Original Article

Anatomy

Comparative Study of Hepatic Serum Enzymes in Albino Rats after treatment with Tamoxifen and Taurine

1. Abid Ali 2. Kausar Tasneem 3. Naikzada 4. Qaisar Inayat

1. Prof. of Anatomy, KMC, Peshawar. 2. Assoc. Prof. of Biochemistry, KMC, Peshawar

3. Asstt. Prof. of Anatomy, SMU, Swat 4. Prof. of Anatomy, KMC, Peshawar

ABSTRACT

Objective: to compare the mean values of serum hepatic enzymes of albino rats after treatment with tamoxifen and taurine.

Study Design: A prospective experimental study.

Place and duration of study: This study was conducted in the Department of Anatomy, Khyber Medical College, Peshawar from July 2011 to December 2011.

Materials and Methods: 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 and end of the study period. At the end of the respective period of treatment the animals were sacrificed under deep ether anesthesia. By a midline incision, the anterior abdominal wall was opened. Blood samples were collected with 5cc syringes by cardiac puncture for estimation of serum hepatic enzymes (serum glutamic pyruvate transaminase-SGPT, Serum glutamic oxaloacetic transaminase (SGOT), and serum alkaline phosphatase (ALP). The livers were removed, washed with normal saline, and their weights were recorded. Formalin fixed, paraffin embedded 4 micron thick sections were stained with H&E for histopathological examination.

Results: The mean values (I.U/L) of SGPT levels of albino rats in control groups A1, A2 and A3 were 40.83±1.62, 42.02±0.95 and 41.09±0.87 respectively, and in the tamoxifen-treated groups B1, B2 and B3 were 155.2±12.47, 243.60±11.96 and 277.60±12.84 respectively. This data show a highly significant increase ($P<0.001$) in the SGPT levels in all the B-subgroups (tamoxifen – treated) albino rats, in comparison with the control subgroups. The mean values (I.U/L) of SGPT in the tamoxifen plus taurine treated groups C1, C2 and C3 were 67.99± 1.89, 107.77± 2.28 and 137.93±8.29. Compared to the corresponding B subgroups, the data show highly significant decrease ($P<0.001$) in SGPT levels in groups C1 and C3, and moderately significant decrease ($P<0.01$) in SGPT levels in group C2.

The mean values of serum levels (I.U/L) of SGOT in control groups A1, A2 and A3 were 38.66±0.89, 37.12±1.15 and 38.52±1.74 respectively. The mean values of SGOT in subgroups B1, B2 and B3 were 73.60±4.98, 152.48±13.00 and 247.40±18.53 I.U/L respectively. Thus there was moderately significant increase ($P<0.01$) in SGOT levels in B1, and highly significant increase ($P<0.001$) in B2 and B3 as compared to the control subgroups. The mean values of SGOT in subgroups C1, C2 and C3 were 65.20±3.15, 109.20±4.83 and 124.98 ± 5.83 respectively. The data show insignificant decrease in SGOT level in subgroup C1 ($P>0.05$), significant decrease in C2 ($P<0.05$), and moderately significant decrease ($P<0.01$) in C3 as compared to subgroups B1, B2 and B3, respectively.

The mean values (I.U/L) of serum ALP in the control groups A1, A2 and A3 were 146.60±17.73, 196.40±22.47 and 164±24.60 respectively, and in the tamoxifen-treated groups B1, B2 and B3 were 436.80±30.92, 467.80±15.43 and 684.20±18.64 respectively. This shows highly significant increase in the serum levels of ALP ($P<0.001$) in all B subgroups. The mean values (I.U/L) of serum ALP in subgroups C1, C2 and C3 were 394.20±20.79, 376.60±20.02 and 420.00±14.66 respectively. This data show that there was insignificant decrease ($P>0.05$) in serum ALP in C1, moderately significant decrease ($P<0.01$) in C2 and highly significant decrease ($P<0.001$) in C3, when compared with B1, B2, and B3, respectively.

Conclusion: Concomitant taurine administration lowers the mean values of serum hepatic enzymes in albino rats treated with heavy doses of tamoxifen.

Key words: tamoxifen, taurine, SGPT, SGOT, serum ALP.

INTRODUCTION

Drug induced liver damage (drug induced hepatotoxicity-DIH) is an important public health problem[1]. There are about 900 medications which are potentially hepatotoxic[2]. The drugs notorious for predictable hepatotoxicity include acetaminophen, tetracyclines, antineoplastic agents, Amanita phalloides toxin, carbon tetrachloride, and alcohol; many others,

such as chlorpromazine, suphonamides, methyldopa, and allopurinol, cause idiosyncratic reactions[3].

Tamoxifen citrate is a non steroidal anti-estrogen drug used for the treatment and prevention of breast cancer[4]. 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[5,6]. 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[7]. It appears that tamoxifen causes hepatic steatosis by causing mitochondrial dysfunction; this leads to impaired β-oxidation of fatty acids, production of reactive oxygen species and depletion of ATP[8]. Also its metabolic product, alpha–hydroxytamoxifen, is conjugated to form the sulfate esters and is genotoxic hepatocarcinogen[9].

A number of biological agents 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 non essential amino acid found in many tissues; it is synthesized in the liver as an end product of L-cystein metabolism[13]. It has been shown to protect many tissues from oxidative injury by cell membrane stabilization, osmoregulation, neuromodulation, and bile acid conjugation[14,15].

In rats taurine attenuates the 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], acetaminophen[15], 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 serum hepatic enzymes as markers of hepatic injury, and to study the beneficial role, if any, played by taurine, a sulphur containing amino acid, against this injury, in albino rats..

MATERIALS 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, B, C and D, each group comprising of fifteen rats. Each group was then divided into three subgroups (1, 2, and 3), according to the period of treatment (one, three and six weeks, respectively). All the animals were fed on the standard laboratory chow and kept under natural environment.

Group-A (subgroups A1, A2 and A3) received no treatment. Group-B (subgroups B1, B2 and B3, each comprising of 5 albino rats), received tamoxifen orally in a daily dose of 45 mg per kilogram body weight[24]. Group-C (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[19, 24]. Group-D (subgroup D1, D2 and D3) received only 1% taurine solution as the sole source of drinking water.

On next day of the last dose in different subgroups, the rats, after recording the body weight of each animal, were anaesthetized by deep ether anesthesia. Blood samples were collected by cardiac puncture using a 5 cc disposable syringe and were sent to the laboratory for estimation of serum liver enzymes (SGPT, SGOT and ALP) through analyses kit provided by Human Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany.

Livers were removed and their weights recorded. They were fixed in 10 % formalin and embedded in paraffin. H&E stained 4 micron thick sections were made 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.

Serum analysis of hepatic enzymes: The serum analysis of liver enzymes, serum glutamic-pyruvic transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT), and alkaline phosphatase between the treated and control groups during variable periods of time was done for assessment of hepatic tissue injury and its correlation with morphological changes.

Observations on SGPT Level (Tables 1 and 2):

The mean values (I.U/L) of SGPT levels of albino rats in control groups A1, A2 and A3 were 40.83±1.62, 42.02±0.95 and 41.09±0.87 respectively. The mean values (I.U/L) of SGPT in the tamoxifen treated groups B1, B2 and B3 were 155.2±12.47, 243.60±11.96 and 277.60±12.84 respectively. This data show that there was highly significant increase ($P<0.001$) in the SGPT

levels in all subgroups of tamoxifen - treated (group B) albino rats in comparison with the control subgroups.

The mean values (I.U/L) of SGPT in the tamoxifen and taurine treated groups C1, C2 and C3 were 67.99± 1.89, 107.77± 2.28 and 137.93±8.29. This data shows a highly significant increase (P<0.001) in SGPT levels in Groups C1, C2 and C3 animals in comparison to the corresponding subgroups of control animals. The data shoes that there was highly significant decrease (P<0.001) in SGPT levels in groups C1 and C3, and moderately significant decrease (P<0.01) in SGPT levels in group C2 when compared to the corresponding subgroups of tamoxifen treated group B animals.

Table No.1: *Mean Levels of SGPT (I.U/ L) in different groups at variable time interval

Groups	Week 1	Week 3	Week 6
A	40.83±1.63	42.02±0.95	41.09 ±0.87
(n=15)	(n=5)	(n=5)	(n=5)
B	155.2 ±12.47	243.6 ±11.96	277.6 ±12.84
(n=15)	(n=5)	(n=5)	(n=5)
C	67.99±1.89	107 ±2.28	137.93 ±8.29
(n=15)	(n=5)	(n=5)	(n=5)

*Mean±SEM. Normal value: 0-45 I/U

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

Table No.2: Statistical analysis of differences in mean serum levels of SGPT across different groups

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

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

Observations on SGOT Level (Tables 3 and 4):

The mean values of serum levels (I.U/L) of SGOT in control groups A1, A2 and A3 were 38.66±0.89, 37.12±1.15 and 38.52±1.74 respectively. The mean values of SGOT in the tamoxifen treated groups B1, B2 and B3 were 73.60±4.98, 152.48±13.00 and 247.40±18.53 I.U/L respectively. The data indicate a moderately significant increase (P<0.01) in B1, and highly significant increases (P<0.001) in SGOT in B2 and B3 as compared to the corresponding control groups.

Table 3: *Mean Levels of SGOT (I.U/ L) in different groups at variable time interval

Groups	Week 1	Week 3	Week 6
A	38.66 ±2.75	37.12 ±1.15	38.52 ± 1.74
(n=15	(n=5)	(n=5)	(n=5)
B	73.6 ±4.98	152.48 ±13.00	247.40± 18.53
(n=15)	(n=5)	(n=5)	(n=5)
C	65.20 ±3.15	109.20±4.83	124.20 ± 5.83
(n=15)	(n=5)	(n=5)	(n=5)

*Mean±SEM Normal value: 5-45 I/U

Table No.4: Statistical analysis of differences in mean serum levels of SGOT across different groups

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

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

Table No.5: *Mean Serum Levels of ALP (I.U/ L) in different groups at variable time interval

Groups	Week 1	Week 3	Week 6
A	146.6 ±17.73	196.4 ±22.47	164 ±24.60
(n=15	(n=5)	(n=5)	(n=5)
B	436.8 ±30.92	467.8 ±15.43	684.2 ±18.64
(n=15)	(n=5)	(n=5)	(n=5)
C	394.2 ±20.79	376.6 ±20.02	420 ±14.66
(n=15)	(n=5)	(n=5)	(n=5)

*Mean±SEM Normal value: 80-306 I/U

The mean values of SGOT in tamoxifen plus taurine treated groups C1, C2 and C3 were 65.20±3.15, 109.20±4.83 and 124.98 ± 5.83 respectively. This data shows that there was a highly significant increase in the levels of SGOT in these groups when compared to the corresponding control groups. In comparison to groups B1, B2 and B3, the difference was insignificant in C1 (P>0.05), significant decrease in group C2 (P<0.05), and moderately significant decrease (P<0.01) in group C3.

Table No.6: Statistical analysis of differences in mean serum levels of ALP across different groups

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

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

Observations on Serum Alkaline Phosphatase (ALP) Level (Table 5 and 6):

The mean values (I.U/L) of serum ALP in the control groups A1, A2 and A3 were 146.60±17.73, 196.40±22.47 and 164±24.60 respectively. The mean values (I/U) of serum ALP in the tamoxifen treated groups B1, B2 and B3 were 436.80±30.92, 467.80±15.43 and 684.20±18.64 respectively. This data shows that there was a highly significant increase in the serum levels of ALP (P <0.001) in all the group C subgroups when compared with the corresponding control subgroups.

The mean values (I.U/L) of serum ALP in the tamoxifen plus taurine treated groups C1, C2 and C3 were 394.20±20.79, 376.60±20.02 and 420.00±14.66 respectively. Compared with tamoxifen treated subgroups B1, B2 and B3, the data show that there no significant difference between Tamoxifen treated subgroup B1 and tamoxifen plus taurine treated group C1, while there was a moderately significant decrease in the serum levels of ALP (P<0.01) in tamoxifen plus taurine treated subgroup C2 and a highly significant decrease (P<0.001) in subgroup C3.

DISCUSSION

The significant increase in the levels of SGPT, SGOT and serum ALP groups B and C could be attributed to tamoxifen-induced damage to the hepatocytes which 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 conformity with El-Beshbishy (2005)[24], Gudbrandsen et al (2006)[25] and Czerny et al (2003)[26]. El-Beshbishy observed that the administration of high dose of tamoxifen citrate elicited a dramatic increase in the activity of SGPT and SGOT. According to Gudbrandsen et al, serum transaminases are regarded as reliable markers of hepatic steatosis and liver damage, and indeed, tamoxifen treatment increased the serum levels of SGPT and SGOT. D'Mello et al (1999)[27] observed an increase in the serum GPT, GOT and ALP due to tamoxifen treatment in female Wistar rats.

The significant decrease of these enzymes in the group-C animals represent the membrane protection offered by taurine administration. Dorgru-Abbasoglu et al (2001)[21] noted that concomitant taurine treatment diminished the severity of thioacetamide-induced liver injury by decreasing oxidative stress due to its possible scavenger effect, in rats. Waters et al (2001)[15] observed that when taurine was given to rats 12 hours before or simultaneously with acetaminophen, there was greatest significant improvement of SGOT, SGPT and serum ALP compared with the acetaminophen-only group. The results in the present study are in agreement with the findings of these authors. Our results are also in conformity with those of Kerai et al (1999)[23], who confirmed the protective role of taurine in ethanol – induced hepatic steatosis. They noted that the effects of taurine on reversing hepatic steatosis may be due to the enhanced secretion of hepatic triglycerides, and that increased bile flow as a result of taurine treatment may contribute to the removal of lipid peroxides.

CONCLUSION

The study data suggest that concomitant taurine administration can significantly lower the mean values of elevated serum levels of hepatic enzymes due to tamoxifen treatment in albino rats. Further studies are needed to confirm the results of this study and to see if these can be generalized to the humans also.

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Address for Corresponding Author:
Prof. Dr. Abid Ali,
Anatomy Department, Kabir Medical College,
Peshawar.
Cell # 0300-5980992
Email: faqir_abid@yahoo.com