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COMPARISON OF BODY AND LIVER WEIGHTS IN ALBINO RATS AFTER TREATMENT WITH TAMOXIFEN AND TAURINE

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ABSTRACT

Objective: To compare the mean body weights and mean absolute and relative liver weights (in Grams) of albino rats after tamoxifen and taurine treatment.

Material and Methods: This prospective experimental study was conducted in the Department of Anatomy, Khyber Medical College, Peshawar from July 2011 to December 2011. Sixty adult female albino rats were divided into four groups (A-control, B-tamoxifen-treated, C tamoxifen plus taurine-treated, and D-taurine treated). Each group was further divided into three subgroups (1, 2, 3) according to the period of treatment which was one, three and six weeks, respectively. Body weights were recorded at the commencement of the study. At the end of the respective period of treatment, the body weights were recorded again and the animals were sacrificed under deep ether anesthesia. By a midline incision, the anterior abdominal wall was opened. The livers were removed, washed with normal saline, and their weights were recorded.

Results: The data show a moderately significant increase in final body weight in control groups A1 and A2 ($P<0.01$) and a highly significant increase ($P<0.001$) in final body weight in control group A3, respectively, as compared to the initial weight in these groups. There was a highly significant decrease ($P<0.001$) in the final body weight of groups B1, B2 and B3 when compared with the initial body weights within the group and with the control groups A1, A2 and A3 respectively. The data shows a moderately significant increase ($P<0.01$) in final body weights of C1 and C3 when compared with B1 and B3 respectively and a significant increase ($P<0.05$) in the final weights of C2 when compared with tamoxifen treated group B2. The mean absolute liver weight showed significant increase (P value < 0.05) in group B1 and B3, and a moderately significant increase ($P<0.01$) in group B2, when compared with corresponding control groups A1, A2 and A3. There was insignificant decrease in the mean absolute liver weight in group C1 ($P>0.5$), highly significant decrease ($P= 0.001$) in group C2 and highly significant decrease ($P<0.001$) in group C3 when compared with groups B1, B2 and B3 respectively. The mean relative weight was increased significantly (P value <0.05) in group B1 and B2 and highly significantly (P value <0.001) in B3, when compared to control groups A1, A2 and A3 respectively. Compared to groups B1, B2 and B3, the mean relative liver weight was decreased moderately significantly ($P<0.01$) in group C1, significantly ($P<0.05$) in C2 and highly significantly ($P<0.001$) in C3, respectively.

Conclusion: Dietary taurine supplementation reduces the loss in mean body weight and decreases the increase in absolute and relative liver weights brought about by tamoxifen treatment in albino rats.

Key Words: Tamoxifen, Taurine, Mean absolute body weight, Liver weight.

INTRODUCTION

Liver damage caused by medication ingestion, also known as drug induced hepatotoxicity (DIH) has become an important public health problem[1]. Predictable drug reactions are ascribed to acetaminophen, tetracycline, antineoplastic agents, Amanita phalloides toxin, carbon tetrachloride, and alcohol; many others, such as chlorpromazine, suphonamides, methyldopa, and allopurinol, cause idiosyncratic reactions[2].

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Cases of tamoxifen- induced hepatotoxicity have been described, including toxic hepatitis, massive hepatic steatosis or multifocal hepatic fatty infiltration, submassive hepatic necrosis and even cirrhosis in humans[3,4]. Tamoxifen-induced fatty liver is observed in more than 30% of breast cancer patients who receive adjuvant tamoxifen treatment[5]. Tamoxifen initiates the process of lipid peroxidation by subtraction of hydrogen from unsaturated fatty acids to form carbon-centered lipid radicals; the addition of molecular oxygen to the carbon centered lipid radicals form lipid peroxy radicals[6].

Tamoxifen is a potent hepatocarcinogen in both male and female rats[7,8]. Tamoxifen itself is not genotoxic but is activated in the liver to alpha–hydroxytamoxifen. This is further conjugated to form

the sulfate esters as the putative reactive intermediates[9]. A number of biological agents have been found to protect the liver from the harmful effects of drugs used for the treatment of different disease conditions. Zinc alleviates the toxic effects of nickel in rat liver[10]. Antioxidants such as vitamin E and C attenuate hepatic fibrosis in biliary-obstructed rats[11]. Selenium, a dietary micronutrient, is particularly protective in limiting the action of diethylnitrosamine during the initiation phase of hepatocarcinogenesis[12].

Taurine (2-aminoethanesulfonic acid), is a suphur-containing non essential amino acid found in many tissues; it is synthesized in the liver as an end product of L-cysteine metabolism[13]. Its protective role against tissue injury has been reported by many authors and the physiological actions attributed to taurine include cell membrane stabilization, antioxidation, osmoregulation and bile acid conjugation[14,15]. In rats, taurine attenuates the oxidative stress and injury in the urinary bladder and kidney induced by nicotinamide[16]. It reduces the severity of cyclophosphamide - induced hemorrhagic cystitis[17], and ameliorates the hypoxia induced lactic acidosis in brain, liver and heart[18].

In liver taurine attenuates the injury induced by agents such as cyclosporine A[19], carbon tetrachloride[20] and thioacetamide[21]. Its restorative role in experimentally induced non - alcoholic steatohepatitis has been observed[22]. 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[23]. The purpose of this work was to study the effects of heavy doses of tamoxifen on the total mean body weights, mean absolute liver weight and mean relative liver weight as markers of toxicity in albino rats, and to study the beneficial role, if any, played by taurine, a sulphur containing amino acid, on these effects.

MATERIAL AND METHODS

Sixty healthy adult female albino rats 90-120 days of age and 200-300 gram in weight were divided into four groups (A-control, B-tamoxifen-treated, C-tamoxifen plus taurine-treated, and D-taurine treated), and each group was divided into three subgroups (1, 2 and 3), on the basis of period of treatment (one, three and six weeks, respectively). Each subgroup comprised of 5 animals.

All subgroups A1, A2 and A3 were fed with the standard laboratory chow and water ad libitum and received no treatment. All subgroups B1, B2 and B3, received tamoxifen orally in a daily dose of 45 mg per kilogram body weight[6]. All Subgroups C1, C2 and C3 received tamoxifen orally in a daily dose of 45 mg per kilogram body weight, plus 1% taurine solution as the sole source of drinking water. All subgroup D1, D2 and D3 received 1% taurine solution as the sole source of drinking water.

The body weights of animals were recorded at the start of treatment and at the time of sacrifice. On next day of the last dose in different subgroups, the rats were sacrificed by deep ether anesthesia. Blood samples were collected by intra cardiac puncture for estimation of serum liver enzymes (SGPT, SGOT and ALP). Livers were removed and their weights recorded. They were fixed in 10% formalin and embedded in paraffin. 4 micron thick sections were stained with hematoxylin and eosin for microscopic examination. Statistical analysis of the data was done and results tabulated.

RESULTS

In all parameters the results were similar and comparable between group A (control) and D (taurine treated) with no significant differences across the groups. So the data is given only for groups A, B and C.

Mean absolute body weight: The mean values of initial body weight in control groups A1, A2 and A3 were 265.40 ± 6.02, 278.60 ± 3.00 and 260.20 ± 2.15 grams respectively. The mean values of final body weight in control groups A1, A2 and A3 were 274.60 ± 6.02, 285.20 ± 2.08 and 271.40 ± 2.2.44 grams respectively. The data show a moderately significant increase in final body weight in control groups A1 and A2 ($P<0.01$) and a highly significant increase ($P<0.001$) in final body weight in control group A3 respectively as compared to the initial weight in these groups, respectively.

The mean values of initial body weight in tamoxifen treated groups B1, B2 and B3 were 265.00 ± 6.72, 273.60 ± 6.49 and 269.60 ± 9.08 grams respectively. The mean values of final body weight in groups B1, B2 and B3 were 232.80 ± 7.05, 233.20 ± 5.21 and 230.40 ± 7.06 gram respectively. The data show a highly significant decrease ($P<0.001$) in the final body weight of groups B1, B2 and B3 when compared with the initial body weights within the group and with the control groups A1, A2 and A3 respectively (Table 1).

The mean values of initial body weights of tamoxifen plus taurine treated groups C1, C2 and C3 were 271.80 ± 8.35, 271.20 ± 7.47 and 269.40 ± 8.41 grams respectively. The mean values of the mean final body weight in groups C1, C2 and C3 were 259.80 ± 7.84, 258.20 ± 8.19 and 259.20 ± 9.81 grams respectively. When compared with their initial body weights there was highly significant decrease ($P<0.001$) in the mean final body weights of groups C1, C2 and C3.

The data show a moderately significant increase ($P<0.01$) in mean final body weights of C1 and C3 when compared with B1 and B3 respectively, and a significant increase ($P<0.05$) in the mean final weights of C2 when compared with group B2. The data also show that there was insignificant decrease ($P>0.5$) in the final body weights in groups C1 and C3 and a

Table 1: Statistical Analysis of differences in mean body weight across different groups

Groups	P-Value
A1vs B1	P<0.01***
A1vs C1	P>0.05*
B1vs C1	P<0.05**
A2 vs B2	P<0.001****
A2 vs C2	P<0.05**
B2 vs C2	P<0.01***
A3 vs B3	P<0.01***
A3 vs C3	P>0.05*
B3 vs C3	P<0.05**

Key (P value):*Insignificant **Significant ***Moderately significant ****Highly significant

Table 2: Statistical analysis of mean absolute weight of liver between different groups

Groups	P-value
A1 vs B1	<0.05**
A1 vs C1	>0.05*
B1 vs C1	<0.05**
A2 vs B2	<0.01***
A2 vs C2	<0.05**
B2 vs C2	=0.001****
A3 vs B3	<0.05**
A3 vs C3	<0.01***
B3 vs C3	<0.001****

Key: *Insignificant **Significant ***Moderately significant ****Highly Significant

moderately significant decrease (P<0.01) in group C2, when compared with the corresponding control groups A1, A3 and A2 respectively.

Mean absolute liver weight: The mean values of absolute liver weight in control groups A1, A2 and A3 were 10.66 ± 0.50, 10.50 ± 0.20 and 10.70 ± 0.13 grams respectively. The mean values of absolute liver weights in tamoxifen treated group B1, B2 and B3 were 11.12 ± 0.31, 12.25 ± 0.12 and 11.25 ± 0.30 grams respectively. This data show that there was significant increase (P<0.05) in the absolute liver weights of tamoxifen treated groups B1and B3 in comparison with

Table 3: Statistical Analysis of mean relative weight of liver between different groups

Groups	P-Value
A1 vs B1	P<0.05**
A1 vs C1	P>0.05*
B1 vs C1	P<0.01***
A2 vs B2	P<0.05**
A2 vs C2	P>0.05*
B2 vs C2	P<0.05**
A3 vs B3	P<0.001****
A3 vs C3	P=0.001***
B3 vs C3	P<0.001****

Key (P value): *Insignificant **Significant ***Moderately significant ****Highly Significant

control group A1 and A3 respectively, while there was a moderately significant increase (P<0.01) in the mean absolute liver weight in the tamoxifen treated group B2 as compared to control group A2 (Table 2).

The mean values of absolute liver weight in the tamoxifen plus taurine treated group C1, C2 and C3 were 10.11 ± 0.37, 10.01 ± 0.13 and 9.11 ± 0.17 grams respectively. This data shows insignificant decrease in mean liver weight (P>0.05) in group C1, a significant decrease (P<0.05) in group C2 and moderately significant decrease (P<0.01) in mean liver weight in group C3 in comparison with the corresponding control groups A1, A2 and A3, respectively, while there was significant decrease (P<0.05) in group C1, highly significant decrease (P= 0.001) in group C2 and highly significant decrease (P<0.001) in the mean absolute liver weight in group C3 when compared with the corresponding tamoxifen treated groups B1, B2 and B3 respectively, as shown in Table 3.

Mean relative liver weight: The mean values of relative liver weight in control group A1, A2 and A3 were 3.87 ± 0.10, 3.66 ± 0.05 and 3.93 ± 0.02 respectively. The mean values of relative liver weight in tamoxifen treated groups B1, B2 and B3 were 4.24 ± 0.08, 4.99 ± 0.30 and 4.88 ± 0.01 grams respectively. The data show that there was significant increase (P <0.05) in the relative liver weights in group B1, and B2 and a highly significant increase (P <0.01) in B3, when compared to the corresponding control groups A1, A2 and A3 respectively, as shown in Table 4.

The mean values of relative liver weight in tamoxifen plus taurine treated groups C1, C2 and C3 were 3.89 ± 0.07, 3.57 ± 0.06 and 3.31 ± 0.09 grams respectively. This data show that there was insignificant decrease (p>0.05) in mean relative liver

weight in groups C1 and C2 and highly significant decrease (P=0.001) in group C3, when compared with the corresponding control groups A1, A2 and A3 respectively, while there was moderately significant decrease (P<0.01) in mean relative liver weight in group C1, significant decrease (P<0.05) in C2 and highly significant decrease (P<0.001) in C3 when compared to the tamoxifen treated groups B1, B2 and B3 respectively.

DISCUSSION

The body weights of animals decreased significantly in groups B and C. This was due to the decreased food intake in the animals of these groups and was in conformity with the findings of Lelliott et al (2005)[25]. They observed that tamoxifen was known to reduce food intake and body weight in rats. Lopez et al also observed that tamoxifen causes a tremendous decrease in appetite via its direct action on the ventromedial nucleus of the hypothalamus. The authors noted a decrease of 5 gram in body weight over a period of only 5 days in rats treated with tamoxifen in a dose as low as 0.5 mg per kg per day[26]. Hard et al (1993)[27], noted progressive decrease in body weights starting from 5th week through a study period of 15 months[27]. Greaves et al in his work on the two year carcinogenicity study of tamoxifen in rats, noted that right from week one, growth of all rats given tamoxifen was impaired compared with controls and this was associated with lower food consumption with males generally more affected than females.

In the present study, the animals in group C showed significant increase in body weight in comparison with group B animals in the three-week study while moderately significant increase was noted in the one-week and six-weeks study groups. This was because of the reduction of tamoxifen toxicity due to taurine treatment, as was evident from the other observations in this study. Hwang et al in their study on the effect of taurine on the toxicity of oxidized fish oil in rats, suggested that taurine could improve the decrease in body weight and in the glutathione level in the liver, and that taurine may play an important role in reducing the toxic effects of oxidized fish oil in rats[28].

The increase in the absolute and relative liver weight in animals of group B was due to the toxicity of tamoxifen causing hypertrophy of hepatocytes and accumulation of fat. This finding matches with the study of Hard et al who noted a significant increase in both absolute and relative liver weight in rats treated with tamoxifen over a 12 months, although the authors referred this to the presence of masses in liver.

The significant decrease in absolute and relative weight of liver in group-C animals as compared to the group-B can well be due to the protection provided by 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. This finding of decrease in the absolute and relative liver weight is in agreement with the work of Chen et al The authors observed that taurine treatment resulted in a significant decrease in liver weight, serum transaminase activities, serum triglycerides, fasting plasma glucose and oxidative stress, in experimentally induced non-alcoholic steatohepatitis in rats[22].

CONCLUSION

The study data suggest that concomitant taurine administration can significantly improve the loss of body weight due to tamoxifen treatment in albino rats. It also suggests that the increase in the absolute and relative liver weights can be reversed/ attenuated in these animals by taurine supplementation of their diet. Further studies are needed to confirm these findings and to generalize their application to the humans.

REFERENCES

1. Bonacini M, Miyashita L. Drug induced Hepatotoxicity cases on the rise: Expanding pharmaceuticals and alternative medicines contribute to the problem [letter]. Liver GI Rev 2002: 10.
2. Kumar V, Abbas AK, Fausto N, Mitchel RN. Robbin's basic pathology 8th ed. Philadelphia: W.B. Saunders, 2007: pp 652-53.
3. Nemoto Y, Saibara T, Ogawa Y, Zhang T, Xu N, Ono M, et al. Tamoxifen-induced nonalcoholic steatohepatitis in breast cancer patients treated with adjuvant tamoxifen. Internal Medicine 2002: 41: 345-50.
4. Pratt DS, Knox TA, Erban J. Tamoxifen induced steatohepatitis [letter]. Ann Intern Med 1995; 123: 236.
5. Tabassum H, Rehman H, Banerjee BD., Raisuddin S, Parvez S. Attenuation of tamoxifen induced hepatotoxicity by taurine in mice, Clinica Chimica Acta 2006; 370: 129-136.
6. El-Beshbishy H. Hepatoprotective effect of Green tea (Camellia sinensis) extract against tamoxifen-induced liver injury in rats. J Biochem Mol Biol; 2005; 5: 563-70.
7. Greaves P, Goonetilleke R, Nunn G, Topham J, Orton T. Two year carcinogenicity study of tamoxifen in Alderly Park Wistar-derived rats. Cancer Res; 1993; 53: 3919-24.
8. Davis W., Hewer A, Rajkowski KM, Meinl W, Glatt H, Phillips DH. Sex differences in the activation of tamoxifen to DNA binding species in rat liver in vivo and in rat hepatocytes in vitro: role of sulfotransferase induction. Cancer Res; 2000; 60: 2887-91.
9. White IN. Tamoxifen: Is it safe? Comparison of activation and detoxification mechanisms in rodents and in humans, Curr drug metabol 2003; 4: 223-39.

10. Sidhu P, Garg ML, Dhawan DK. Protective role of zinc in nickel induced hepatotoxicity in rats, Chem-Biol Interact 2004; 150: 199-209.

11. Soylu AR, Aydogdu N, Basaran UN, Altaner S, Tarcin O, Gedik N, et al. Antioxidants vitamin E and C attenuate hepatic fibrosis in biliary obstructed rats, World J Gastroenterol. 2006 12: 6835-41.

12. Thirunavukkarasu C., Jagadeeswaran R., Babu E., Sakthisekaran D. Inhibitory effect of selenium on N-nitrosodiethylamine and Phenobarbital-promoted rat liver carcinogenesis, J Clin Biochem and Nutr 2000; 28: 69-80.

13. 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.

14. Mozaffari,M.S. Taurine modulation of renal excretory function, Thai J Physiol Sci 2003; 16: 83-90.

15. 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: 1274-79.

16. Sener G, Sehirli O, Ipçi Y, Centinel S, Cikler 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.

17. 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.

18. 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.

19. Hagar HH. The protective effect of taurine against cyclosporine A – induced oxidative stress and hepatotoxicity in rats. Toxicol Letter, 2004; 151: 335-43.

20. Nabila, Hassan S, Nagla, Abbas F. Hafiza, Sharaf A. 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.

21. Dorgru- AS, 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.

22. 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.

23. Kerai MD, Waterfield CJ, Kenyon HK, 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.

24. You JS, Chang KJ. Taurine protects the liver against lipid peroxidation and membrane disintegration during rat hepatocarcinogenesis. Adv Exp Med Biol. 1998; 442: 105-12.

25. Lilliott CJ, Lopez M, Curtis RK, Parker N, Laudes M, Yeo G et al. Transcript and metabolite analysis of the effects of tamoxifen in rat liver reveals inhibition of fatty acid synthesis in the presence of hepatic steatosis, FASEB J 2005; 19: 1108-19.

26. Lopez M., Lelliott CJ, Tovar S, Kimber W. Tamoxifen induced anorexia is associated with fatty acid synthase inhibition in the ventromedial nucleus of the hypothalamus and accumulation of malonyl- CoA, Diabetes 2006; 55: 1327-36.

27. Hard GC, Iatropoulos MJ, Jordan K, Kaltenberg OP, Imondi AR Williams GM. Major difference in the hepatocarcinogenecity and DNA adduct forming ability between toremifene and tamoxifen in female rats. Can Res 1993; 53: 4534-41.

28. Hwang DF, Hour JL, Cheng HM. Effect of taurine on toxicity of oxidized fish oil in rats, Food Chem toxicol 2000; 38: 585-91.

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