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Research Article

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Taurine as a Drug for Protection of Liver and Kidney against Toxicity of Dinitrotoluene on Male Rats (Applicable Study)

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ABSTRACT

Technical grade dinitrotoluene (tg-DNT) [CH3C6H3 (NO2)2] nitroaromatic agents which are manufactured in the industries and applied in both commercial and military in all over the world and Egypt. DNT causes malfunctions in kidneys, heart, liver, testes and mammary glands in animals and human beings, which may be considered as carcinogenic in experimental animals and human. Moreover, taurine, a free β-amino acid with remarkable antioxidant activity, has important beneficial effects on the human body; hepatoprotection, nephroprotection, cardiovascular protection, hypoglycemic impact and hypolipidemicaction. Currently, taurine level in the serum is used as early marker of breast, endometrial and colon cancers. The current research was aimed to explore the potential impacts of the antioxidant properties of taurine as a protecting material on tg-DNT induced toxicity in the liver and kidney in male rats. 100 apparently healthy male rats in 4 groups were included; the first is Frank control group, mouth feeding via gavage with distilled water; the other groups were administered as following, taurine alone, tg-DNT alone (toxic group), taurine + tg-DNT (protective group) in the second, third and fourth groups, respectively. In these groups, blood biochemistry and taurine concentrations in serum were measured for all animals. Furthermore, histopathological examination studies for liver and kidney were done for all groups. The results showed that, the protective group has marked improvement in most biochemical parameters than the toxic group. Histological studies revealed a significant marked disturbance in the histopathological architectures of the kidney and liver in all toxic rats. However, marked improvements in histological architectures were observed in protective group. The results support the ameliorative effect of taurine as a protective agent against tg-DNT toxicity in experimental rats.

Key words: *Technical Grade Dinitrotoluene (Tg-DNT), Hepatoprotection, Nephroprotection, And Antioxidant Taurine.*

INTRODUCTION

Dinitrotoluenes (DNT) are nitroaromatic elements that are created industrially and discharged into the environment as a consequence of mixing toluene with mixed acids (sulfuric &nitric acids). DNTs exist as a combination of 2 to 6 isomers in the form of pale-yellow crystales [1, 2], tg-DNT consists of about 20% 2, 6-DNT isomer, 75% 2, 4-DNT isomer and 5% of the further DNT isomers. In the industries dinitrotoluenes are manufactured for many purposes, in ammunitions as smokeless propellant powder, in the preparation of dyes, gelatinizing and in manufacturing of plasticizing materials in mutually marketable and military explosive structures [3], in the production of polyurethane during the processes of synthesis of the organic intermediate toluene diamine, and used in the air bags of automobiles [4]. DNT can reach the human or animals through different routes: ingestion, inhalation, and skin or eye contact, and can be absorbed from the surface of skin during manufacturing, transporting and using [5, 6].Some authors reported that liver is the main target organ of toxicity of some compounds [7].

Hepatotoxic effects have consistently been demonstrated in dogs, mice, and rats administered with oral DNT. The carcinogenicity of 2, 6-DNT has been confirmed based on hepatic tumor initiation-promotion experiments [6]. So that tg-DNT is tumor-promoting, and 2, 6-DNT is a potent hepatocarcinogen and has the principal part in tg-DNT in inducing cancers and their activities [8].

The present work was carried out on 14 patients diagnosed as renal cancers and 6 patients with urothelial cancer from a total of 3000 workers which were working in mines had used tg-DNT for induction of explosions in the mountains [9]. Another survey was carried out in the copper-mining industry of the German Democratic Republic during the period from 1984 to 1997. 14 patients of renal cell cancer and six patients of urothelial cancer were found in a group of 500 underground mining workers working at environment exposed to high rate of explosions by using technical dinitrotoluene in explosions [10].

Taurine, are found normally in all animal tissues in most species of animals. The taurine can be synthesized from cysteine or methionine principally in the hepatic tissues, or it can be supplied from external food sources, where the taurine is found with high concentrations in the white blood cells, central nervous system and skeletal & the heart muscles. Taurine has important beneficial effects on the human body. With respect to the beneficial effects of taurine it was found that taurine has been used in protection against many diseases such as protection of hepatic tissues from injuries [11, 12], nephroprotection [13], cardio protection [14], hypoglycemiceffect [15], hypolipidemic effect [16]. Moreover, medicinally, it is used in the treatment of anemia due to iron-deficiency [17]. Currently, the level of taurine in the serum can be used as indicator for detection of colon, breast and endometrial cancers [18-20].

However, this study was aimed to explore the impacts of taurine on physiological and pathological disorders induced by tg-DNT in male rats to study the probable protective effect against highly toxic effects of tg-DNT compound.

MATERIALS AND METHODS

Animals and Protocol

100 healthy male albino rats (Rattusrattus), 4-5 weeks old with body weight between 100-120 gm. were randomly selected from a large population of animals. The animals were fed on a standard laboratory rodent diet and fresh tap water. The male rats were classified into four groups as follow:

- **Group 1- control group: 20** rats were gavaged with distilled water all through the experiment period.
- **Group 2- taurine group: 20** rats were daily gavaged with 500 mg/Kg B.wt. taurine dissolve in water during experiment period.
- **Group 3- toxic group: 30** rats were daily gavaged (35 mg/Kg B.wt.) with tg-DNT dissolve in corn oil during experiment period.
- **Group 4- protective group: 30** rats were treated with taurine in the doses as in taurine group (500mg/Kg B. wt) two weeks befor experiment, and were treated with tg-DNT in the doses as toxic group (35 mg/Kg B.wt.) during experiment period.

The doses of tg-DNT and taurine were selected according to previous studies, [21, 22]. The duration of the experiment was selected as 52 weeks to cover all expected deterioration in liver, kidney, and cardiovascular system, which were documented in previous studies [8-10].

Chemicals

Technical Dinitrotoluene (tg-DNT) was obtained from Abu-Zaabal Company for Specialized chemicals (M.F. 18) (Egypt). Taurine ≥ 99% and 1-Fluoro-2,4-dinitrobenzene ≥ 99% (DNFB) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Acetonitrile, methanol and deionized water all HPLC grade purchase from Merck-Millipore (Merck KGaA, Darmstadt, Germany) all other chemicals and reagents were of analytical grade purchase from El-Gomhouria CO. (Cairo, Egypt).

Collection of samples

All rats were sacrificed under light chloroform anesthesia. Blood samples were drawn, in clean and sterile serum tubes. Blood samples were centrifuged for 10 minutes, at 3000 r.p.m. within an hour of the blood collection and the sera were obtained. Sera were divided into small fractions and kept in deep freezer under -20 ˚C for analysis of taurine concentration in serum, while biochemical parameters have been done before freezing for all animals [23, 24].

Biochemical analysis and taurine determination

Serum samples were analyzed with biochemical analyzer (Humalyzer 3000) according to the manufacturer's guidelines. Urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin and creatinine phosphokinase (CPK) were analyzed. High performance liquid chromatography (HPLC) with precolumn derivatization were used for determination of taurine [25].

Histopathological Studies

Necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was sacrificed at the end of stage or experiment. Autopsy samples were taken from the liver and kidney of rats in different groups and fixed in 10% saline and, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination [26].

Statistical Analysis

After confirmation of normal distribution for all variables, the significance of differences was evaluated by paired t-test or analysis of variance (ANOVA). Relationships between variables were analyzed by simple correlation analysis. Data are expressed as mean ±SD, and a value of $P<0.05$ was the criterion for statistical significance. All statistical analyses were performed using SPSS computer program.

RESULTS

Liver enzymes (Biochemica Liver Profile) and histopathology

Tables (1a& b), clearly showed that all serum concentration of liver enzymes ALT, AST, ALP, and bilirubin, still were at healthy level with insignificant change between control and taurine treated rats along the period of experiment, but there is high to extremely significant elevation in liver enzymes in toxic group (tg-DNT treated rats) than control and taurine alone treated rats, however in protective group these increases were obviously reduced than those documented in toxic rats. As a conclusion, impacts of taurine were observed in protective group at week 13, 26, and 52, and the liver enzymes revealed a significant reduction in their levels paralleled to toxic rats.

Table 1a: Serum levels of ALT&AS

		week 13	week 26	week 52
AST U/L	Control group	68.16±9.65	71.16±11.44	80.26±5.44
	Taurine group	65±15.75 a^{ns}	68±5.8 a^{ns}	75.6±12 a^{ns}
	Toxic group	320±54 a, b***	663±65 a, b***	705±50 a, b***
	Protective group	120+25.5 a, b, c***	130+25.5 a, b***	263+45 a*, b**, c***
ALT U/L	Control group	36.50±4.57	34.50±5.57	40.50±2.53
	Taurine group	30±6.5 a^{ns}	31±16.5 a^{ns}	30.5±4 a^{ns}
	Toxic group	210±69.5 a, b***	566±70.5 a, b***	620±72.5 a, b***
	Protective group	90±23.6 a, b, c***	105±15 a, b**, c***	366±30.5 a*, b**, c***

Table 1b: Serum levels of ALP & total bilirub

		week 13	week 26	week 52
ALP U/L	Control group	90±35	120±50	220±70
	Taurine group	80±11.2 a^{ns}	100±30 a^{ns}	110±40 a^{ns}
	Toxic group	280±75 a, b***	340±125 a, b***	420±130 a, b***
	Protective group	130+65 a, b*, c***	150+110 a, b*, c***	320+43.5 a*, b**, c***
Bili	Control group	0.28±0.02	1.08±0.12	0.98±0.92
	Taurine group	0.19±0.12	0.89±0.02	0.08±0.02

		a^{ns}	a^{ns}	a^{ns}
	Toxic group	2.5±0.8 a, b***	4.5±2.8 a, b***	5±1.2 a, b***
	Protective group	1.5±0.52 a, b*, c***	2.3±1.2 a, b*, c***	2.5±0.58 a, b*, c***

Data are expressed as mean ±SD.

(a): refers to control group, (b): refers to taurine group, (c): refers to toxic group. Where, p value > 0.05, Non-significant (ns), p value 0.01 to 0.05 Significant *, p value 0.001 to 0.01 High significant **, p value 0.0001 to 0.001 Extremely significant ***.

Histopathological findings: Histopathological findings met the biochemical findings, as follow:

At 13-Week (90 days) stage (figure 1 A-D), at control and taurine groups, liver of the rats had normal structure lobules with normal hepatic cords with normal hepatocytes, blood sinus and normal Kupffer and epithelium cells, there is no difference between control and taurine groups, Whereas, in toxic group the hepatotoxic effects of tg-DNT resulted in deterioration of liver tissue forming clear liver fibrosis accompanied with hepatocyte cytomegaly, inflammatory cells infiltration, ,cytoplasmic vacuolization, karyomegaly, bile duct hyperplasia and centrilobular necrosis. But in Protective group liver showed normal structure of hepatic lobules, normal hepatocytes shape and size with hepatic cordes radial arranged, normal kupffer and epethilial cells without abnormality.

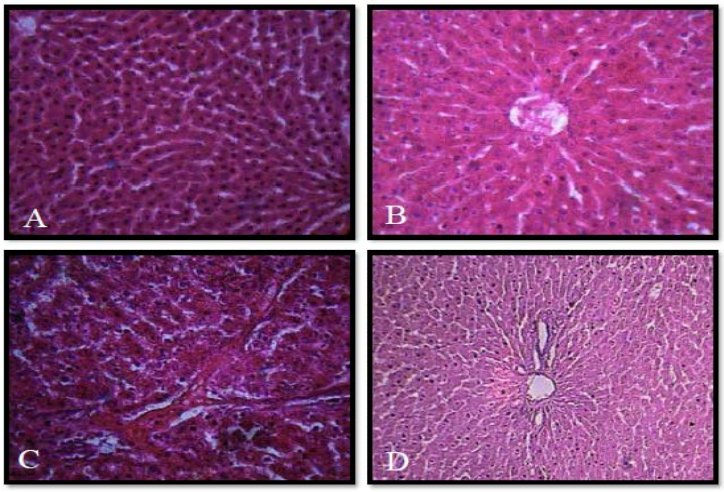

Figure 1: Histomicrograph of liver in 13-week: (A) Control rats with normal organization of hepatic cords with normal hepatocytes. (B) taurine treated rats with normal liver, (C) toxic group with liver fibrosis, inflammatory cells infiltration and congestion of portal vein. (D) protective group with normal hepatocytes (shape and nuclei) with radial expression of hepatic cords.

All slides are stained by Hematoxylin eosin (H&E), original magnification power X200.

But, in 26-Week (180 days) stage (figure 2 A-D), control and taurine groups still revealed normal architecture of the hepatic lobules and hepatic cords radiated from CV, with normal hepatocytes, normal blood sinus and Kupffer cells; there was no necrosis or apoptosis or vacuolation of hepatic cells, whereas, in toxic group rats the liver revealed cirrhotic feature in which fibrosis expands to cover portal area and spreads over hepatic lobules and cords, and hepatic architecture mild to severe disturbance, but the Liver of protective group showed normal organization with mainly normal blood sinusoid, with only few vacuolated cells and rare inflammatory cells around the central vein (CV).

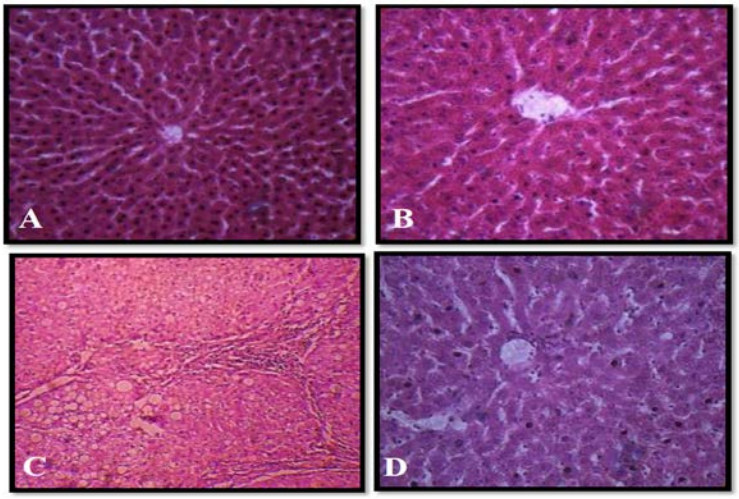

Figure 2: Histomicrograph of liver of 26-week: (A) control group with normal liver architecture with normal hepatic lobules, (B) taurine group with normal healthy liver, C) liver of toxic group shows cirrhotic liver alteration, (D) shows liver of protective group with normal liver organization, few vacuolated cell and one dilated sinus were found.

All slides are stained by Hematoxylin eosin (H&E) original magnification power X20.

Finally, at 52-Week (365 days) stage (figure 3A-D), the histomicrograph of liver of control and taurine groups show the architecture of the hepatic lobes was still intact, hepatic cords radiated from CV, hepatocytes arranged in neat rows with clear cell nuclei. But in toxic group liver showed severe loss of architecture with disorganization of hepatic cords and the thickness of the cord is becoming many cell envelopes in one sheet separated with tissue, hepatocytes pleomorphism with abnormal nuclei and cytoplasm spread over tissue, nuclear pleomorphism and hyperchromasia are found, epithelium karyomegaly, expands of sinus with bioplastic cells separate between hyperplastic hepatocytes, clear mixed acinar and trabecular carcinoma is found. Whereas, in protective group liver retains not more than liver fibrosis stage with inflammation, (fibrosis in portal area may expands to inter lobular) and some fatty changes, necrosis or apoptosis in hepatic cells appear in solitary cells, no other deterioration is found.

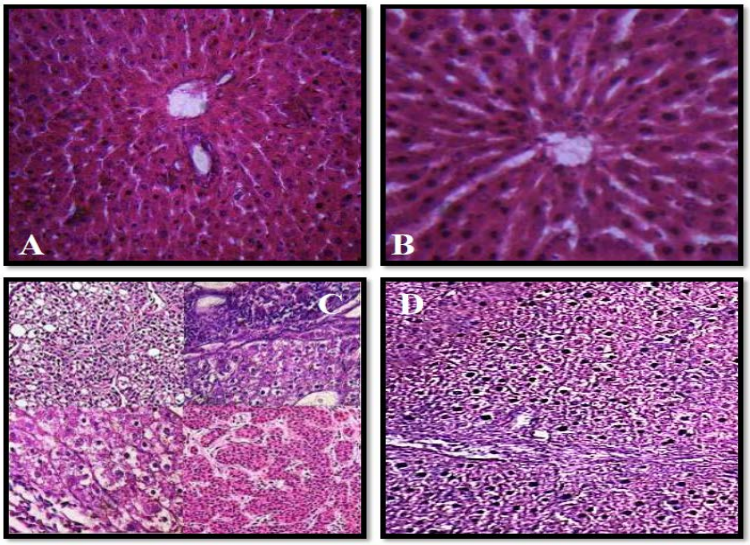

Figure 3: Histomicrograph of the liver of 52-week: (A)-control group shows normal liver architecture with normal hepatic cords, few vacuolated cells are found, (B) liver histomicrograph of 52-week taurine group shows normal liver architecture with normal hepatic cords, no abnormality are found (C)-histomicrograph of the liver of toxic group shows, hepatocytes polymorphism, many cords envelopes in one sheet separated with fibrous tissue, mixed acinar and trabecular carcinoma, (D)-Liver of the protective group shows fibrotic and dilated portal area expands to interlobular, Dilation of some sinus, vacuolated cytoplasm, inflammation and necrosis of few hepatocytes.

All slides are stained by Hematoxylin and eosin (H&E), original magnification power X 200.

Renal Profil and Histopathological examination of the kidney:

As shown in table (2), Serum concentrations of urea and creatininein control and taurine groups run healthy with in-significant differences between them along the study period, but it was highly elevated to extremely significant in toxic group (tg- DNT treated rats) as compared to control and taurine treated group. However, serum concentrations of creatinine and urea revealed significantly clear improvement in protective rats than that toxic rats.

Table 2: Serum concentrations of both urea and creatinine.

		week 13	week 26	week 52
S. Urea mg/dl	Control group	36±21.2	39±5.7	40±5.7
	Taurine group	20.9±11.2 a-^{ns}	37.9±15.3 a^{ns}	35±11.7 a^{ns}
	Toxic group	375±120 a, b***	495±106 a, b***	685±150 a, b**
	Protective group	200+60 a, b**, c***	290+102 a, b, c***	320+96 a, b, c***
S. Creatinine mg/dl	Control group	0.64±0.2	0.68±0.17	0.91±0.2
	Taurine group	0.56±0.18 a^{ns}	0.86±0.21 a^{ns}	0.51±0.3 a^{ns}
	Toxic group	2.26±0.8 a, b***	3.16±1.8 a, b***	8.3±2.2 a, b***
	Protective group	1.01±1.2 a, b^{ns}, c**	2±1.02 a, b**, c***	4.16±1.8 a, b, c***

Data are expressed as mean ±SD.

a: refers to control group, b: refers to taurine group, c: refers to toxic group.

p value > 0.05 Non-significant ns, p value 0.01 to 0.05 Significant *, p value 0.001 to 0.01 High significant **, p value 0.0001 to 0.001 Extremely significant ***.

All findings of the present investigation are in the same line with histological findings as mentioned later in week (13) (90 days) stage (figure 4 A-D). In control and taurine groups histological slides of the control and taurine rats revealed: normal kidney architecture, normal glomerulus which bounded by the Bowman's capsule, devoid of any inflammation in proximal or distal convoluted tubules or pathological alterations, in toxic rats kidney is showing inflammatory infiltration in interstitiumand variable degree of fibrosis with dilated and atrophic of some renal tubules, thickening of glomerulus wall with some glomerulus – amyloidal changes, degeneration of the scattered glomeruli; this findings are not present in protective group as follow: the kidney of rat's protective group showed glomeruli of normal size and shape, normal capillary Lumina and Bowman's space, tubules are normal and no detected pathology.

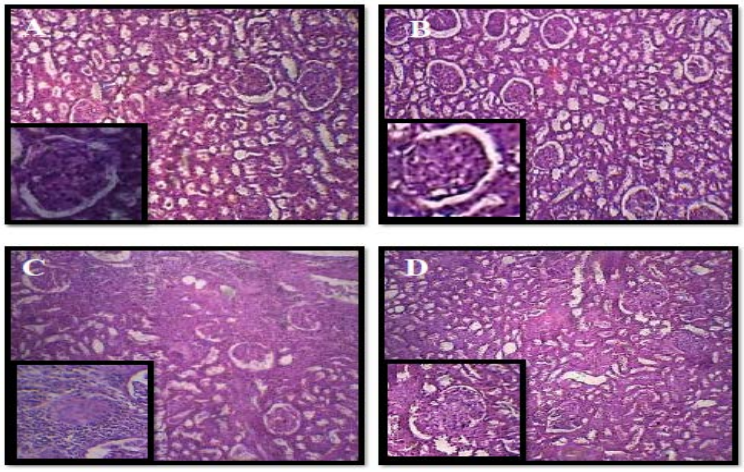

Figure 4: Histomicrograph of the kidney of 13-week: A) control group with normal kidney and normal renal architecture, (B) taurine group normal kidney with normal renal architecture, (C) kidney of toxic group rat showed inflammation, fibrosis and amyloid changes, (D) kidney of protective group rat showed normal kidney feature.

All slides are stained with Hematoxylin and eosin (H&E) original magnification power X 200.
Whereas, in 26-Week (180 days) stage (figure5 A-D), histomicrograph of the kidney of control and taurine groups showed normal kidney architecture with normal glomerular histology, and glomeruli are of normal shape and size. Whereas, in toxic group kidney shows atrophic renal tubules with chronic inflammatory cells infiltrate and hemorrhage in fibrous stroma; hyaline accumulation was recruit in glomeruli of the kidneys and the epithelial tubules. But, in protective group kidneys showed normal renal histology without inflammation or fibrosis changes, few tubules may show mild dilation and scattered glumeruli shrunken of capillary tuft.

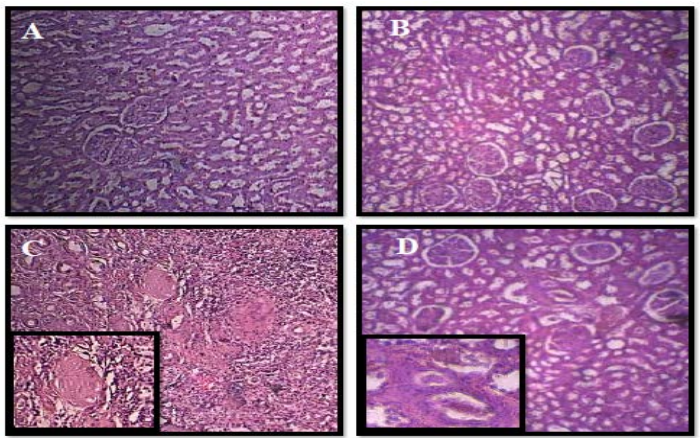

Figure 5: Kidney histomicrograph of 26-week: (A) control group with normal kidney architecture, glomeruli are of normal shape and size, (B) show taurine group with normal kidney architecture, (C) kidney of toxic group shows atrophic renal tubules with chronic inflammation, fibrosis and glomerulosclerosis is found, (D) protective group shows normal feature, fibrosis and inflammation is absent, dialeted and congested few blood vessels can be seen.

All slides are stained with Hematoxylin and eosin (H&E) original magnification power X 200.
Rat kidney at 52-Week (365 days) stage (figure 6 A-D) of control and taurine groups showed glomeruli of normal size and shape, normal capillary lumina and opened bowman's space, tubules are intact, with no casts dilatation or atrophy, and thick walled blood vessels can be seen. Whereas, histomicrograph of kidney of toxic group slides give all feature of renal carcinoma which includes disorganization of renal architecture, and proliferation of cells with pleomorphic nuclei appears clearly (renal carcinoma). But in protective group slide the kidney architecture view is still well expressed, the changes in kidney is limited, fibrosis, inflammatory cell infiltrate, hyaline accumulation in scattered glomeruli with no more pathological deterioration or cell alterations could be observed.

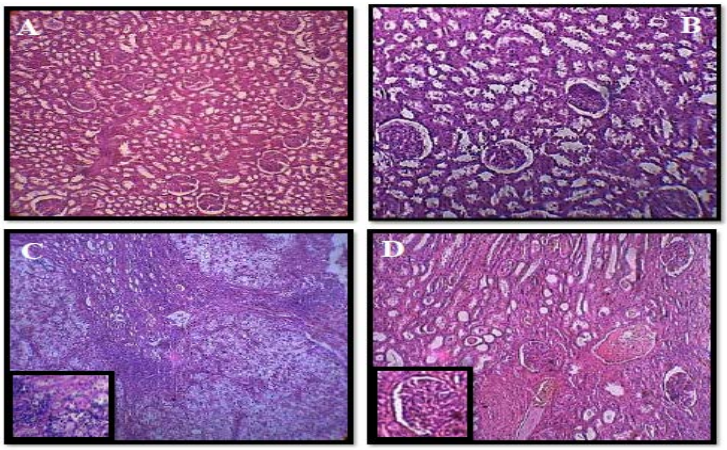

Figure 6: Histomicrograph of kidney at 52-week:(A) Control group with normal kidney, (B) taurine group with normal kidney feature, (C) Toxic group slides show renal cell carcinoma with dis-organization of renal architecture and renal cell pleomorphism, (D) Protective group showed renal architecture well expressed, scattered fibrosis, hyaline changes and inflammation are found, no malignant changes were found.

All slides are stained with Hematoxylin and eosin (H&E) original magnification power X 200

Serum CPK (creatinine phosphokinase) levels.

As shown in table (3) serum concentrations of taurine in taurine group is raised significantly with control group along the study period, but it was extremely depleted in toxic group (tg- DNT treated rat) as compared to control and taurine treated groups.

However, serum concentration of taurine showed significantly noticeable improvement in protective rats than that detected in toxic rats.

As shown in table (4) CPK showed highly significant elevation in tg-DNT treated group (toxic group) than control and taurine groups. However, in protective group were evidently fewer than those verified in toxic rats. Though, impacts of taurine were detected clearly in protective rats at week 26. At week 52 the heart CPK enzyme level showed a moderate significant decrease in protective group than toxic group.

Table 3: Serum Taurine levels

Taurine			
	week 13	week 26	week 52
Control group	70.36±4.21	65.97±8.59	63.47±6.53
Taurine group	79.83±7.91 a**	76.05±6.42 a*	72.98±8.35 a*
Toxic group	40.00±4.65 a, b***	34.55±2.43 a, b***	20.63±2.22 a, b***
Protective group	50.00+6.80 a, b***, c**	45.48+4.78 a, b***, c*	30.76+1.94 a, b***, c*

Table 4: Serum CPK (creatinine phosphokinase) levels

CPK			
	week 13	week 26	week 52
Control group	76.01±17.61	86.91±14.33	179.68±49.44
Taurine group	89.03±28.48 a^{ns}	104.89±11.72 a^{ns}	124.89±37.22 a^{ns}
Toxic group	285.00±103.84 a, b***	736.23±44.34 a, b***	1945.60±142.7 a, b***
Protective group	148.01+41.81 a*, c***, b^{ns}	295.88+45.54 a, b, c***	726.47+50.70 a, b, c***

Data are expressed as mean ±SD.

a: refers to control group, b: refers to taurine group, c: refers to toxic group.

p value > 0.05 Non-significant ns, p value 0.01 to 0.05 Significant *, p value 0.001 to 0.01 High significant **, p value 0.0001 to 0.001 Extremely significant ***.

DISCUSSION

Technical grades of dinitrotoluene (tg- DNT) has many biological hazards, and affects many organs of the body such as heart, liver, kidney, spleen and gonads; briefly tg-DNT increases the mortality from heart diseases, liver and renal dysfunctions. There is a relationship between exposure to DNT and cancer of the liver and biliary. High exposure to DNT might be associated with urothelial tumor formation [10, 27, 28].

Additionally, there is no previous study that determines the exact protective effect of taurine against toxicity of tg-DNT on liver and kidney. Therefore, the objective of the present work was to examine the defending effect of taurine alongside toxic action of tg-DNT on liver, kidney and heart of male rats. The results can then be generalized to human beings to evaluate the possible risks in the human inhabitants owing to exposure to tg-DNT.

The sample size in the present study included 100 male albino rats divided into 4-groups; control group, taurine group, tg-DNT, toxic group and protective group, throughout the 52 week of the experiment; doses of tg-DNT and taurine were carefully chosen depending on previous reports [21, 22]. After 13 and 26 weeks, some animals were sacrificed to obtain blood and tissue samples for histopathological examinations. After 52 weeks, all survived rats were sacrificed to achieve the objectives of this work. Studies were extended to examine liver enzymes, serum ALT, AST, ALP and bilirubin, also, kidney functions (profile), serum urea, creatinine, as well as heart enzyme CPK and taurine values. In addition, liver and kidney tissue samples were taken for microscopic studies and histopathological examinations.

Liver enzymes and the histopathological examinations.

In the present study all data at weeks 13, 26 and 52 showed significant elevation in all serum liver enzymes AST, ALT, ALP and bilirubin in tg-DNT toxic group when compared to control group, that hence leads to severe damage in liver tissues which increases with increasing the period of the experiment. These findings agree with previous studies [29-32] which demonstrated that, serum enzymes ALT and AST are one of the most sensitive indicators of the hepatotoxicity diagnosis. Moreover, according to [29-32] serum AST, ALT, ALP is related to a marked hepatic tissue injury. Furthermore, [33] mentioned that, the increase in the level of enzymes in the serum has been recorded in conditions of tissue injury owing to the use of several chemicals and drugs, as the liver is considered the main center in the body responsible for vital processes such as detoxification and biotransformation of foreign materials and toxins.

Thehistopathological examination: As it was shown in the present study, in liver of control and taurine groups structure lobules, hepatic cords, hepatocytes, blood sinus, Kupffer cells and the epithelium cells are normal, there is insignificant difference between control and taurine groups along the study period, but in toxic group hepatotoxic effects of tg-DNT on rats liver leads to deterioration of liver tissue forming clear liver fibrosis accompanied with hepatocyte cytomegaly and karyomegaly, inflammatory cells infiltration, centrilobular necrosis, cytoplasmic vacuolization, and hyperplasia in the bile ducts. At week-13 this effect is deteriorate to become cirrhotic feature in which fibrosis expands to cover portal area and spreads over hepatic lobules and cords, hepatocytes with abnormal vacuolated cytoplasm, blood sinus expanded with hyperplasia of Kupffer cells, there were necrosis or apoptosis of many cells forming focal lesion. At the end of week-26, hepatic architecture showed mild to severe disturbance, then liver showed severe loss of architecture with disorganization of hepatic cords and the thickness of cord is becoming many cell envelopes in one sheet separated with tissue and hepatocytes.

Pleomorphism with abnormal nuclei and cytoplasm spread over tissues, nuclear pleomorphism and hyperchromasia were found. Epithelium karyomegaly, expands of sinus with bioplastic cells separate between hyperplastic hepatocytes, and clear mixed acinar and trabecular carcinoma was found. However, in protective group, results showed normal liver at week-13, then the Liver showed only few vacuolated cells and rare inflammatory cells around the central vein (CV) at week-26. Whereas, liver retained not more than liver fibrosis stage without any other deterioration at the end of the study at week 52. The data of the present study agree with previous studies which mentioned that these deteriorations, and tumor formation may have been induced by tg-DNT [31, 32, 34]. The histopathological findings in the liver, included hyperbasophilia, megalocytosis of hepatocytes increase in hepatocellular carcinomas, neoplastic nodules, Cholangiocarcinomas, biliary hyperplasia with atypia of the bile duct epithelium, vacuolation and necrosis of hepatocytes is in accordance with the findings of [34].

According to data obtained from the present study we can conclude that oral taurine treatment reduces oxidative stress, increases taurine accumulation, and prevents development of hepatotoxic effects of tg-DNT, and also taurine is known as cytoprotective in different patterns [35], antihypertensive [36], and hepatoprotective [37]. Many investigations reported and confirmed the hepatoprotective effects of taurine in different clinical conditions [38-43].

Taurine was found to protectively attenuates or prevent the hepatic damage, necrosis and fibrosis induced by carbon tetrachloride toxicity [44-48]. Then, the decrease in hepatic tissue impairment hypothetically reduced infiltration of inflammatory cells and platelet agglomeration, and also transformation of myofibroblasts should be reduced, and hepatic cirrhosis or fibrosis should be diminished [48].

Kidney functions (Renal Profile) and histopathological examinations:

Kidney functions were assessed through the measurements of urea and creatinine at 13, 26, and 52 weeks. The level of urea in the circulation was generally elevated significantly along theentire experimental period paralleled to the control group and taurine values. Generally, urea is present in the liver due to degradation of proteins and it is considered as an indicator of renal function [49]. The significant increased in serum urea estimated in the current work may be due to deficiency in its synthesis as a result of reduced liver functions, decrease in protein metabolism, and reduction in its excretion due to low rate of filtration through the kidneys. Frthermore, the creatinine values were significantly raised along the whole period of experiment, and this could be explained through the impairment of renal functions, with damage to functioning nephrons. This comes in agreement with [50-52] who menthioned that, such raise in creatinine levels may indicate a pre-renal problem. They also mentioned that, as the kidneys is impaired for any reason, a rise in blood creatinine level is observed.

However, Serum concentrations of both creatinine and blood urea displayed obvious improvement in protective rats compared to toxic rats as a marker of taurine enhancement of body protection against tg-DNT damage effect on kidney tissues. This is due to, taurine free radical scavenger, action transport modulator, and an osmoregulator which reduce the damage degree of the kidneys [53]. In addition, Bruninget al. [10, 27] concluded that, exposure to DNT might be associated with urothelial tumor formation and signs of subclinical renal damage.

Moreover, renal dysfunctions with diabetes mellitus, aging, and hypertention are inversely liked to body taurine level [54-56**]**. Therefore, addition of taurine could avoid age-linked renal impairment [57]. Moreover, taurine accesses the ability of kidney tissues to overcome toxic effect of tg-DNT, this is because taurine is playing a significant role in regulation of cell volume [58-60]. In addition, taurine can stop programmed cell death (cell apoptosis) via different tools, comprising suppression of the generation of nitric oxide (NO), tumor necrosis factor alpha (TNF-alpha), reactive oxygen species (ROS), and regulation of intracellular calcium flux [61, 62]. Mutagenicity or genotoxicity of tg-DNT has been evaluated in vitro and in vivo test systems. Results from experimental animal studies showed that tg-DNT increased the incidence of multiple tumor types in rats [28]. The histopathologic examinations were in accordance with the results of the biochemical study.

Serum creatinine phosphokinase (CPK):

In the current work, taurine-treated toxicated rats exhibited a significant enhancement in the CPK levels. This indicates that taurine prevents cardiac damage against the toxicity of tg-DNT. The decreases in CPK levels in toxicated rats treated with taurine suggests that taurine reduce the risk of disorders associated with toxicity. This comes in agreement with Howard-Alpeetal [63] who reported that elevation in levels of CPK (circulating cardiac damage marker), denote a potent and sensitive predictor of enlarged cardiac problems.

CONCLUSION

From the previous studies and the current results, we can conclude that taurine can inhibit and minimize the degree of hepatic fibrosis, cirrhosis and tumors. In addition, taurine can protect or ameliorate the liver against hepatotoxic effect of tg-DNT.

Furthermore, can summerise that:

1. This dose of tg-DNT caused severe injury in liver and kidney of rats and taurine ameliorate its effect.
2. Chronic administration of taurine ameliorates the toxic effects of tg-DNT on liver and kidney.

REFERENCES

1. Wiley-VCH, Verlag-Gmbh, and Kgaa, CO. (2002): Ullmann's Encyclopedia of Industrial Chemistry, Nitro Compounds, Aromatic Gerald Booth, 15 JUN 2000
2. Grayson M. (2009): Kirk-Othmer concise encyclopedia of chemical technology. Abridged version, 3rd ed. New York, NY. John Wiley & Sons
3. Etnier, E. (1987): Water Quality Criteria for 2,4-Dinitrotoluene and 2,6-Dinitrotoluene. Final Report ORNL-6312.Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA.
4. ASTDR (2009): Agency for Toxic Substances and Disease Registry, Division of Toxicology and Environmental Medicine, Atlanta, GA 30333.
5. NIOSH. (1994): NIOSH pocket guide to chemical hazards. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 94-116.
6. OSHA. (1994): Computerized information system. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration.
7. Ayaz N.O. (2018). Beneficial impacts of N-3 polyunsaturated fatty acids on gold nanoparticles – induced liver injury by activating PPARγ and NRF2/HO-1 signaling pathway. Pharmacophore, 9(1): 1-9.
8. EPA. (2008): Document Number: 822-R-08-010 January, Drinking Water Health Advisory for 2,4-Dinitrotoluene and 2,6-Dinitrotoluene.

9. Bruning T.; Chronz, C.; Thier, R.; Havelka, J.; Ko, Y.; Bolt, HM. et al. (1998) Technical dinitrotoluene (tg-DNT): a potential carcinogenic and nephrotoxic hazard in humans? Toxicology Letters, Volume 95, Supplement 1, July 1998
10. Bruning, T; Chronz, C; Thier, R; Havelka, J; Ko, Y; Bolt, HM. (1999): Occurrence of urinary tract tumors in miners highly exposed to dinitrotoluene. J Occup Environ Med 41: 144-149.
11. Ghosh, J.; Manna, P.; Sil, PC. (2008): "Taurine provides antioxidant defense against NaF-induced cytotoxicity in murine hepatocytes". Pathophysiology 15 (3): 181–90
12. Shanmugam, Lakshmi Devi, Carani V. Anuradha. (2010): Oxidative and nitrosative stress in experimental rat liver fibrosis: Protective effect of taurine Original Environmental Toxicology and Pharmacology, Volume 29, Issue 2, Pages 104-110.
13. Winiarska K', Szymanski K, Gorniak P, Dudziak M, Bryla J. (2009): "Hypoglycaemic, antioxidative and nephroprotective effects of taurine in alloxan diabetic rabbits". Biochimie, 91(2): 261-270.
14. Militante J, Lombardini J, Schaffer, S. (2000) The role of taurine in the pathogenesis of the cardiomyopathy of insulin-dependent diabetes mellitus. Cardiovasc Res 46: 393 - 402.
15. KatarzynaWiniarska, Konrad Szymanski, PatrykGorniak, Marta Dudziak, Jadwiga Bryla. (2009): Hypoglycaemic, antioxidative and nephroprotective effects of taurine in alloxan diabetic rabbits Biochimie, Volume 91, Issue 2, Pages 261-270
16. Elvevoll EO, Eilertsen KE, Brox J, Dragnes BT, Falkenberg P., Olsen JO., Kirkhus B., Lamglait A., Osterud B. (2008): Seafood diets: Hypolipidemic and antiatherogenic effects of taurine and n-3 fatty acids. Atherosclerosis, 200(2):396-402.
17. Sirdah M. M. 1, El-Agouza I. M. A., Abu Shahla A. N. K. (2002): Possible ameliorative effect of taurine in the treatment of iron-deficiency anaemia in female. University students of Gaza, Palestine
18. Chromik AM1, Daigeler A, Hilgert C, Bulut D, Geisler A, Liu V, Otte JM, Uhl W, Mittelkötter U. (2007): Synergistic effects in apoptosis induction by taurolidine and TRAIL in HCT-15 colon carcinoma cells, J InvestSurg: 20(6):339-48.
19. EL-Agouza and EL-Nashar. (2011): Serum Taurine as a Marker of Endometrial Cancer. The Open Women's Health Journal, 2011, 5, 1-6.
20. El-Agouza, I.; Eissa, S. S.; El-Houssini, M. ML.; El- Nashar, D.E., Abd El-Hameed, O. M. (2011): Taurine: A novel tumor marker for enhanced detection of breast cancer among female patients. Angiogenesis, 14:321-330.
21. Hazleton Laboratories. (1982): 104-week chronic study in rats. Dinitrotoluene. Final report Volume I of II. Submitted to Chemical Industry Institute of Toxicology, Research Triangle Park, NC. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. Document No. 878212041. OTS0205947.
22. Tamamura Ryo, BagciCahit, et al. (2010): Tumor-specific mutation and downregulation of ING5 detected in oral squamous cell carcinoma." International journal of cancer. Journal international du cancer 127 (9): 2088-94. doi:10.1002/ijc.25224.
23. Wintrobe M. (1965): The problems of drug toxicity in man--a view from the hematopoietic system. Ann N Y Acad Sci. Mar 12;123: 316-25.
24. Hepler CD. (1966): A study of direct labor costs in hospital pharmacy systems using the model concept. Am J Hosp Pharm. 1966 Dec;23(12):645-61."
25. Yang Z., Zhang P., Chin. J. (1998): Health Res. 27 (3) 192
26. BancrofetJ, and Stevens A. (1977): Theory and practice of histological techniques. Chuchil L, Ed. Inburgh-London and New York: USA Press.; pp. 98-115.
27. Bruning, T; Thier, R; Mann, H; Melzer, H; Brode, P; Dallner, G; Bolt, HM. (2001). Pathological excretion patterns of urinary proteins in miners highly exposed to dinitrotoluene. J Occup Environ Med 43: 610-615.
28. NTP (National Toxicology Program). (2011). Report on carcinogens: Twelfth edition. Washington, DC: U.S. Department of Health and Human Services. http://ntp.niehs.nih.gov/ntp/roc/twelfth/roc12.pdf
29. Tos-Luty S, Obuchowska-Przebirowska D, Latuszynska J, Tokarska-Rodak M, Haratym-Maj A. (2003) Dermal and oral toxicity of malathion in rats. Ann Agric Environ Med., 10: 101-6.
30. Akhtar N., Srivastava M.K. and Raizada R.B. (2009): Assessment of chlorpyrifos toxicity on certain organs in rat, Rates norvegicus. Journal of Environmental Biology 30 (6): 1047-1053

31. Ambali S.F., Shuaib K., Edeh R., Orieji B.C., Shittu M., Akande M. (2011): Hyperglycemia induced by subchronic co-administration of chlorpyrifos and lead in Wistar rats: Role of pancreatic lipoperoxidation and alleviating effect of vitamin C. Article Biology and Medicine 3 (1): 6-14
32. Newairy A. A. and Abdou H. M. (2013): Effect of propolis consumption on hepatotoxicity and brain damage in male rats exposed to chlorpyrifos. African Journal of Biotechnology 12 (33): 5232-5243.
33. Mansour S.A. and Mossa A.H. (2010): Adverse effects of lactational exposure to chlorpyrifos in suckling rats. Human and Experimental Toxicology 29 (2): 77-92.
34. Bannasch, P; Moore, MA; Klimek, F; Zerban, H. (1982): Biological markers of preneoplastic foci and neoplastic nodules in rodent liver. ToxicolPathol 10: 19-34. http://dx.doi.org/10.1177/019262338201000204
35. Bullock R., Maxwell WL., Graham DI., Teasdale GM., Adams JH. (1991): Glial swelling following human cerebral contusion: an ultrastructural study. J NeurolNeurosurg Psychiatry 54:427–434
36. Dawson R., Lius S., Juny B., Messina S., Eppler B. (2000): Effects of high salt diets and taurine on the development of hyper tension in the stroke-prone spontaneously hypertensive rats. Amino Acids; 19: 643-65.
37. Dogru-Abbasoglu S, Kanbagli O, Balkan J, Cevikbas U, Aykac-Toker G, Uysal M (2001): The protective effect of taurine against thioacetamide hepatotoxicity of rats. Hum ExpToxicol 20:23–27
38. Park T and Lee K, Um Y. (1998): Dietary taurine supplementation reduces plasma and liver cholesterol and triglyceride concentrations in rats fed a high-cholesterol diet. Nutrition Research. 18(9):1559-1571.
39. Yokogoshi H. and Oda H. (2002): Dietary taurine enhances cholesterol degradation and reduces serum and liver cholesterol concentrations in rats fed a high-cholesterol diet. Amino Acids 23(4): 433–439
40. Chen, Y. X.; Zhang, X. R.; Xie, W. F. and Li, S. (2004): Effect of taurine on proliferation and apoptosis of hepatic stellate cells in vitro. Hepatobiliary Pancreatic Dis. Int., 3(1):106-109.
41. Castor, E.; Sola.S.; Ma. X.; Ramalho, M.; Kren, T.; Steer, J. (2005): A distinct microarray gene expression profile in primary rate hepatocytes incubated with unsodeoxycholic acid. J. Hepatol. 42(6):897-906.
42. Liang, J.; Zhang, L.; Yang, Y.; Pang, S.; Yuan, F.; Liang, S. and Huang, B. (2005): Observation of the promotion effect taurine on hepatic stellate cell's apoptosis in rat hepatic fibrosis model. Sichuan Da XueXueBao Yi Xue Ban, 36(3):365-367.
43. Warskulat U, Borsch E, Reinehr R, Heller-Stilb B, Mnnighoff I, Buchczyk D et al (2006) Chronic liver disease is triggered by taurine transporter knockout in the mouse. FASEB J 20(3):574–576
44. Waterfield C, Turton J, Scales M, Timbrell J. (1993): The correlation between urinary taurine and liver damage. Toxicology; 77: 1-5.
45. EI Agouza I, Rawi S, Lashin, S. (1995): Effect of taurine on rat liver injured by chronic carbon tetracholride treatment. Egypt J Med Sci 1995; 16: 185-98.
46. Chen, Y.; Li, S. and Zhang, X. (1999): Taurine inhibit deposition of extracellular matrix in experimental liver fibrosis in rats. Zhonghua Yi XueZaZhi, 7 (3): 165-167.
47. Wu QD, Wang JH, Fennessy F, Redmond HP, Bouchier-Hayes D. (1999): Taurine prevents high-glucose-induced human vascular endothelial cell apoptosis. Am J Physiol 277 (6 Pt 1): C1229–1238
48. Dincer, S.; Ozenirler, S.; Oz, E. (2002): The protective effect of taurine pretreatment on carbon tetrachloride - induced hepatic damage, a light and electron microscopic study. Amino Acids, 22(4):417-426.
49. Debra Manzella R.N. (2008): Kidney disease in diabetes, http://diabetes about.com/od/preventingcomplications/ p/kidney disease.htm.
50. Zama D., Meraihi Z., Tebibel S., Benayssa W., Benayache F., Benayache S., Vlietinck A.J. (2007): Chlorpyrifos-induced oxidative stress and tissue damage in the liver, kidney, brain and fetus in pregnant rats: The protective role of the butanolic extract of Paronychia argentea L. Indian Journal of Pharmacology 39 (3): 145-150
51. Delanghe J., De Slypere J.P., De Buyzere M., Robbrecht J. M Wieme R. and Vermeulen A. (1989): Normal reference values for creatine, creatinine and carnitine are lower in vegetarians. Clinical Chemistry 35 (8): 1802-1805.

52. Ambali S.F., Abubakar A.T., Shittu M., Yaqub L.S., Anafi S.B. and Abdullahi A. (2010): Chlorpyrifos-induced alteration of hematological parameters in Wistar rats: Ameliorative effect of Zinc. Journal of Environmental Toxicology 4: 55-66.
53. Han X. and Chesney RW. (2012): The role of taurine in renal disorders. Amino Acids 43:2249–2263
54. Cruz CI., Ruiz-Torres P., del Moral RG, Rodriguez-Puyol M, Rodriguez-Puyol D. (2000): Age-related progressive renal fibrosis in rats and its prevention with ACE inhibitors and taurine. Am J Physiol Renal Physiol 278:F122–F129.
55. Eppler B. and Dawson R. Jr. (2001): Dietary taurine manipulations in aged male Fischer 344 rat tissue: taurine concentration, taurine biosynthesis, and oxidative markers. Biochem Pharmacol 62: 29–39
56. Militante JD., and Lombardini JB. (2002): Treatment of hypertension with oral taurine: experimental and clinical studies. Amino Acids.;23 (4):381-393.
57. Dawson R Jr, Liu S, Eppler B, Patterson T. (1999): Effects of dietary taurine supplementation or deprivation in aged male Fischer 344 rats. Mech Ageing Dev 107:73–91
58. FalktoftB.and Lambert IH. (2004): Ca2+-mediated potentiation of the swelling-induced taurine efflux from HeLa cells: on the role of calmodulin and novel protein kinase C isoforms. J MembrBiol 201:59–75.
59. Molchanova S, Oja SS., Saransaari P. (2004): Characteristics of basal taurine release in the rat striatum measured by microdialysis. Amino Acids 27:261–268.
60. Huang Z., Hou Q., Cheung N.S., Li QT. (2006): Neuronal cell death caused by inhibition of intracellular cholesterol trafficking is caspase dependent and associated with activation of the mitochondrial apoptosis pathway. J Neurochem 97 :280–291.
61. Gurujeyalakshmi G, Wang Y, Giri SN (2000): Suppression of bleomycin-induced nitric oxide production in mice by taurine and niacin. Nitric Oxide 4:399–411.
62. Huang XM., Zhu W H., Kang ML. (2003): Study on the effect of doxorubicin on expressions of genes encoding myocardial sarcoplasmic reticulum Ca2+ transport proteins and the effect of taurine on myocardial protection in rabbits. J Zhejiang UnivSci 4:114–120.
63. Howard-Alpe GM, Sear JW, Foex P. (2006): Methods of detectingatherosclerosis in non-cardiac surgical patients; the role of biochemical markers. Br J Anaesth.; 97:758-69.