HEPATOPROTECTIVE AND HYPOLIPIDEMIC EFFECTS OF BIS [4-(4'-HYDROXY-3'-METHOXYBEZYLIDINEAMINOPHENYL)]TELLURIDE (R_2TE) AGAINST SODIUM NITRITE INTOXICATION IN MALE ALBINO RATS.

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ABSTRACT

Sodium nitrite is widely used as a color fixative and preservative in meat and fish. Impairment of hepatic function and disturbances in lipid metabolism are well recognized adverse effects of sodium nitrite. The aim of this study is to investigate the role of bis [4-(4'-hydroxy-3'-methoxybezylidineaminophenyl)]telluride, a novel compound, in preventing the hepatic damage and disturbances of lipid metabolism induced by sodium nitrite toxicity in male albino rats. The estimated LD_{50} of [4-(4'-hydroxy-3'-methoxybezylidineaminophenyl)]telluride in adult male albino rats is 218.7 mg/kg body weight. Rats given sodium nitrite (0.2%) in the drinking water showed a significant increase in serum ALT, AST, ALP, Total cholesterol, TG. LDL and VLDL while HDL significantly reduced. These changes are reversed by administration of bis [4-(4'-hydroxy-3'-methoxybezylidineaminophenyl)]telluride in a dose of 11mg/kg body weight corresponding to 1/20 LD_{50}. It is concluded that bis [4-(4'-hydroxy-3'-methoxybezylidineaminophenyl)]telluride is effective in preventing hepatic damage and dislipidemia in sodium nitrite intoxicated male rats.

INTRODUCTION

Sodium nitrite ($NaNO_2$) is inorganic salt with wide spread applications in food industry as color fixative and preservative in meat and fish (1) in medicine as antidote for cyanide poisoning (2). The major metabolites of $NaNO_2$ are nitric oxide and nitrosamine (3). The later is highly carcinogenic and associated with high risk of stomach; liver and esophageal carcinomas (4). A moderate and significant acceleration of leukemia development was observed in sodium nitrite treated mice (5). Sodium nitrite in blood is highly reactive with hemoglobin, thus affecting hematopoiesis. A major concern considering the toxicology of $NaNO_2$ is the induction of methemoglobinemia, a condition in which there is a reduction in oxygen transport ability of hemoglobin (6). It has been well recognized that sodium nitrite administration in rats leads to impairment of hepatic function (7, 8) and disturbances in lipid metabolism (9). Various medicinal plants and vitamins protect liver from damage induced by sodium nitrite administration in rats (10, 11). Moreover, some organotellurium compounds also possess hepato-protective effect which could be attributed to their antioxidant activity (12). The present study was designed to investigate the role of bis[4-(4'-hydroxy-3'-methoxybezylidineaminophenyl)]telluride in preventing the hepatic damage and disturbances in lipid metabolism induced by sodium nitrite toxicity in male albino rats.

MATERIALS AND METHOD

Chemicals:

Sodium nitrite **(**$NaNO_2$) was applied as a freshly prepared solution and given in a dose of 0.2% (2 g/L) in the drinking water. R_2Te, organo-tellurium compound, bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride was synthesized and characterized according to the method described by Kadhum *et al.* (13). Bis[4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride was administered as suspension in corn oil by gavages at a dose of 11mg/kg body weight corresponding to 1/20 of its LD_{50} (14, 15).

Experimental animals:

One hundred and two adult male albino rats were used in the study, seventy animal weighted (250±25g) are used to determine LD_{50} of bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride and thirty two weighted (300±25g) are used to study its hepatoprotective effect in $NaNO_2$ intoxicated rats. Rats were kept under standard environmental conditions at temperature 24-28°C and 12 hr photoperiod. They were acclimatized for 2 weeks before the start of the experiment and housed in polyethylene cages with wire mesh, 5 rats per cage. They fed standard rat pellet and fresh clean water was provided *at libitum.*

LD_{50} experiment:

LD_{50} of bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride was determined according to the method of Miller and Tainter (16). After a pilot study in which a small number of animals (2 each dose) are used in order to determine the range of doses used to estimate the LD_{50}, the rats divided into 7 groups (10 rats in each). Rats in the control group administered 0.5ml corn oil orally by gavage, whereas those in groups 1, 2, 3, 4, 5 and 6 were administered 100, 175, 250, 325, 400, and 500 mg /kg body weight of bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride) in 0.5ml corn oil respectively. Food was removed from animal's cage 4 hours before oral dosing. The animals were observed daily for a period of 72 hours for acute toxicity signs. After 72 hours, the number of deceased rats was counted in each group and percentage of animals that had died at each dose level was transformed to probits (17). The percentage dead for 0 and 100 are corrected before determination of probits as following: for 0% dead=100 (0.25/n), and for 100% dead=100(n-0.25/n). Where (n) is number of animal in each group**.** The probit values are plotted against log- doses and the dose corresponding to probit 5, i,e., 50% is found out (18).

Experimental design:

Rats are divided into 4 equal groups (8 rats in each group) as following:

1. Control group: rats were administered orally 0.2ml corn oil by gavage daily.
2. $NaNO_2$ group: rats were given 0.2% $NaNO_2$ in the drinking water and administered orally 0.2 ml corn oil by gavage daily.

3. Treated group: rats given 0.2% $NaNO_2$ in the drinking water and administered 11mg/kg body weight (1/20 LD_{50}) of bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride orally as suspension in 0.2ml corn oil daily.
4. R_2Te group: rats administered 11mg/kg body weight of bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride orally as suspension in 0.2ml corn oil daily.

At the end of the experimental period (1 month), the rats were sacrificed under light chloroform anesthesia; a 'Y' shaped cut in the rat abdomen was done. Blood were collected from posterior vena cava as it enters the right ventricle (19); then transferred into plain tubes and centrifuged at (3000 rpm for 15 minute) to obtain the serum which stored at -4ºC till used for measurement of different parameters.

Biochemical tests:

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are determined by colorimetric method (20). Serum alkaline phosphatase (ALP) was determined also by colorimetric method (21, 22). Total cholesterol estimated by enzymatic method (23). Triglyceride (TG) was measured according to the method described by Fossati and Prencipe (24), associated with Trainder reaction (25). HDL-cholesterol obtained in supernatant after precipitation of LDL, VLDL, and chylomicrons from specimens by phosphotungstic acid (PTA) and magnesium chloride was measured with total cholesterol reagent (26). Serum very low density lipoprotein (VLDL) was calculated by dividing serum triglyceride by five (27), where as serum LDL was calculated according to Friedewald formula (27): LDL-C = TC – HDL-C – TG/5

Statistical analysis:

Statistical analysis was performed by a one-way ANOVA (followed by LSD test). Data were expressed as mean ± SDM. Statistical significance was set at $P \leq 0.05$.

RESULTS

The results of LD_{50} determination showed 0 (0%), 4 (40%), 6 (60%), 8 (80%), 9 (90%) and 10 (100%) deaths in groups 1, 2, 3, 4, 5 and 6 respectively within a period of 72 hours post-administration of bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride and no mortality recorded in control group (Table:1). The log dose corresponding to probit 5, i.e., 50% is found out from the Figure (1) and it was 2.34 and the LD_{50} which is equal to the antilog of 2.34 is 218.7 mg/kg body weight.

The obtained results in Table (2) revealed that administration of sodium nitrite was associated with significant increase in serum level of ALT, AST and ALP when compared with the control group. Elevated serum ALT, AST and ALP enzymes are reduced and they became insignificantly different from the control by treatment with bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride. Serum ALT, AST and ALP were not affected by separate administration of bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride.

The results in Table (3) revealed that HDL-cholesterol was significantly decreased while total cholesterol, TG, LDL, and VLDL significantly increased in $NaNO_2$ group compared with control group. These changes in lipid profile are reversed and their values became close to values in control group by administration of bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride. Lipid profile were not affected by separate administration of bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride.

Table (1) The results of the lethal doses of (R_2Te) for the determination of LD_{50} in rats after oral administration.

Group	Dose R_2Te(mg/kg)	Log dose	Total No.	No. death	% mortality	*Corrected % mortality	Probit units
Control	0	-	10	0	0	0	0
1	100	2	10	0	0	2.5	3.04
2	175	2.243	10	4	40	40	4.75
3	250	2.397	10	6	60	60	5.25
4	325	2.51	10	8	80	80	5.84
5	400	2.6	10	9	90	90	6.28
6	500	2.698	10	10	100	97.5	6.96

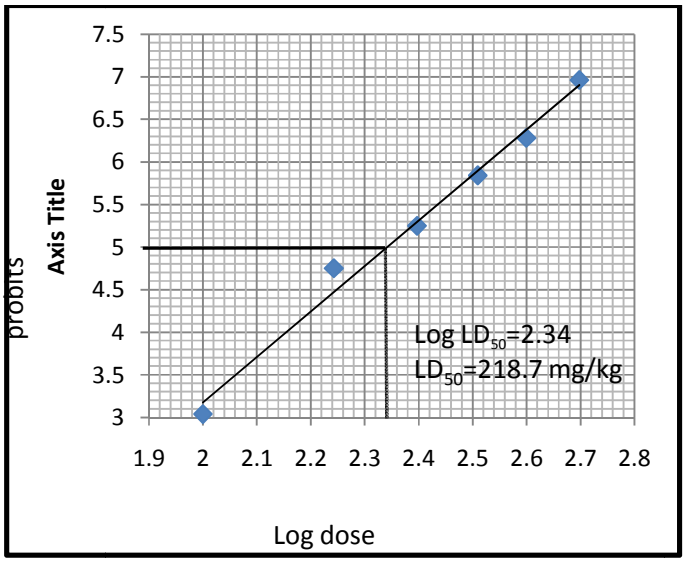

Figure (1) plot of log doses versus probits from Table (1) for calculation of oral LD_{50} of R_2Te in rats.

Table (2) Effects of $NaNo_2$ and R_2Te on serum ALT, AST and ALP enzymes (Means ± SD)

Enzyme / Group	ALT u/L	AST u/L	ALK IU/L
Control group	8.5±1.2 b	17.63±1.77 b	55.68±6.29 b
$NaNO_2$ group	17.13±0.99 a	41.5±4.6 a	97.94±14.86 a
Treated group	9.5±.177 b	19.38±1.77 b	51.77±6.6 b
R_2Te group	9.25±1.83 b	20.75±4.03 b	57.71±15.97 b
LSD	2.06	4.63	40.22

Different small letters represent significant difference at (P≤0.05).

Table (3) Effects of $NaNo_2$ and R_2Te on lipid profile (Means ± SD).

Group	Total CHOL mg/dL	Triglyceride mg/dL	HDL mg/dL	VLDL mg/dL	LDL mg/dL
Control group	59.13±6.13 b	78.5±9.68 b	57.25±1.98 a	15.73±1.94 b	19.6±3.48 b
$NaNO_2$ group	73.25±2.49 a	98.38±6.02 a	41.75±2.43 b	19.68±1.2 a	41.82±3.35 a
Treated group	60±3.07 b	77.5±15.35 b	57.38±3.29 a	15.5±3.07 b	17.16±3.07 b
R_2Te group	61.5±4.54 b	83.83±7.44 b	54.88±2.95 a	16.68±1.49 b	19.95±6.44 b
LSD	11.75	12.5	13.125	3	21.8

Different small letters represent significant difference at (P≤0.05).

DISCUSSION

Bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride is a novel compound and no data available regarding its toxicity, therefore the experiment focused to determine its acute toxicity by measuring its LD_{50} in adult's male albino rats. There are

a number of methods used for LD_{50} determinations. The simpler ones are not very precise and often do not provide adequate information. In this study, acute toxicity study was carried out by measuring the median lethal dose (LD_{50}) utilizing the graphical method (18, 28). Although this method requires large number of animals but it's less time consuming and gives more accurate result with least degree error and does not require complex calculations (29). Lack of death of rats in control group showed that there was no factor that caused death in the test animals other than the tested compound. According to Hodge and Sterner scale (30) and WHO scale (31), the estimated value of 218.7 mg/kg body weight of R_2Te as the LD_{50} in our study rated the compound moderately toxic.

Data in the present study revealed that serum liver enzymes (ALT, AST and ALP) increased in rats administered sodium nitrite compared with control (Table: 2). The present results are in agreement with the results of previous studies (7, 32, 33, and 34). Impairment of hepatic function has been recognized in rats administered sodium nitrite (7 and 8). The high levels of AST and ALT in serum are usually indicative of liver damage in animals (35). Orally administered sodium nitrite reaches the liver through the portal vein and may cause destructive changes in hepatic cells leading to the release of the enzymes from the cytoplasm to the circulation after rupture of the plasma membrane and cellular damage (36). The increase serum level of AST, ALT and ALP enzymes in sodium nitrite treated rats could be attributed to hepatic necrosis induced by the toxic effect of nitroso-compounds, formed in the acidic environment of the stomach (37). It is known that these enzymes are mainly found on the liver in high concentration, the high values of the activities of serum transaminases, alkaline phosphatases and γ-GT of nitrite treated rats relative to control values are indicative of severe intra-hepatic cell damage due to the compound administered (33). Data in the present study revealed that bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride reduces elevated liver enzymes in sodium nitrite intoxicated rats, this could be due to cell membrane stabilizing and hepato-protective activity against free radical-mediated liver cell toxicity by attenuation of sodium nitrite induced oxidative stress. Other organotellurium compounds like DPTVP (diethyl-2-phenyl-2-tellurophenyl vinylphosphonate) also have been found to posse's antioxidant and hepatoprotective activity (12).

Concerning lipid metabolism, the results demonstrated that total cholesterol, triglycerides, VLDL, and LDL were increased while HDL was decreased in response to sodium nitrite administration to the rats (Table: 3). The results of this study are in agreement with the results of Sidoriak and Volgin (9). Hypercholesteremia,

hypertriglyceridemia and reduction in HDL-C are well recognized consequences of sodium nitrite intoxications in rats (32, 38, 39, 40 and 41). Nitrite induced hypercholesterolemia may be attributed to peroxidation of cell membrane lipids and mobilization of free fatty acids from the adipose tissue to the blood stream and increase level of acetyl CoA, leading to increase in the synthesis of cholesterol (42). It has been found that feeding graded amounts of nitrites to rats resulted in hypothyroidism caused by competitive inhibition of iodide transport by nitrite and manifested by altered metabolism of thyroid hormones as indicated by decreased serum concentrations of free T4 and increased serum concentrations of TSH (41). Moreover, hypertriglyceridemia and elevated TC/HDL-C ratio have been reported in middle-aged women with subclinical hypothyroidism (43). This effect could be related to a reduced removal rate of triacyglycerols from plasma in hypothyroidism (44). Thyroid hormone induces increased numbers of LDL receptors on the liver cells, leading to rapid removal of LDL from the plasma by the liver and subsequent secretion of cholesterol in these lipoproteins by the liver cells, consequently decreased thyroid secretion greatly increases the plasma concentrations of cholesterol, phospholipids, and triglycerides and almost always causes excessive deposition of fat in the liver as well (45, 44). The organo-tellurium compound, 1-buthyltelurenyl-2-methylthioheptene, induced a significant reduction in serum triglyceride in rats (46). The changes in lipid profile in this study may be attributed to peroxidation of cell membrane lipids and mobilization of free fatty acids from the adipose tissue to the blood stream resulted from nitrite induced oxidative stress and free radical generation. The nitrite induced changes in lipid profile were ameliorated when rats treated by bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride, this hypolipemic effect of bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride could be related to its antioxidant and free radical scavenging activity.

Conclusion:

Although bis [4-(4'-hydroxy-3'-methoxybenzylideneaminophenyl)]telluride is moderately toxic compound, it did not affect the liver adversely when administered in a dose of 1/20 of its LD_{50}. Moreover, it protect the liver from damage induced $NaNO_2$ and modulated the changes in lipid profile caused by sodium nitrite administration**.**

التاثيرات الواقية للكبد والخافضة لدهون الدم لمركب بس ٤-(٤´-هايدروكسي-٣َ- ميثوكسي بنزلدين امينوفينايل) تلوراید في ذكور الجرذان البيضاء المسممة بنايترايت الصوديوم.

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الخلاصة

يستخدم نايترايت الصوديوم على نطاق واسع كمثبت للون وكمادة حافظة للحوم والاسماك. تدهور وظائف الكبد واضطرابات التمثيل الغذائي للدهون من الاثار السلبية المعروفة جيدا لهذه المادة. تهدف الدراسة الى التحقق من دور مركب بس ٤-(٤´-هايدروكسي-٣َ- ميثوكسي بنزلدين امينوفينايل) تلوراید في منع تلف الكبد والاضطرابات في تأييض الدهون الناجمة عن سمية نايترايت الصوديوم في ذكور الجرذان البيضاء. ان الجرعة القاتلة للنصف قيست في ذكور الجرذان البيضاء وكانت ٢١٨,٩ملغم/ كغم من وزن الجسم. وأظهرت الفئران التي أعطيت ٠,٢٪ نايترايت الصوديوم في مياه الشرب زيادة معنوية بانزيمات الكبد والكوليسترول والدهون الثلاثية ولايبوبروتين واطئ الكثافة واللايبوبروتين واطئ الكثافة جدا بينما الليبروبروتين عالي الكثافة قل معنويا. هذه التغيرات عكست بتجريع مادة بس ٤-(٤´-هايدروكسي-٣َ- ميثوكسي بنزلدين امينوفينايل) تلوراید بجرعة ١١ ملغم/ كغم من وزن الجسم واللتي تعادل ٢٠/١من الجرعة القاتلة للنصف. يستنتج من الدراسة بان المركب الجديد بس ٤-(٤´-هايدروكسي-٣َ- ميثوكسي بنزلدين امينوفينايل) تلوراید له القابلية على حماية الكبد وخفض الدهون في ذكور الجرذان البيضاء المسممة بنايترايت الصوديوم.

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