Original Article

The Protective Role of Taurine in Oxytetracycline - Induced Hepatotoxicity in Albino Rats

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ABSTRACT

Objective: To study the protective effects of taurine in oxytetracycline induced hepatic steatosis in albino rats.
Study Design: Prospective Experimental Study.
Place and Duration of Study: This study was conducted in the Department of Anatomy, Khyber Medical College, Peshawar from July 2011-December 2011.
Materials and Methods: Four groups of male albino rats, each comprising 8 animals, were treated for 21 days as follows: Group A served as control, group B treated with oxytetracycline 120 mg/kg body weight intraperitoneally for three consecutive days, group C treated with oxytetracycline 120mg/kg body weight intraperitoneally for three consecutive days plus taurine 1% solution as their sole source of drinking water for 21 days, and group D treated with taurine 1% solution alone as their sole source of drinking water for 21 days.
The animals were weighed at the start and end of treatment and were sacrificed on 22nd day of start of treatment under deep ether anaesthesia. Blood samples were collected for enzyme study by intracardiac puncture. The livers were removed, washed in normal saline and weighed. They were fixed in 10% formalin and embedded in paraffin. 4 µ thick sections were cut and processed for H & E staining, and were examined microscopically. Statistical analysis of the data was done and results were tabulated.
Results: Groups A (data not shown) and D showed normal results with no significant differences across the groups. The relative liver weights in group B increased significantly (P value <0.05) in comparison with group C and D. The relative liver weights decreased significantly (P value <0.05) in group C and D as compared to group B. There was moderately significant decrease (P value <0.01) in the values of serum hepatic enzymes (SGPT, SGOT, and ALP) in the animals of group C as compared to group B. Histologically the livers of group B animals showed generalized microvesicular steatosis. In group C the fatty change was much less pronounced as compared to group B.
Conclusion: The data show that the hepatic steatosis induced by oxytetracycline can be reversed / attenuated by taurine supplementation for 21 days, in albino rats.
Key Words: Oxytetracycline, Hepatic Steatosis, Taurine.

INTRODUCTION

Oxytetracycline is a broad spectrum antibiotic from the tetracycline group active against many gram-positive and gram-negative bacteria including anaerobes, reckettsiae, chlamydiae, mycoplasmae and protozoa. It is very effective against vibrio cholera, and in combination with other drugs, against Helicobactor Pylori[1].

Tetracyclines in excessive doses are hepatotoxic and are known to induce microvesicular steatosis in liver, the underlying mechanism being deficient ß-oxidation of fatty acids[2,3,4]. In different studies it has been used to create experimental hepatic steatosis in rats[4,5,6]. Hepatic steatosis can progress into steatohepatitis, cirrhosis and hepatocellular carcinoma[7].

Taurine (2-aminoethanesulfonic acid), is a non essential, sulphur containing amino acid found in many tissues and synthesized in the liver as an end product of L-cystein metabolism[8]. The protective role of taurine against tissue injury has been reported by many authors, and the physiological actions attributed to taurine include antioxidation, cell membrane stabilization, neuromodulation, osmoregulation and bile acid conjugation[9, 10, 11].

In rats taurine attenuates the oxidative stress and injury in the urinary bladder and kidney induced by nicotinamide[12]. It reduces the severity of cyclophosphamide - induced hemorrhagic cystitis[13], and ameliorates the hypoxia induced lactic acidosis in brain, liver and heart [14].

In the liver taurine attenuates the injury induced by agents such as cyclosporine A[15], carbon tetrachloride[16], acetaminophen[11], and thioacetamide[17]. Its restorative role in experimentally induced non - alcoholic steatohepatitis has been observed[18]. It has been suggested that taurine reverses hepatic steatosis by enhancing the secretion of hepatic triglycerides and enhances the removal lipid peroxides by increasing the flow of bile[19].

The purpose of this study was to find the histological and biochemical evidence of the hepatotoxicity caused

by overdose of oxytetracycline in rats; and to find if taurine, a sulphur containing amino acid, can offer any protection against such toxicity.

MATERIALS AND METHODS

This study was conducted in the Department of Anatomy, Khyber Medical College, Peshawar. Oxytetracycline (STAR Pak) and taurine (GNC, USA) were purchased from the local market.

Thirty two healthy adult male albino rats 90-120 days of age and 200-300 gram in weight were selected for this study. They were fed on the standard chow and were divided into four groups with eight animals in each group. They were treated for 21 days as follows:

Group A served as control and were injected normal saline 1 cc intraperitoneally for three consecutive days. They were allowed to have free access to drinking water and were kept for 21 days.

Group B animals received injection oxytetracycline 120mg per Kilogram body weight, intraperitoneally for three consecutive days, with free access to drinking water for 21 days. This dose regime of oxytetracycline has been shown to induce fatty change in rat liver [5, 6].

Group C animals received injection oxytetracycline 120mg per Kilogram body weight intraperitoneally for three consecutive days and 1% taurine solution as their sole source of drinking water for 21 days[15,18].

Group D received normal saline 1 cc intraperitoneally for 3 consecutive days, and 1% taurine solution as their sole source of drinking water[15, 18] for 21 days.

On the 22nd day of the start of the treatment, all the animals were sacrificed under deep ether anaesthesia. Blood samples for liver enzymes were collected by heart puncture. Enzyme studies (Serum gltutamic pyruvate transaminase -SGPT, Serum glutamic oxaloacetic transaminase -SGOT, Alkaline phosphatise -ALP) were done to measure the amount of liver injury and for correlation with morphological findings. Livers were removed, washed with normal saline and weighed. They were fixed in 10% formalin and embedded in paraffin. 4µ thick sections were stained with hematoxylin and eosin, and examined microscopically for cell morphology and lobular architecture. Histological diagnosis was made and the results were tabulated.

RESULTS

The findings in all parameters in group A (control, data not shown) and group D (taurine only) were comparable without any significant difference across the groups. The relative liver weight in group B (Oxytetracycline group) increased significantly (P value <0.05) in comparison with group C (Oxytetracycline plus taurine treated) as shown in table 1. The relative liver weights in group C was comparable to group D with an insignificant increase (p value >0.05), but the relative liver weights of animals of group C decreased significantly (P value <0.05) in comparison with group B. These findings are shown in table 2.

Table No.1: Comparison of *Mean relative liver weight (G/100G) between groups B, C and D

Relative liver weight	Group B	Group C	Group D
	4.99±0.30	3.66±0.05	3.58±0.06

*Mean +SEM

Table No.2: Statistical analysis of differences in mean relative liver weight between different groups

Groups	P-Value
B vs C	P<0.05**
B vs D	P<0.05**
C vs D	P>0.05*

Key (P-value): Insignificant* Significant** Moderately Significant***

The mean values (IU/L) of serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) and serum alkaline phosphatise (ALP) levels of albino rats in group B were raised moderately significantly (P value <0.01) as compared to both group C and D, but were decreased moderately significantly (P value <0.01) in group C in comparison with group B (table 3).

Table No.3: Comparison of Mean (Mean±SEM) serum levels of liver enzymes in different groups

Liver Enzymes	Normal value	Group B	Group C	Group D	P Value	
SGPT (ALT)	0-45 IU	107±2.28	67.99±1.89	42.02±0.95	P1	<0.01***
					P2	<0.05**
SGOT (AST)	5-45 IU	124.20±5.83	65.20±3.15	45.31±4.14	P1	<0.01***
					P2	<0.01***
Alk Phosphatase	80-306 IU	376.6±20.02	196.4±22.47	146.6±17.73	P1	<0.01***
					P2	<0.01***

Key (P Value): Insignificant* Significant** Moderately Significant*** P1=Group B versus Group C P2=Group C versus Group D

The livers of animals in groups A and D exhibited normal histological features in the H&E stained sections (Figure-1). In group B there was some distortion of lobular architecture and dilatation of central vein (Figure-2). Swelling of hepatocytes and narrowing of sinusoids were seen. Hepatocytes showed

microvesicular steatosis with plenty of fatty vacuoles. Increase in the number of mononuclear cells in the region of portal triad was observed. Few ballooned hepatocytes were seen in the acinar zone III.

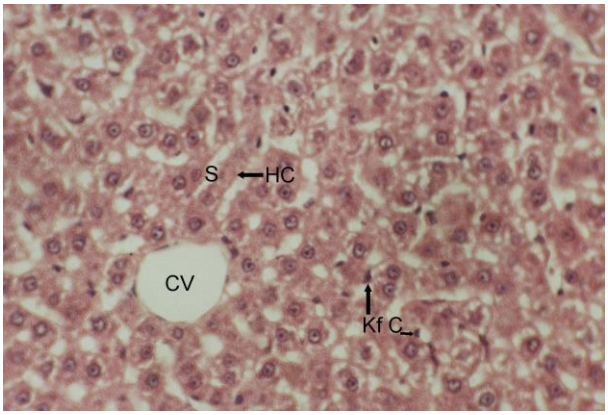

Figure No.1: Photomicrograph of H&E stained section of rat liver (control) (CV- central vein, HC – hepatic cords, S – sinusoids, Kf C- Kupffer cells x400).

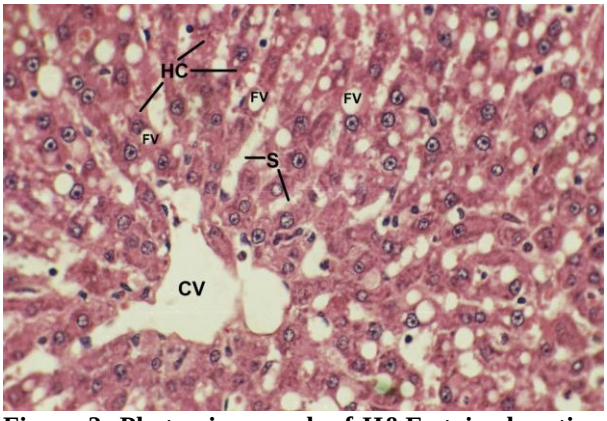

Figure 2: Photomicrograph of H&E stained section of rat liver treated with oxytetracycline (CV- central vein, HC – hepatic cords, FV – fat vacuoles, S – sinusoids, x400).

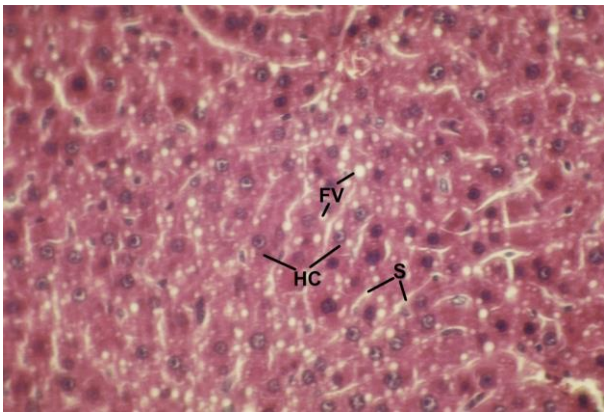

Figure No.3: Photomicrograph of H&E stained section of rat liver treated with oxytetracycline and taurine (HC – hepatic cords, FV – fat vacuoles, S – sinusoids, x400)

In group C (figure 3), the H&E stained sections showed markedly reduced fatty change in comparison to group B. There was marked decrease in the size of fatty vacuoles. The lobular architecture showed little distortion and the arrangement of cells in cords was obvious. The central vein showed less dilatation, and the walls were not distorted.

DISCUSSION

Drug-induced liver diseases are clinicopathologic patterns of liver injury related to drugs; about 900 medications have been identified as potentially hepatotoxic, ranging in severity from mild toxicity to fatal injury[20].

Fatty liver (hepatic steatosis) is a condition in which fat accumulates in liver cells[5]. This condition can progress into steatohepatitis, cirrhosis and hepatocellular carcinoma[7,21].

The protective role of taurine against various hepatotoxic agents has been observed in a number of studies. In a study, Kerai et al (1999)[19], demonstrated for the first time that hepatic steatosis and lipid peroxidation, occurring as a result of chronic alcohol consumption can be reversed by administration of taurine to rats. Waters et al (2001)[11] demonstrated that taurine supplementation attenuates lipid peroxidation and hence the liver injury by acetaminophen, represented by significant decrease in serum hepatic enzymes and reduced hepatocyte apoptosis and necrosis[11].

Keeping in view the different studies wherein the protective effects of taurine against drug-induced hepatotoxicity has been documented, it was considered worthwhile to try to find out if taurine can offer any protection against the oxytetracycline-induced fatty liver in albino tats.

The increase in relative liver weight in oxytetracycline treated (group B) animals was due to hypertrophy of hepatocytes and accumulation of fat as described in the morphological findings. These findings coincide with the study of Huang et al (2011)[22] who noted significant increase in the relative liver weight in rats with fatty liver induced by high fat diet.

The significant decrease in relative weight of liver in group-C animals as compared to the group-B can well be due to the antioxidant effect of taurine administration. In this group there was a reduction in the deposition of fat (fatty change) in liver and a decrease in the swelling of hepatocytes. Chen et al (2006)[18] observed that taurine treatment resulted in a significant decrease in liver weight, liver index and plasma lipid and glucose levels, and oxidative stress in experimentally induced non-alcoholic steatohepatitis in rats.

The serum levels of liver enzymes (SGPT, SGOT, ALP) were found to be significantly (P value < 0.01) raised in both groups B and C as compared to group D.

The damage to hepatocytes increases the permeability of the cell membrane with the resultant leakage of the cytosolic enzymes into the sinusoids and thence into circulation. The increase in serum hepatic enzymes is in agreement with the findings of Helal et al (2011)[5,6]. The authors observed that the administration of oxytetracycline caused highly significant increase in the activity of SGPT and SGOT. The elevated values of serum hepatic enzymes also correlated with the histological findings in the present study.

The significant decrease (P value <0.01) of these enzymes in the group-C animals speak of the membrane protection offered by taurine administration and are in conformity with the findings of Waters et al (2001)[11], Dorgru-Abbasoglu et al (2001)[17], and Jagadeesan and Pillai (2007)[24]. They noted significant decrease in the serum hepatic enzymes, after taurine treatment, in the liver toxicity induced by acetaminophen, thioacetamide, and mercury, respectively.

The morphological examination of H&E stained sections of liver in group-B animals demonstrated microvesicular fatty change and swelling of hepatocytes. Some ballooned hepatocytes were also seen. The findings coincide with a number of studies[4, 5, 6], where oxytetracycline has been used to induce experimental steatosis in rats.

The morphological examination of H&E stained sections of liver in group-C demonstrated that hepatic lobular architecture was comparable to control. The fatty change was of a much lesser degree than in group B and the fat vacuoles were very much reduced in size. These findings can be attributed to the antioxidant, membranoprotective and detoxifying properties of taurine. These findings match with the findings of Chen et al[18], who observed significant improvement in both histological and biochemical parameters in the experimentally induced nonalcoholic steatohepatitis in rats fed on high fat diet. Kerai et al[19] suggested that the taurine-induced reversal of hepatic steatosis in the ethanol treated rats is due to increased triglycerides secretion from the liver. They also suggested that an increased bile flow enhances the removal of peroxides.

CONCLUSION

This study suggests that the attenuation of fatty change by taurine administration is a finding of great importance. As it has been shown that non alcoholic fatty liver disease can progress into steatohepatitis and cirrhosis, dietary supplementation of taurine to patients receiving drugs such as oxytetracyclie can save them from fatty liver disease. Further studies are needed to confirm the findings on this topic.

REFERENCES

1. Katzung B, Masters S, Trevor A. Basic and Clinical Pharmacology. 11th ed. Lange: Mc Graw Hill; 2009.p.596-97.
2. Sturgll MG, Lambart GH. Xenobiotic-induced hepatotoxicity: mechanisms of liver injury and methods of monitoring hepatic function. Clin Chem 1997; 43(8B):1512–26.
3. Pari L, Gnanasoundari M. Influence of naringenin on oxytetracycline mediated oxidative damage in rat liver. Basic and Clinical pharmacol & toxicol 2006; 98: 456-61.
4. Saraswat B, Viseu PKS, Patnaik GK, Dhawan BN. Protective effects of picroliv active constituent of picroorhiza kurrooa against oxytetracycline induced hepatic damage. Indian J Exp Biol 1997; 35:1302-5.
5. Helal EG, Abd El-Wahab SM, Zedan GA. Effect of curcuma longa L. On fatty liver induced by oxytetracycline in albino rats. The Egyptian J Hosp Med 2011; 43:109-20.
6. Helal EG, Abd El-Wahab SM, Sharaf AM, Zedan GA. Effect of Cichorium intybus L. On fatty liver induced by oxytetracycline in albino rats. The Egyptian J Hosp Med. 2011; 45:522–35,
7. Wong VW, Vergniol J, Wong GL, Foucher J, Chan HL, Bail BL, et al, Victor de Ledinghen. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in non alcoholic fatty liver disease. Hepatology 2010; 51: 454-62.
8. Das J, Ghosh J, Manna P, Sil PC. Protective role of taurine against arsenic-induced mitochondria-dependent hepatic apoptosis via the inhibition of PKC§-JNK Pathway, PloS ONE 5:(9):e 12602.
9. Mozaffari MS. Taurine modulation of renal excretory function. Thai J Physiol Sci 2003;16(3): 83-90.
10. Sato S, Kurasaki M. The physiological role of taurine in tissues and organs, especially in the liver and kidney, Foods. Food Ingr J Jpn 2003:208(2).
11. Waters E, Wang JH, Redmond HP, Wu QD, Kay E, Bouchier-Hayes D. Role of taurine in preventing acetaminophen-induced hepatic injury in the rat, Am J Physiol Gastrointest Liver Physiol 2001; 280:1274-9.
12. Sener G, SehiriliO, Ipci Y, Centinel S, Cickler E, Gedik N, et al. Protective effects of taurine against nicotine-induced oxidative damage of rat urinary bladder and kidney. Pharmacol 2005; 74:37-44.
13. Abdallah AR, Gado AM, Al-Majed AH, Al-Yahya AAA, Al-Shabanah AO. Protective effect of taurine against cyclophoshamide induced urinary bladder toxicity in rats. Clin Exp Pharmacol Physiol 2005; 32: 167-72.
14. Mankovskaya IN, Serebroyskaya T, Swanson JR, Vavilova LG, Kharlamova ON. Mechanisms of taurine antihypoxic and antioxidant action, High Alt Med Biol 2000;1:105-10.
15. Hagar HH. The protective effect of taurine against cyclosporine A – induced oxidative stress and

hepatotoxicity in rats. Toxicol Letter 2004; 151: 335-43.
16. Nabila, Hassan S, Nagla F, et al. Histopathological and histochemical studies on the effect of taurine in preventing carbon tetrachloride –induced hepatic injury in the albino rat. The Egyptian J Hosp Med 2003;10:52–65.
17. Dorgru-Abbasoglu S, Kanbagli O, Balkan J, Cevikbas U, Aykac-Tokeri, Uysall. The protective effect of taurine against thioacetamide hepatotoxicity of rats, Hum Exp Toxicol 2001; 20:23-27.
18. Chen SW, Chen YX, Shi J, Lin Y, Xie WF. The restorative effect of taurine on experimental nonalcoholic steatohepatitis. Dig Dis Sci 2006; 51: 2225-34.
19. Kerai MDJ, Waterfield CJ, Kenyon SH, Asker DS, Timberell JA. Reversal of ethanol-induced hepatic steatosis and lipid peroxidation by taurine: a study in rats. Alcohol & Alcoholism 1999;34:.529-41.
20. Das SK, Vasudevan DM. Drugs and non-alcoholic steatohepatitis, Ind J Pharmacol 2006; 38:238-42.
21. Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. Hepatology 2010;51: 1972-8.
22. Huang Y, Fu JF, Shi HB, Liu LR. Metformin prevents non-alcoholic fatty liver disease in rats: role of phospholipase A2/lysophosphatidylcholine lipoapoptosis pathway in hepatocytes. Zhonghua Er Ke Za Zhi 2011;49(2):139-45.
23. Jagadeesan G, Pillai SS. Hepatoprotective effects of taurine against mercury induced toxicity in rats. J Environ Biol 2007; 28(4):753-6.

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CORRIGENDUM

The name of the College / Hospital mentioned with the names of Abid Ali, Assoc. Prof of Anatomy and Iftikharud Din, Assoc. Prof. of Pharmacology appeared at Sr. Nos. 1 & 3 respectively in the article "Protective Role of Taurine on Tamoxifen-induced liver damage in Rats: A Morphological Study", published in the Medical Forum Monthly, in the month of August 2012 at pages 6-9 may be read as Kabir Medical College, Peshawar instead of Khyber Medical College, Peahawar.

Chief Editor.