the oxidation of hemoglobin in vitro and preventing the disturbances in lipid metabolism, and liver enzymes induce by sodium nitrite toxicity in rat.

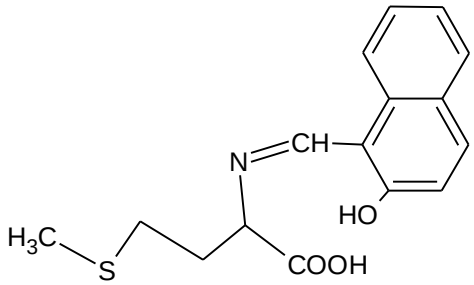

Figure 1: Chemical structure of methionine derivative

EXPERIMANTAL

Materials and methods

Sodium nitrite-induces met hemoglobin in hemolyseate

Blood samples were collected from healthy volunteers, and centrifuged (2500 rpm × 20 min) then remove plasma and the Buffy coat of white cells by micro pipette, the erythrocytes obtained were washed thrice with phosphate-buffered saline, the washed cells were lyses by suspending in 20 volumes, of 20 mM phosphate buffer, PH 7.4 , the hemolysate was centrifuged at 2500 rpm × 20 min for 30 min to remove the membrane, and then diluted to give a 150 µM concentration of oxyhemoglobin. The reaction was initiated by the addition of sodium nitrite (final conc. 0.6mM) to the solution of hemolysate and the formation of met hemoglobin was measured by monitoring absorbance at 631 nm using a Shimadzu Graphicord UV 240 Spectrophotometer. New Schiff base compound [MDJ] was synthesized and characterized according to the method described by Azzouz *et al* [8]**.** The synthesized compound was dissolved in DMSO and injected intrapretonially of rat at dose of 8.213 mg/kg of body weight corresponding to 1/10 of its LD_{50}, and added either before or at various time -interval after the addition of sodium nitrite. The control experiments were conducted without base synthesized compound and all experiments were triplicate and were repeated many times. The data given are for one set [9].

Antioxidant *in vivo*.

Animals of the study

This study was carried out on 24 young male and female rats (*Ratus norvegicus*) weighing about 240-250 gm. They were maintained at standard experimental condition, rats were housed in the animal house of College of Veterinary Medicine / University of Basrah, and they were kept under good hygienic conditions. The rats were (12-14) weeks of age with an average weight ranged between (230-250gm). Animals were placed in polypropylene cages (30 × 25 × 17 cm). The animals were maintained under laboratory conditions at temperature of (22-24 ºC) and exposed to a photoperiod of 12 hrs light followed by 12 hrs of darkness.

They were fed standard rat pellets and fresh clean water was provided (*at libitum)* throughout the experimental period. Animals were acclimated to the laboratory conditions for about 10 days before the application of experimental protocols.

Experimental Design of Antioxidant *In Vivo*.

This study was carried out on 24 young male and female rats (*Ratus norvegicus*) weighing about 240-250 gm. They were maintained at standard experimental condition, rats were housed in the animal house of College of Veterinary Medicine / University of Basrah, and they were kept under good hygienic conditions.

After ten days of acclimatization young mal rat were divided in to 4 groups (6 in each group) as following.

A- Control group: Rats were injected daily with DMSO intraperitoneally by 0.5ml for 21 days.
B- Second group: Rats were injected daily with $NaNO_2$intraperitoneally by 0.5 ml at dose of 120 mg/kg. [1.5 mg/rat/day] for 21 days.
C- Third group: Rats were injected daily with 1/10 LD_{50} of synthesized compound (MDJ) intraperitoneally by 0.5 ml at dose of 47.7 mg/kg for 21 days.
D- Fourth group: Rats were injected daily with $NaNO_2$ intraperitoneally by 0.5 ml at dose of 120 mg/kg [1.5 mg/ rat/day] then after one hour the rats **were** injected with1/10 LD_{50} of synthesized compound (MDJ), intraperitoneally by 0.5 ml at dose of 47.7 mg/kg for 21 days.

At the end of experimental period (21 days) the rats were sacrificed under general anesthesia by placing them in tightly closed glass container which contain cotton soaked with chloroform as inhaled anesthesia, After that the abdominal cavity was opened and a “Y" shaped cut the rat abdomen was done, blood was collected via direct heart[10] by using 5ml disposable syringe. Blood samples were transferred into plain tubes centrifuged at (3000 rpm for 15 minutes) to obtain the serum which then transferred into numerous eppendorf tubes to use in analyses of different parameters and stored at - 4°C.

Biochemical test

The biochemical tests were done in central research unit of veterinary medicine college-Basrah university by using autoanalyzer (Serial No.20628, Human Star, Germany). The device has 54 wells numbered from 1 to 54. The Samples were placed in each specific well of the device. The reagent was put it in special container beside the wells, the serum biochemical parameters estimated by this instrument included: total cholesterol, triglyceride, HDL**,** AST, and ALT.

Statistical Analysis

The results of the present three experiments were analyzed by univalent analysis of variance (ANOVA) by using computerized SPSS (Statistical Packages for the Social Sciences) V.13 program. $P<0.05$ was considered to be the limit of significance. The data were expressed as mean ± standard deviation (mean ± SD). Least significant difference test (LSD) was used to test the difference between groups

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RESULTS AND DISCUSSION

In vitro antioxidant activity of MDJ (inhibition of nitrite induced methemoglobin formation by MDJ)

The present study shows that sodium nitrite caused a rapid oxidation of hemoglobin to methemoglobin. The oxidation process was delayed by the addition of different concentrations ofsynthesized compound (MDJ) (5, 10 and 20µM) in a concentration dependent manner. The time required to convert 50% of the available hemoglobin to methemoglobin was increased from 11 to 20 and 73 minutes by the addition of (5,10 and 20µM) of test compound (MDJ) respectively.

Figure 2 describes the effect of synthesized compound on the time –course of nitrite oxidation of hemoglobin. Without synthesized compound, the time- course oxidation slow initial transformation followed by a rapid autocatalytic process. When the synthesized compound was added along with sodium nitrite at 0 min, the formation of met hemoglobin was inhibited to great extent. However, when the synthesized compound added at the end of autocatalytic stage, i.e. at 10 min no protection was observed. The best result in our study at 20 µM concentration of synthesized compound which protect hemoglobin from oxidation by sodium nitrite Fig 4. Since the synthesized compound [MDJ] is newly prepared by Al-Masoudi *et al* [11] with LD_{50} (477 mg/kg), therefore the experiments focused to determine its antioxidant activity. The present study has showed that synthesized compound can protect hemoglobin from oxidation by oxidation by sodium nitrite both in hemolyseate and in intact erythrocytes. However, it did not revere the effect of sodium nitrite if added at a later stage. It is well established that oxidation of hemoglobin takes place in two stage. There is slow initial stage followed by a rapid autocatalytic stage, which carries the reaction to completion [12].

Synthesized compound is able to prevent the onset of autocatalytic stage. Since superoxide is implicated in autocatalytic stage [12].

The fact that synthesized compound is potent scavenger of superoxide [14].

Direct interaction between nitrite and synthesized compound a reason for protection is ruled out because the concentration of synthesized compound causing protection 20µM compared to the nitrite concentration (0.6mM).This result was agreement with Doyle *et al* [12], who used curcumin analogue as antioxidant and recorded a 20 µM . Although synthesized compound can reduce ferric ions Fe^{3+} to the ferrous state Fe^{2+}, it fails to reverse the oxidation of hemoglobin, suggesting that protection is not due to reduction of met hemoglobin to hemoglobin. Many antioxidants like ascorbic acid, vitamin B1 analogue, protect hemoglobin from oxidation by nitrite**.** These antioxidants also inhibit the onset of the autocatalytic stage of nitrite if added at a later stage [15].

Thus, the effect of synthesized compound may be similar to this antioxidant in protecting hemoglobin from nitrite ions. The best result in our study at (20µm) concentration which protects hemoglobin from oxidation by sodium nitrite, Figure 4.

In vivo study

Effect of sodium nitrite and (M DJ) on lipid profile

It is clear from the results that sodium nitrite caused a significant increase ($P<0.05$) in TGs, LDL and VLDL values. Whereas, it causes in significant changes in HDL value (Table:1).

Nitrite induced hypercholesterolemia may be attributed to oxidation of lipids in the cell membrane and mobilization of free fatty acids from the adipose tissue to the blood and increase level of acetyl CoA, leading to increase in the hepatic synthesis of cholesterol[16]. These changes in lipid profile may be attributed to peroxidation of cell membrane lipids and lipolysis consequent to sodium nitrite induced oxidative stress and free radical generation, and hypolipidemic effect of synthesized compound could be related to its free radical scavenging activity and protection of cell membrane lipids from peroxidation.

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It has been observed that methionine has a significant effect on the liver health and its natural detoxification functions. This sulfur-containing amino acid helps liver in processing fats, which prevents fatty liver disease. Also by increasing glutathione levels, methionine helps the liver to effectively neutralize toxins. Methionine naturally supports cellular health with its powerful antioxidant activities [17].

Table 1: Effects of NaNO2 and MDJ on serum lipid profile

Parameter / Groups	TC Mg/dl	TG Mg/dl	LDL-c Mg/dl	VLDL-c Mg/dl	HDL-c Mg/dl
Control DMSO 0.5ml	45.3 ± 4.7 a	55.50 ±22.10 b	8.23 ± 2.35 b	4.40 ± 3.81 C	34.15 ± 5.90 C
$NaNO_2$ 120mg/kg	56.10 ±4.90 a	80.45 ±10.76 a	17.30 ± 6.40 a	12.12 ± 0.93 b	34.85 ± 3.61 C
MDJ 48mg/kg	49.95 ± 5.10 a	11.05 ± 0.05 C	5.47 ± 6.58 b	14.40 ± 0.89 a	37.10 ± 3.34 b
$NaNO_2$+ MDJ	53.02 10.76 ± a	38.85 ± 19.16 b	9.07 ± 6.34 b	7.72 ± 3.85 b	39.95 ± 4.69 a
LSD	24.95	24.95	7.72	4.40	0.80

The different letter denote the significant differences between groups ($P \leq 0.05$)

Effect of sodium nitrite and (MDJ) on liver enzymes

According to (Table: 2), it seems that $NaNO_2$ has a significant effect on AST and ALT value. The AST and ALT values have risen significantly, due to the $NaNO_2$ injection compared with AST and ALT control groups. Offering of MDJ alone to the treated rats reduced the AST value significantly compared with control group. Whereas ALT value of MDJ group rats was not affected by the injection of MDJ compared with the control group. Injection of MDJ after an hour of treated rats with $NaNO_2$, led to significant declined of AST, but still less significantly compared with control group. The administration of MDJ after an hour of injection $NaNO_2$ reduced ALT value significantly, and got it back close to its normal value compared with the control group.

AST and ALT enzymes act as indicators of liver functions. The results showed that AST and ALT was significantly increased ($P<0.05$) compared with controle group following administration of $NaNO_2$ (Table:2), which led to acute hepatocelular damage and extra hepatic obstructions [18]. In this study data showed that MDJ improve liver function and restoration of normal levels of AST enzyme compared to control group, and it was able to get back normal hepatic cell functions. The results came in accordance with those obtained by Mori *et al* [19], who demonstrated that methionine is a protective factor against various types of liver damage

Table 2: Effects of NaNO2 and MDJ on serum alanine transaminase (ALT) and aspartate transaminase (AST)

Parameters / Groups	AST (GOT) U/l	ALT (GPT) U/l
Control DMSO 0.5ml	220.33 ± 8.38 b	31.73 ± 8.64 b
NaNO2 120mg/kg	323.0 ± 51.56 a	50.4 ± 8.37 a
MDJ 48mg/kg	173.75 ± 55.75 c	42.47 ± 14.32 b
NaNO2 + MDJ	126.85 ± 49.85 d	25.1 ± 4.96 b
LSD	46.5	18.37

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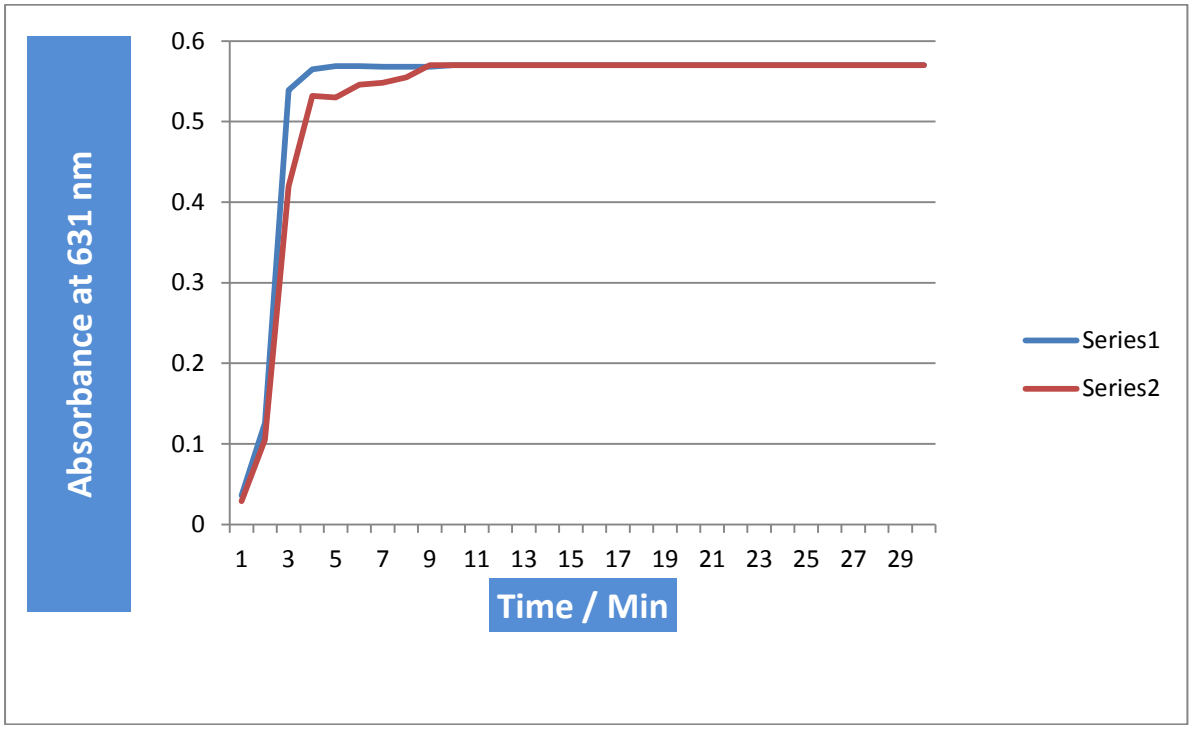

Figure 2: Antioxidant activity *in vitro* of synthesized compound (MDJ) at 5 µM

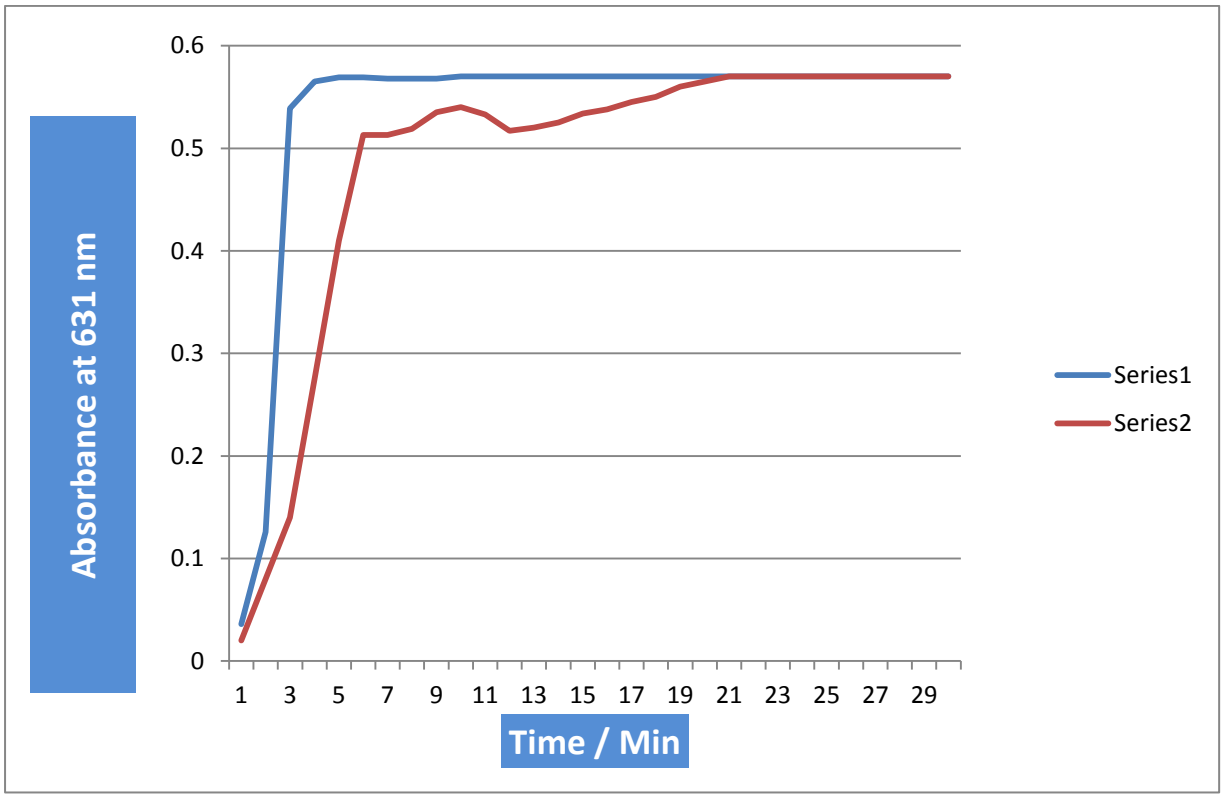

Figure 3: Antioxidant activity *in vitro* of synthesized compound (MDJ) at 10 µM

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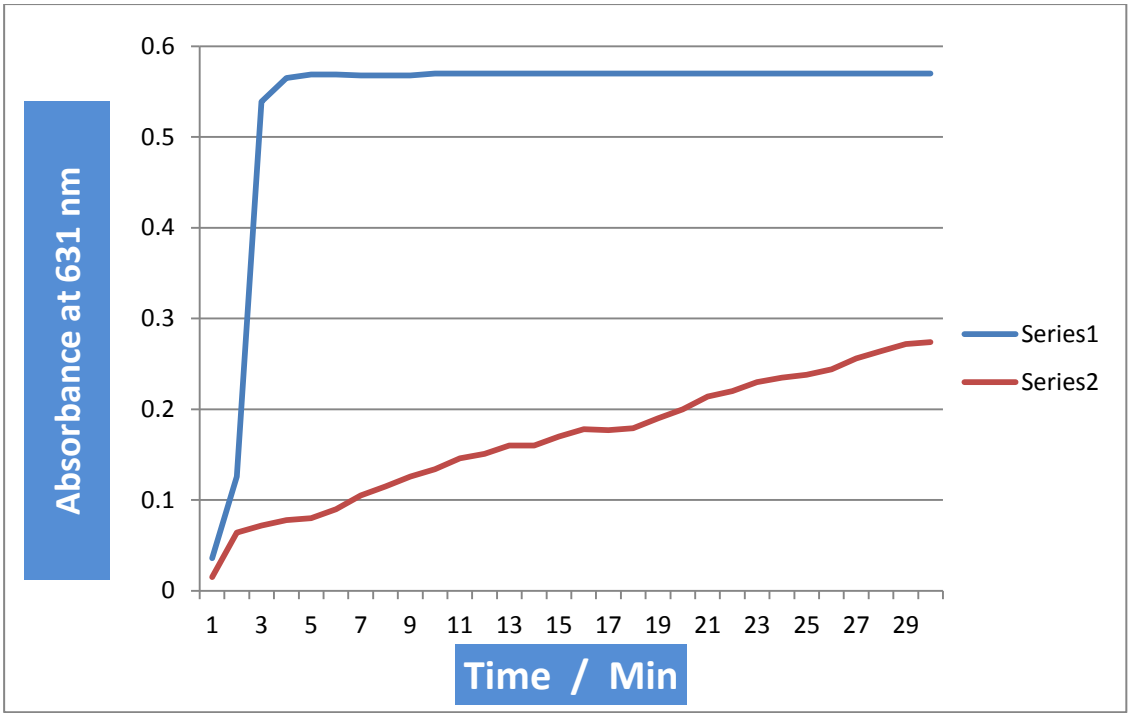

Figure 4: Antioxidant activity *in vitro* of synthesized compound (MDJ) at 20 µM

CONCLUSION

Due to the hazardous effect of food additives as sodium nitrite, it is recommended that the use of sodium nitrite as food additives must be limited and Schiff base derived from methionine has the antoxidant ability to prevent its toxic effect.

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