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RESEARCH PAPER

THE THERAPEUTIC EFFECTS OF TAURINE AGAINST HEPATOTOXICITY INDUCED THROUGH CARBON TETRA CHLORIDE IN RATS

Lubna Naz Assistant Professor Department of Physiology University of Karachi, Karachi, Pakistan.

Tabassum Mahboob Professor Department of Biochemistry University of Karachi Karachi, Pakistan.

ABSTRACT The present study explores the protecting functions of Taurine on lipid-peroxidation and antioxidant enzymes' activity in Carbon tetrachloride induced liver fibrosis and cirrhosis in experimental rat models. Study included 24 age matched male Albino Wistar rats divided (n=6) into; Group I =healthy untreated rats, Group II & III = CCl4 in a dose of 0.8 ml/Kg body weight, subcutaneously two times per week for 60 days, group III & IV= in addition received1% w/v taurine in drinking water daily for 8 weeks. Biochemical investigations included MDA, bilirubin, hepatic and antioxidant enzymes. CCl4 produced liver toxicity as shown by increased total bilirubin, hepatic enzymes (SDH, ALT, ALP & AST) and MDA concentrations while decline in activity of antioxidant enzymes; catalase, GSH, SOD, PON-1 & CP. Taurine supplementation in rats noticeably reduced the levels of AST, ALT, ALP, total bilirubin & SDH and resumed the levels of Catalase, GSH, SOD, MDA, PON-1 & CP. The histopathololgic findings showed mild portal and periportal inflammatory changes and fibrosis alongwith degenerated hepatocytes rats treated with CCl4. Rats treated with taurine along with CCl4 showed slight periportal fibrosis without degenerated hepatocytes suggesting that taurine mitigates the liver toxicity created by CCl4 in rats.

KEY WORDS : Antioxidant, CCl4, Cirrhosis, Hepatotoxicity, Taurine.

INTRODUCTION

Taurine, 2-aminoethylsulfonic acid has been shown to maintain liver health and detoxify toxins within tissues (Balkan, 2002; Refik et al., 2004; Waterfield et al., 1991). Several studies have claimed that it counteracts alcohol, avert cholestasis and diminish the consequences of DNA break induced by aromatic-amine compounds. (Kerai, et al., 2005 & Refik et al., 2004). Taurine has been derived fromcysteine, which is a sulfur-containing amino acid occurs particularly in sea food and meat. It is the major plenteous amino acid present in hepatic, nervous, ocular, peri renal, white and brown adipose tissue and epididymis (Tappaz et al., 1998 & Ide et al., 2002). The amount of intracellular taurine is comparatively increased as compared with other amino acids chiefly in liver where it is produced as terminal product during the metabolism of amino acids that contain sulfur (Hosokawa et al., 1990; Kaisaki et al., 1995; Reymond et al., 1996 & Tappaz et al., 1999). Taurine is believed to be concerned in cellular defense, growth, nutrition, endurance and regulation of growth and segregation (Harris & Wen, 2012). It raises expression and actions of antioxidants for instance superoxide dismutase, catalase and glutathione reductase (Jang et al., 2009). It also has been shown to counteract oxidative stress by restricting the accessibility of lipids intended for lipid peroxidation. Data from research studies propose that taurine intake may avoid oxidative stress caused by toxins, alcohol and injury induced by ischemia, reperfusion and mechanical trauma (Murakami et al., 2010 & Silva et al., 2011).

Various studies have accepted the defending roles of taurine in opposition to damage induced by ROS, toxins and carcinogens (Sinha et al., 2009 & Erman et al., 2004). The antioxidant potential of taurine is attributed to its ability to inhibit lipid peroxidation via scavenging ROS as well as through maintaining membrane permeability altered by oxidative stress (Koch et al., 2004). Research studies have established that the byproducts of taurine such as taurolidine and taurochloramine exhibit anti-neoplastic property in vitro as well as in vivo through devastating cellular proliferation and amplification of tumor cell apoptosis (Duffy, 2006).

Liver is prone to the toxicity produced by chemicals and toxins during their course of elimination therefore precise monitoring of hepatotoxicity is necessary in scientific trials to build up strategies and avert eventual hepatic malfunction.

Chronic liver damage (CLD) may lead to liver cirrhosis as its final worst stage thereby increasing morbidity and mortality. In cirrhosis replacement of normal hepatocytes through regenerative nodules, fibrotic and scar tissue occur that ultimately leads to liver collapse. Liver serves via detoxifyng toxins trough their metabolism and conversion to intermediary metabolites for the successive excretion. During the metabolic pathway toxins can be transformed to reactive radicals that can damage hepatic parenchyma leading to degeneration, necrosis and atrophy of hepatocytes thus creating hepatotoxicity. Among the mechanisms of liver cirrhosis examined by various researchers in the course of development of numerous experimental animal models, bile duct ligation, alcoholism, carbon tetrachloride and thioacetamide administration are prominent. CCl_4, a chlorinated hydrocarbons is responsible to cause harmful effects on diverse body tissues. It has been extensively used to persuade liver injury, and to set up investigational hepatotoxic models. Exposure to elevated concentrations of CCl_4 can cause degeneration of CNS, hepatic and renal tissues that may escort to even loss of consciousness and fatality (Raja et al., 2007).

The neuroprotecting, osmoregulating and cellular protective roles of taurine have been investigated widely but statistics allied to therapeutic function in the management of drug induced hepatic toxicity is inadequate. Further no study to date has reported estimation of paraoxonase, ceruloplasmin & sorbitol dehydrogenase along with regular liver functioning tests and histologic investigations to evaluate the protecting role of taurine against liver damage induced by CCl_4. Therefore this study was designed to investigate the defensive effects of taurine against hepatic toxicity caused by CCl_4 in wistar rats.

MATERIALS & METHODS

Ethical guidelines

The study was carried out at Department of Physiology, University of Karachi,Karachi, Pakistan. The experimental phase was carried out in accordance with ethical guiding principle of ERB (Ethical Review Board), University of Karachi and globally accepted ethics for laboratory use and care in animal research (Health Research Extension Act of 1985).

Study design

Study included age matched male Albino Wistar rats acclimatized

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*Corresponding Author Lubna Naz

Assistant Professor Department of Physiology University of Karachi, Karachi, Pakistan. ,lunasaf@yahoo.com

and caged in a calm temperature restricted animal house (23±4°C). Rats were kept free to access water and typical rat diet. Age and body weight matched rats were divided in to four groups (n=6).

Group I : Untreated control rats
Group II : CCl4 treated
Group III : CCl4 + taurine treated
Group IV : Taurine treated

The experimental phase consisted of 60 days. Group I rats remained untreated and received 2 mL/kg normal saline solution for 60 days. Group II & III received CCl_4 subcutaneously, twice a week for 60 days at a dose of 0.8 ml/kg b.w. Group III & IV rats were given 1% taurine alongwith drinking water daily for 60 days. Experimental phase ended at 60th day and 24 hours after the last dose, rats were sacrificed to get blood and tissue samples for biochemical and histological analysis.

Biochemical analysis

Plasma Alanine aminotransferase (ALT) (Reitman etal.,1957), Aspartate transaminase (AST) (Wilkinson etal.,1972), Alkaline phosphatase (ALP) (Tietz et al.,1983) and total and direct bilirubin (Dangerfield et al.,1953) were analyzed using commercially prepared reagent kits from Randox. Hepatic concentration of Paraoxonase (PON-1), Sorbitol dehydrogenase (SDH) & Ceruloplasmin (CP) were estimated through commercially prepared ELISA kits.

Histopathological examination

Liver tissue samples were consecutively fixed, embedded, sectioned and stained for microscopic examination. The extent of liver injury was estimated from the histologic sections through numerical scores as described by French et al. 2000.

Score 0 = no visible damage
Score 1 = focal hepatocyte damage on less than 25% of the tissue
Score 2 = focal hepatocyte damage on 25-50% of the tissue
Score 3 = extensive, but focal hepatocyte lesion
Score 4 = global hepatocyte necrosis

Statistical analysis

Results are reported as mean± standard deviation. Data was analyzed through Statistical Package for the Social Sciences (SPSS) version 16. Statistical comparisons and difference between group means were estimated by applying one-way analysis of variance (ANOVA) followed by the LSD (Least Significance Difference Post hoc multiple comparison test) test and were considered significant when p<0.05.

RESULTS

Table 1 : Comparison of body weight, liver weight and relative liver weight in Control, CCl_4 treated, CCl_4 +Taurine treated &Taurine treated groups

	Control (n=6)	CCl_4[1] (n=6)	CCl_4 + Taurine[1,2] (n=6)	Taurine[1,2,3] (n=6)
Initial body weight	214.66±4.50925	212.67±2.5166	209.33±1.1547	204±3.6056
Final body weight	237.33±11.1505	121.667±7.63[d]	183.3±2.88[b,c]	219.33±9.0185[a,n,b]
Weight Gain	22.67±6.6412			15.33±5.4129
Weight Loss		91.003±5.1204	26±1.7321	
Liver Weight	4.85±0.6403	7.42±1.4056[a]	4.725±0.2872[n,a]	4.525±0.4113[n,a,n]
Relative liver weight (g liver /100 g body)	2.119±0.2444	6.486±1.411[b]	2.6201±0.2017[a,b]	2.0843±0.2404[n,b,a]

The data is expressed as mean ± standard deviation.
1= As compared with control
2= As compared with Ccl_4
3= As compared with CCl_4+Taurine
a= P<0.05, b= P<0.01, c= P<0.001, n= P>0.05(Non-significant)

Table 2 : Comparison of Plasma enzymes & Bilirubin levels in Control, CCl_4 treated, CCl_4 +Taurine treated & Taurine treated groups

	Control (n=6)	CCl_4[1] (n=6)	CCl_4 + Taurine[1,2] (n=6)	Taurine[1,2,3] (n=6)
AST (U/l)	8.5±0.212	25.16±5.48[c]	10.5±0.96[n,b]	7.17±4.49[n,c,a]
ALT (U/l)	17.5±3.27	24.62±3.68[b]	2.98±2.03[c,c]	3.48±0.44[c,c,n]
ALP (U/l)	290.70±111.43	683.56±219.52[a]	546.6±486.26[n,n]	452.09±309.43[n,n,n]
Bilirubin (mg/dl)	0.4±0.0967	2.03±0.53[c]	0.566±0.04[n,c]	0.45±0.208[n,c,n]
PON-1 (ng/ml)	103.9 ± 2.46	80.06 ± 4.53 [c]	102.1 ± 6.11[n b]	81.68 ± 9.602[b n b]
CP(ng/L)	238.66 ± 31.7	143.33 ± 66.5 [a]	204.33 ± 91.34 [n n]	355.66 ± 100.45 [n a n]
SDH(ng/ml)	12.33 ± 2.57	17.6 ± 2.264 [b]	10.733 ± 2.023 [n a]	18.33 ± 1.457 [b n c]

The data is expressed as mean ± standard deviation.
1= As compared with control
2= As compared with Ccl_4
3= As compared with CCl_4+Taurine
a= P<0.05, b= P<0.01, c= P<0.001, n= P>0.05(Non-significant)

Table 3 : Comparison of Liver antioxidant enzymes and MDA activity in Control, CCl_4 treated, CCl_4 +Taurine treated & Taurine treated groups

	Control (n=6)	CCl_4[1] (n=6)	CCl_4 + Taurine[1,2] (n=6)	Taurine[1,2,3] (n=6)
Catalase (µmol/g tissue)	17.31±2.18	4.831±0.68[b]	14.63±4.76[n,b]	1.28±0.17[c,n,c]
SOD (Unit/g tissue)	1.436±0.63	0.422±0.25[a]	1.7±0.25[n,a]	6.36±1.43[c,c,c]
GSH (Unit/g tissue)	0.811±0.08	0.0214±0.0015[c]	0.03±0.003[c,n]	0.08±0.002[c,n,n]
PON-1(ng/ml)	197.06±33.99	128.2 ±9.634 [b]	102.866 ±16.07 [a a]	203.63 ±45.44 [n b b]
CP(ng/L)	344.66±57.14	224.66 ±18.58 [a]	251.66 ± 34.03 [a n]	260 ±44.44[n n n]
SDH(ng/ml)	8.33± 1.35	15.56± 3.95 [a]	8.73 ±0.98 [n a]	9.83± 5.26 [n a n]
MDA (µmol/g tissue)	0.906±0.32	1.710±0.71[a]	1.51±0.61[a,n]	0.806±0.205[n,a,a]

The data is expressed as mean ± standard deviation.
1= As compared with control
2= As compared with Ccl_4
3= As compared with CCl_4+Taurine
a= P<0.05, b= P<0.01, c= P<0.001, n= P>0.05(Non-significant)

Table 4 : Hepatic Histopathological features in Control, CCl_4 treated, CCl_4 +Taurine treated &Taurine treated groups

Histopathological findings	Control	CCl_4	CCl_4 + Taurine	Taurine
Enlargement	0	0	0	0
Paleness	0	0	0	0
Fatty change	0	0	0	0
Hydropic degeneration	0	0	0	0
Portal inflammation	0	2	0	0
Periportal inflammation	0	2	0	0
portal fibrosis	0	2	0	0
Periportal fibrosis	0	2	1	1
Focal lobulitis	0	2	0	0

Total Score	0	10	01	01
Intracellular pigment deposition	absent	present	absent	Absent
Sinusoidal expansion	absent	absent	Marked	mild
Degenerative hepatocytes	absent	30-40-%	absent	Absent
Ballooning degeneration	absent	absent	absent	Absent
Bile duct proliferation	absent	absent	slight	absent

Degree of hepatic injury is expressed as scores observed via light microscopy. Score 0 = no noticeable impairment, Score 1 = focal liver cell destruction (≤25% of the tissue), Score 2 = focal liver cell destruction (25-50% of the tissue), Score 3 = widespread, but focal hepatocyte injury & Score 4 = comprehensive hepatocyte necrosis.

Figure 1 : Hepatic Histological features in Control rats

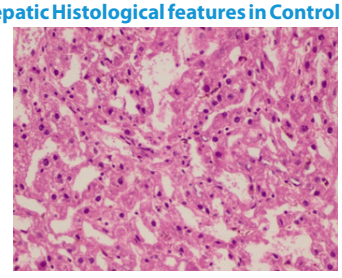


Figure 2 : Hepatic Histological features in CCl_4 treated rats

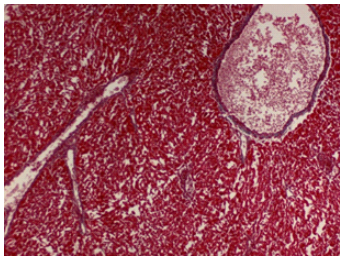


Figure 3 : Hepatic Histological features in CCl_4+ Taurinetreated rats

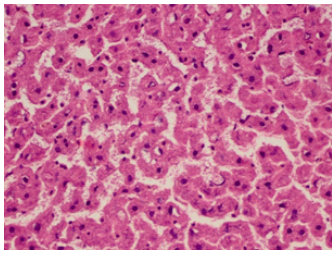
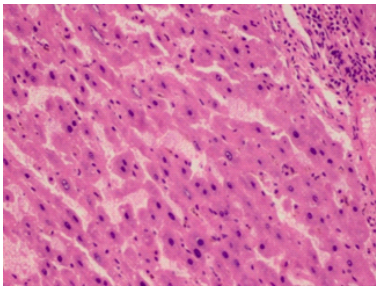


Figure 4 : Hepatic Histological features in Taurine treated rats



DISCUSSION

This study was designed to evaluate the protecting roles of taurine against hepatotoxicity and oxidative stress caused by induction of CCl_4 in experimental rat models.

It is well known that high concentrations of CCl_4 can influence the CNS and cause liver impairment. Metabolism of CCl_4 is carried out by cytochrome P450 and yield trichloromethyl free radical (CCl_3) that can bind with cell proteins and lipids in oxygen rich environment to generate trichloromethylperoxyl radical which is toxic enough to cause hepatic inflammation, lipid peroxidation and fibrosis (Fang et al., 2008). Group II (CCl_4 treated) rats lost their body weight rapidly as compared with healthy untreated rats. The cytotoxic effects of CCl_4 were further endorsed by alterations in biochemical hepatic enzyme levels as membrane disintegration of hepatocytes leads to resultant release of hepatic enzymes in plasma whereas some enzyme levels are proportional to liver injury. A significant reduction in plasma PON-1($P<0.001$) and CP ($P<0.05$) whereas significant increase in plasma bilirubin ($P<0.001$), ALP ($P<0.05$), SDH ($P<0.01$), AST ($P<0.001$) and ALT ($P<0.01$) was observed in CCl_4 treated group as compared with control group (Table II). CCl_4 treatment in rats created oxidative stress and subsequent oxidation induced modification of antioxidant enzyme proteins which was shown by significantly reduced activity of antioxidant enzymes catalase ($P<0.01$), CP ($P<0.05$), SOD ($P<0.05$) and PON-1 ($P<0.01$) as compared with untreated rats (Table III). In addition, metabolism of CCl_4 via hepatocytic Kupffer cells further propagate hepatocytic necrosis and lipid peroxidation (Augustyniak et al., 2005). MDA is a specific marker for lipid peroxidation and the raised levels are associated with consequent damage similarly SDH activity is proportional to disruption of cell membrane followed by cell lysis. A non significant increase in MDA ($P<0.05$) and SDH ($P<0.05$) activity has also been reported in rats treated with CCl_4 (Table III). The histologic results obtained in the present study are in harmony with biochemical fndings. The hepatotoxic evidences of CCl_4 treatment indicate hepatic portal as well as periportal inflammation along with fibrosis, pigment accumulation and degeneration.

Taurine treatment along with CCl_4 has been shown to prevent and reverse the hepatotoxic alterations in rats. The body weight reducing effect of CCl_4 was attenuated when taurine was administered together with CCl_4 in rats (Table I). The preventative effects of taurine were further endorsed by the biochemical results of this study that showed a significant decline in plasma bilirubin ($P<0.001$), AST ($P<0.01$), ALT ($P<0.001$) & SDH ($P<0.05$) whereas non significant decrease in PON-1 and CP plasma levels in CCl_4+taurine treated rats when compared with CCl_4 treated rats (Table II). The synthesis of ceruloplasmin, a glycoprotein with iron is the primary responsibility of liver cells hence, a drop in CP level may be considered as a strong indicator of end stage liver disease except Wilson's Disease (Uhlikova et al., 2008 &Schaff et al., 1991). The oxidative stress played a key role in pathogenesis of hepatotoxicity created via CCl_4. The major therapeutic effectiveness of taurine has been attributed to its antioxidant defense system as shown by significantly raised levels of liver antioxidants SOD ($P<0.05$), catalase ($P<0.01$) and PON-1($P<0.05$) whereas non-significant increase in GSH in CCl_4+taurine treated rats as compared with CCl_4 treated rats (Table III). Our results are supported by some other limited studies which have shown anti-oxidant and anti-inflammatory of taurine (Erman et al., 2004; Sun et al., 2012 & Miyazaki et al., 2005). MDA and SDH are considered as the marker of hepatic damage and lipid peroxidation the level of which were found to be reduced in rats treated with taurine and CCl_4 when compared with CCl_4 treated rats (Table III). The histological features investigated in rats treated with taurine along with CCl_4 showed only minor periportal fibrotic change with bile duct proliferative growth and sinusoidal development without any marked degeneration of hepatocytes (Table IV).

The changes in body weight, biochemical parameters, antioxidant enzyme and histological measures in only taurine treated rats are in accordance with control group (Table I, II, III & IV) that assure the efficacy of taurine without creating any toxicity.

It is therefore, rational to consider the biochemical and histological results of this study that pin point the therapeutic and antioxidant potential of taurine and suggest that it may be taken as an alternative medicine to heal hepatic damage and may be beneficial against oxidative stress induced hepatotoxicity.

CONCLUSION

This study claims taurine as a strong protective agent against

hepatotoxicity caused by CCl_4 and concludes that it may provide a substitute of chemotherapeutic drugs used for the treatment of liver diseases without any side effects.

REFERENCES

1. Balkan J, Kanbagli O, Aykac-Toker G, Uysal M. Taurine treatment reduces hepatic lipids and oxidative stress in chronically ethanol-treated rats. Biol Pharm Bull. 2002: 25:1231–1233
2. Dangerfield WG and Finlayson R. Estimation of bilirubin in serum. J. Clin. Path. 1953: 6, 173.
3. Duffy M. Serum tumor markers in breast cancer: are they of clinical value? Clin Chem: 2006: 52(3):345–351
4. Erman J, Balkan U, CevikbasN, Kocak-Toker M. Uysal M. Betaine or taurine administration prevents fibrosis and lipid peroxidation induced by rat liver by ethanol plus carbon tetrachloride intoxication. Amino Acids F. 2004: 27, 199-205
5. Fang HL, Lai JT, Lin WC. Inhibitory effect of olive oil on fibrosis induced by carbon tetrachloride in rat liver. Clin Nutr. 2008: 27, 900-7.
6. French SW, Miyamoto K, Ohta Y, Geofrion Y. Pathogenesis of experimental alcoholic liver diseasein the rat. Methods Achiev Exp Pathol. 2000: 13:181-207.
7. Harris Ripps, Wen Shen. Taurine: A "very essential" amino acid, Molecular Vision. 2012: 18:2673-2686
8. Hosokawa Y, Matsumoto A, Oka J, Itakura H, Yamaguchi K. Isolation and characterization of a cDNA for rat liver cysteine dioxygenase. Biochem Biophis Res Commun. 1990: 68:473-478.
9. Ide T, Kushiro M, Takahashi Y, Shinohara K, Cha S. mRNA expression of enzymes involved in taurine biosynthesis in rat adipose tissues. Metabolism. 2002: 51(9):1191–1197
10. J.H. Wilkinson, D.N. Baron, D.W. Moss, and P.G. Walker. Standardization of clinical enzyme assays: a reference method for aspartate and alanine transaminases. J.clin.Path. 1972: 25, 940-944.
11. Jang JS, Piao S, Cha YN, Kim Ch. Taurine chloramine activates Nrf2, increases HO-1 expression and protects cells from death caused by hydrogen peroxide. J Clin Biochem Nutr. 2009: 45:37–43.
12. Kaisaki, P.J., Jerkin, A.A., Goodspeed, D.C., Steel, R.D. Cloning and characterization of rat cysteine sulfinic acid decarboxylase. Biochim. Biophys. Acta. 1995:1263(2), 79–82.
13. Kerai MD, Waterfield CJ, Kenyon SH, Asker DS, Timbrell J. Reversal of ethanol-induced hepatic steatosis and lipid peroxidation by taurine: a study in rats. 1999: 34:529–541.
14. Koch OR, Pani G, Borrello S, Colavitti R, Cravero A, Farre S and Galeotti T. Oxidative stress and antioxidant defenses in ethanol induced cell injury. Mol. Asp. Med. 2004: 25, 191-198.
15. Miyazaki T, Karube M, Matsuzaki Y. Taurine inhibits oxidative damage and prevents fibrosis in carbon tetrachloride-induced hepatic fibrosis. J Hepatol. 2005: 43:117–125
16. Miyazaki T, Karube M, Matsuzaki Y. Taurine inhibits oxidative damage and prevents fibrosis in carbon tetrachloride-induced hepatic fibrosis. J Hepatol. 2005: 43:117–125
17. Murakami S, Sakurai T, Toda Y, Morito A, Sakono M, Fukuda N. Prevention of neointima formation by taurine ingestion after carotid balloon injury. Vascul Pharmacol. 2010: 53:177–184.
18. Raja S, Ahmed N, Kumar V, Mukherjee K, Bandyopadhyay A, Mukherjee P. Antioxidant effect of Cytisus scoparius against carbon tetrachloride treated liver injury in rats. J. Ethnopharm. 2007: 109, 41–47.
19. Refik Mas M, Comert B, Oncu K, Vural SA, Akay C, Tasci I, Ozkomur E, Serdar M, Mas N, Alcigir G, Yener N. The effect of taurine treatment on oxidative stress in experimental liver fibrosis. Hepatol Res. 2004: 28(207)–215
20. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am.J Clin. Pathol. 1957: 28 (1): 56-63.
21. Reymond I., Almarghini K., and Tappaz M. Immunocyto chemical localization of cysteine sulfinate decarboxylase in astrocytes in the cerebellum and hippocampus: a quantitative double immunofluorescence study with glial fibrillary acidic protein and S-100 protein. Neuroscience. 1996: 75, 619–633.
22. Schaff Z, Lapis K, Szende B, Jeney A, Gergely P et al. The effect of D-penicillamine on CCl4-induced experimental liver cirrhosis. Exp Pathol. 1991: 43(1-2). 111-120.
23. Silva LA, Silveira PC, Ronsani MM, Souza PS, Scheffer D, Vieira LC, Benetti M, De Souza CT, Pinho RA. Taurine supplementation decreases oxidative stress in skeletal muscle after eccentric exercise. Cell Biochem Funct. 2011: 29:43–49
24. Sinha M, Manna P, Sil PC. Induction of necrosis in cadmium-induced hepatic oxidative stress and its prevention by the prophylactic properties of taurine.J Trace Elem Med Biol. 2009: 23 (4):300-13.
25. Sun M, Zhao Y-M, Gu Y, Xu C. Therapeutic window of taurine against experimental stroke in rats. Transl Res. 2012: 160:223–229.
26. Tappaz M, Reymond I, Bitoun M, Sergeant A. Cysteine sulfinate decarboxylase (CSD): molecular cloning, sequence and genomic expression in brain. Adv Exp Med Biol. 1998: 442:25–32
27. Tappaz M., Bitoun M., Reymond I., and Sergeant A. Characterization of the cDNA coding for rat brain cysteine sulfinate decarboxylase: brain and liver enzymes are identical proteins encoded by two distinct mRNAs. J. Neurochem. 1999: 3, 903–912.
28. Tiez NW, Burtis CA, Duncan P, Ervin K, Petitclerc Cj, Rinker AD, Shuey D, Zygowicz ER. A reference method for measurement of alkaline phosphatase activity in human serum. Clin Chem. 1983: 29(5):751-61.
29. Uhlikova E, Kupcova V, Szantova M, Turecky L. Plasma copper and ceruloplasmin in patients with alcoholic liver steatosis. Bratisl. Lek Listy. 2008: 109(10): 431-433.
30. Waterfield C. J., Turton J. A., Scales M. D. C. and Timbrell J. A. Taurine synthesis in isolated rat hepatocytes in suspension exposed to carbon tetrachloride Biochem. Soc. Trans. 1991: 18; 1218-1219.