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Biochemistry

Assessment of the Activity of Some Biochemical Markers in Serum of Adult Male Wistar Rats Administered with Bromazepam (Lexotan)

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Original Research Article

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Abstract: Bromazepam is one of the most commonly misused benzodiazepines across the globe. The activity of some biochemical parameters in serum of adult male wistar rats administered with bromazepam (Lexotan) was assessed. Sixty (60) wistar rats were used for this study. The rats were divided into five groups (A-E) containing twelve (12) animals. Group A served as control and received only distilled water while groups B-E served as the test groups and received 0.0016mg/kg, 0.0026mg/kg, 0.0036mg/kg and 0.0046mg/kg body weight of bromazepam respectively. The drug was administered orally for a period of four (4) weeks. Blood and liver samples were collected weekly and used for the estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK) and histopathology respectively using standard procedures. The result showed significant ($p<0.05$) difference increase in these parameters in the test groups when compared to the control. However, there was no significant difference ($p> 0.05$) in prostate specific antigen (PSA) in the test groups when compared to the control. Histopathological observation of the liver revealed no pathological alteration for the test groups in weeks 1 and 2 while mild pathological alteration was observed for weeks 3 and 4 when compared to control. From this study, it can be deduced that long time administration of bromazepam did not alter serum activity of PSA. However, prolong exposure to bromazepam may result to hepatotoxicity.
Keywords: bromazepam, lexotan, activity, serum, drug, misused.

INTRODUCTION

Drugs are chemical substances that form the cornerstone of therapy in human diseases. They are generally given for prevention, control or cure of diseases. Most drugs act by interacting with a cellular component called receptor [1]. The efficacy of a drug is measured by the degree of effect it is able to generate at a receptor site. Drugs that produce useful therapeutic effect may also produce unwanted or toxic effects [2].

Bromazepam has been reported to be effective in treating problems such as acute and chronic convulsion, inappropriate elimination associated with anxiety, urine marking or spraying, fear aggression as well as to stimulate appetite [3]. Other therapeutic uses include muscle relaxant, anxiolytic and sedative effects [4]. Unlike other benzodiazepines such as lorazepam, bromazepam has recorded low incidence of unsteadiness after administration of the drug [5]. Long term administration of Bromazepam can lead to adverse effects in the brain which ultimately led to addiction have been widely reported in humans [6] and animals [7]. Other adverse effects include ataxia, neonatal complication in pregnancy, memory impairment, paradoxical stimulant effect, impaired learning capacity, decreased libido and depression [8]. It is the most popular benzodiazepine involved in international poisoning [6]. Acute liver injury may be caused by a direct toxic effect of the drug or its metabolites in the liver cells, generating a dose-dependent effect, or by idiosyncratic drug reactions, which occur in experimental animals exposed to bromazepam [9].

Globally, there is stringent control on the usage of Bromazepam due to the risk it possesses. For instance, the United Kingdom committee on the review of medicine [10] recommended the use of Bromazepam for 2-4 weeks at low dosage. Furthermore, statistics have shown that globally, the use of benzodiazepines (bromazepam) for non-medical purposes is on the increase [11]. This poses a public health threat especially in Nigeria where self-medication is on the high side and many are ignorant of the adverse effects of bromazepam on body tissues.

Despite the increasing use, limited information however exists on the effect of bromazepam on metabolic enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline

phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK) and prostate specific antigen (PSA). Alteration of these enzymes is an indication of cellular changes associated with toxicity of bromazepam on the specific tissues. The aim of this study was to investigate the effects of bromazepam on biochemical markers of the liver (ALT, AST, ALP, LDH), cardiac marker (CK), prostate cancer marker (PSA) in serum as a function of Bromazepam activity.

MATERIALS AND METHODS

Experimental Animals

Sixty adult male wistar rats weighing 100g – 110g obtained from appreciably healthy rats from the animal house, department of biochemistry, University of Port Harcourt were used to investigate the effect of bromazepam on metabolic enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), prostate specific antigen (PSA). Acclimatization was carried out for one week prior to commencement of the study. The rats were kept in well ventilated cages and fed with commercial grower mash, manufactured by Top Feeds Ltd, Sapele, Delta State, Nigeria. Water and feed were administered *ad libitum.*

Experimental Protocol

The sixty male wistar rats used for this study were randomly allocated to five groups (A-E) consisting of twelve rats each. The first group was used as the control while the other groups were administered different doses of bromazepam. The study lasted for four weeks. The groupings are as follows.

Group A rats (control) were administered distilled water, Group B rats were administered 0.0016mg/kg Bromazepam daily, Group C rats were administered 0.0026mg/kg Bromazepam daily, Group D rats were administered 0.0036mg/kg Bromazepam daily and Group E rats were administered 0.0046mg/kg Bromazepam daily.

Drug Administration

Bromazepam tablets from F. Hoffmann-La Roche Ltd., Basel, Switzerland by Roche S.p.A Milan production site, Italy were used for this study. The drugs were orally administered to the rats at varying doses (0.0016 – 0.0046mg/100g body weight) once daily.

Sample Collection/ Preparation

Blood samples were collected from the rats in four phases.

Phase 1: This was carried out one week after treatment.

Phase 2: This was carried out two weeks after treatment.

Phase 3: This was carried out three weeks after treatment.

Phase 4: This was carried out four weeks after treatment.

At the end of each phase, three rats from each group were euthanized under chloroform vapor and dissected. Whole blood sample was collected from the heart cavity with syringe into plain sample bottles; the liver tissues were taken surgically and kept in a plain bottle containing 10% formalin for preservation prior to the histopathological processing. The bottles containing the blood samples were allowed to stand for one hour to enable the blood to clot and therefore centrifuged at 3,000g for 10 minutes. The serum obtained was used for biochemical assay. The samples were then analyzed following the different protocols for each enzyme.

Biochemical Tests

The serum obtained was used to assess aspartate aminotransferase activity using AST Randox test kits, alanine aminotransferase activity using ALT Randox test kits, alkaline phosphatase activity using ALP Randox test kits, creatine kinase activity using CK Randox test kits [12] and prostate specific antigen activity using PSA Accubind Elisa microwells kits [13] according to the manufacturer's instructions.

Histological Analysis

Microscopic observation of the liver of the experimental animals was carried out as described by Baker and Silverton [5].

DATA ANALYSIS

The data generated were analyzed for statistical differences between the different groups treated with different concentration of the drug by means of one-way ANOVA using SPSS software version 20. Differences were considered significant when $p<0.05$. Data were presented as mean ± S.E.M (standard error of mean).

RESULTS

Table-1: Effect of bromazepam on rat serum Alanine aminotransferase (ALT) activity of adult male wistar rats

Serum ALT Concentration [IU/L]

GROUP	WEEK 1	WEEK 2	WEEK 3	WEEK 4
A(Control)	7.00 ± 0.58^{a}	7.33 ± 0.33^{a}	7.00 ± 0.58^{a}	6.67 ± 0.58^{a}
B	12.50 ± 0.87^{b}	13.00 ± 0.58^{b}	11.50 ± 0.28^{b}	12.00 ± 0.00^{b}
C	15.00 ± 0.58^{b}	14.00 ± 0.58^{b}	$14.50 \pm 0.87^{b,c}$	$14.50 \pm 0.29^{b,c}$
D	$16.50 \pm 0.87^{b,c}$	18.00 ± 0.58^{b}	$15.50 \pm 0.87^{b,c}$	$17.00 \pm 0.58^{b,c}$
E	18.00 ± 0.58^{c}	20.00 ± 0.58^{c}	18.00 ± 0.58^{c}	17.83 ± 0.73^{c}

Values are presented as mean ± SEM, n=3 per group. Values on the same column with different superscript letters differ significantly at $p<0.005$

Results in table 1 showed that variations were recorded in enzyme activities among the various groups. Daily administration of bromazepam significantly ($p<0.05$) elevated serum alanine aminotransferase activity values when groups B (0.0016mg/kg bromazepam), C (0.0026mg/kg bromazepam), D (0.0036mg/kg bromazepam) and E (0.0046mg/kg bromazepam) were compared with group A (control rats) from week one to week four. The control group at week one recorded an ALT activity values of 7.00±0.29 IU/L while groups B, C, D and E had ALT activity values of 12.50±0.87, 15.00±0.58, 16.50±0.87 and 18.00±0.58 IU/L respectively. At the end of week four, the control group had serum ALT activity values of 6.67±0.58 IU/L while groups B, C, D and E had ALT activity values of 12.00±0.00, 14.50±0.29, 17.00±0.58 and 17.83±0.73 IU/L respectively.

Table-2: Effect of bromazepam on rat serum aspartate aminotransferase (AST) activity of adult male wistar rats

Serum AST concentration (IU/L)

GROUP	WEEK 1	WEEK 2	WEEK 3	WEEK 4
A (Control)	7.00 ± 0.58^{a}	8.66 ± 0.88^{a}	8.50 ± 0.58^{a}	8.66 ± 0.88^{a}
B	13.00 ± 0.58^{b}	13.00 ± 0.87^{bc}	12.00 ± 0.00^{b}	12.50 ± 0.29^{b}
C	16.00 ± 0.58^{b}	17.00 ± 0.58^{bc}	14.50 ± 0.29^{bc}	15.50 ± 0.29^{b}
D	$17.00 \pm 0.58^{b,c}$	19.00 ± 0.58^{bc}	17.50 ± 0.29^{bc}	17.00 ± 0.58^{bc}
E	20.00 ± 0.58^{c}	21.50 ± 0.58^{c}	19.00 ± 0.58^{c}	17.33 ± 0.58^{c}

Values are presented as mean ± SEM, n=3 per group. Values on the same column with different superscript letters differ significantly at $p<0.005$.

Results as presented in table 2 showed that at week one, the control group recorded a serum AST activity values of 7.00±0.58 IU/L while the other groups recorded 12.50±0.29, 13.00±0.58, 16.50±0.29 and 17.00±0.58 IU/L for B, C, D and E AST activity values respectively. It was also observed that at the end of the study, the control group recorded serum AST activity values of 8.33±0.58 IU/L. The serum activity for B, C, D and E were 12.67±0.67, 13.00±0.00, 15.00±0.58 and 17.00±0.58 IU/L respectively.

Table-3: Effect of bromazepam on rat serum alkaline phosphatase (ALP) activity of adult male wistar rats

Serum ALP concentration (IU/L)

GROUP	WEEK1	WEEK 2	WEEK 3	WEEK 4
A(control)	114.00 ± 0.58^{a}	121.33 ± 0.88^{a}	123.00 ± 1.15^{a}	110.00 ± 0.58^{a}
B	165.50 ± 3.18^{b}	174.00 ± 2.31^{b}	169.00 ± 2.89^{b}	169.33 ± 2.40^{b}
C	197.00 ± 1.70^{bc}	196.00 ± 1.76^{bc}	189.00 ± 5.19^{b}	177.00 ± 1.73^{b}
D	217.00 ± 1.44^{bc}	208.00 ± 4.62^{bc}	203.00 ± 1.73^{b}	190.00 ± 2.30^{b}
E	230.00 ± 11.56^{c}	230.00 ± 5.77^{c}	212.50 ± 1.44^{c}	200.00 ± 2.77^{c}

Values are presented as mean ± SEM, n=3 per group. Values on the same column with different superscript letters differ significantly at $p<0.005$.

Results in table 3 revealed that there were differences in enzyme activities among the different test groups. Bromazepam administration significantly elevated serum alkaline phosphatase activity when groups B, C, D and E were compared with the control group. The study also showed that at week one, the control group recorded a serum ALP activity values of 114.00±0.58 while at week four, the serum ALP activity was 110.00±0.58 IU/L. Furthermore, the ALP activity values of 165.50±3.18, 197.00±1.70, 217.00±1.44 and 230.00±11.56 IU/L; 169.00±2.89, 177.00±1.73, 190.00±2.30 and 200.00±2.77 IU/L were recorded for groups B, C, D and E for weeks one and four respectively.

Table-4: Effect of bromazepam on rat serum lactate dehydrogenase (LDH) activity of adult male wistar rats

GROUP	WEEK 1	WEEK 2	WEEK 3	WEEK 4
A (Control)	177.00 ± 0.58^{a}	189.00 ± 0.88^{a}	174.00 ± 1.54^{a}	161.33 ± 0.88^{a}
B	344.00 ± 13.85^{b}	358.67 ± 11.79^{b}	325.00 ± 14.43^{b}	287.00 ± 7.50^{b}
C	411.00 ± 5.19^{b}	376.33 ± 2.03^{b}	333.00 ± 7.55^{b}	303.00 ± 1.73^{b}
D	418.00 ± 12.70^{bc}	399.00 ± 1.15^{bc}	368.00 ± 4.61^{bc}	336.00 ± 3.46^{bc}
E	522.50 ± 13.86^{c}	410.00 ± 17.32^{c}	390.00 ± 5.77^{c}	360.00 ± 5.77^{c}

Values are presented as mean ± SEM, n=3 per group. Values on the same column with different superscript letters differ significantly at $p<0.005$.

There were marked variations recorded in lactate dehydrogenase (LDH) enzyme activity as shown in table 4. Daily bromazepam administration significantly ($p<0.05$) elevated the serum lactate dehydrogenase activity when groups B, C, D and E

were compared with the control group for week one to four. The study also showed that at week one, the control group recorded a serum LDH activity values of 177.00±0.58 IU/L while at week four, the serum LDH activity values was 161.33±0.88 IU/L. Furthermore, the LDH activity values of 344.00 ± 13.85, 411.00 ± 5.19, 418.00 ± 12.70 and 522.50 ± 13.86; 287.00 ± 7.50, 303.00 ± 1.73, 336.00 ± 3.46 and 360.00 ± 5.77 IU/L were recorded for groups B, C, D and E for weeks one and four respectively.

Table-5: Effect of bromazepam on rat serum creatine kinase (CK) activity of adult male wistar rats

GROUP	WEEK 1	WEEK 2	WEEK 3	WEEK 4
A (Control)	36.00 ± 0.58^{a}	32.00 ± 0.78^{a}	31.00 ± 0.58^{a}	31.00 ± 0.58^{a}
B	48.00 ± 0.58^{b}	52.00 ± 0.58^{b}	45.00 ± 1.44^{b}	45.00 ± 0.00^{b}
C	50.50 ± 1.55^{b}	52.00 ± 0.58^{b}	57.00 ± 1.72^{b}	45.00 ± 0.89^{b}
D	64.00 ± 1.73^{bc}	62.00 ± 1.73^{bc}	45.00 ± 2.58^{bc}	48.00 ± 1.15^{bc}
E	70.00 ± 1.15^{c}	66.00 ± 1.53^{c}	60.00 ± 2.30^{c}	50.00 ± 0.58^{c}

Values are presented as mean ± SEM, n=3 per group. Values on the same column with different superscript letters differ significantly at $p<0.005$.

Results in table 5 showed that there were differences in enzyme activities among the different test groups. Bromazepam administration significantly elevated serum creatine kinase activity when groups B, C, D and E were compared with the control group. The study also showed that at week one, the control group recorded a serum CK activity of 36.00±0.58 while at week four, the serum CK activity was 31.00±0.58 IU/L. Furthermore, the CK activity values of 48.00 ± 0.58, 50.00 ± 1.55, 64.00 ± 1.73 and 70.00 ± 1.15; 45.00 ± 0.00, 45.00±0.89, 48.00±1.15 and IU/L were 50.00 ± 1.15 recorded for groups B, C, D and E for weeks one and four respectively.

Table-6: Effect of bromazepam on rat serum prostate specific antigen (PSA) activity of adult male wistar rats

GROUP	WEEK1	WEEK 2	WEEK 3	WEEK 4
A (Control)	0.27 ± 0.03^{a}	$0.37 \pm 0.0.3^{a}$	0.37 ± 0.03^{a}	0.37 ± 0.03^{a}
B	0.30 ± 0.10^{a}	0.33 ± 0.10^{a}	0.40 ± 0.06^{a}	0.40 ± 0.10^{a}
C	0.30 ± 0.06^{a}	0.30 ± 0.06^{a}	0.37 ± 0.09^{a}	0.40 ± 0.06^{a}
D	0.30 ± 0.06^{a}	0.37 ± 0.03^{a}	0.40 ± 0.12^{a}	0.37 ± 0.07^{a}
E	0.27 ± 0.03^{a}	0.30 ± 0.10^{a}	0.40 ± 0.06^{a}	0.40 ± 0.06^{a}

Values are presented as mean ± SEM, n=3 per group. Values on the same column with different superscript letters differ significantly at $p<0.005$.

There were significant differences recorded in prostate specific antigen (PSA) enzyme activity as shown in table 6. bromazepam administration significantly ($p<0.05$) elevated the serum PSA activity when groups B, C, D and E were compared with the control group for week one to four. The study also showed that at week one, the control group recorded a serum PSA activity of 0.27±0.03 ng/ml while at week four, the serum PSA activity values was 0.37±0.03 ng/ml. Furthermore, the PSA activity values of 0.30±0.10, 0.30±0.06, 0.30±0.06 and 0.27±0.03 ng/ml; 0.40±0.10, 0.40±0.06, 0.37±007 and 0.40±0.06 ng/ml values were recorded for groups B, C, D and E for weeks one and four respectively.

HISTOLOGY RESULTS

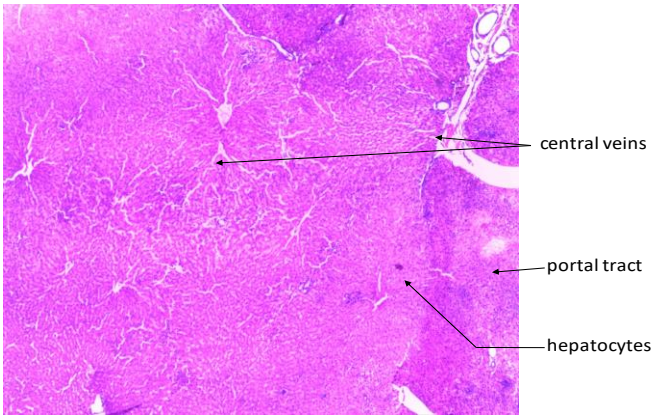

Plate 1: Photomicrograph of histology of liver of control rat (H&E mag. X 200) Result shows normal histology

CONTROL SLIDE SHOWING NORMAL HISTOLOGY

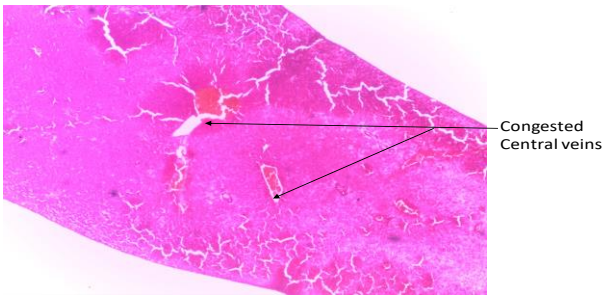

Plate 2 Photomicrograph of histology of liver of male adult rats after week three (H&E mag. X 200)

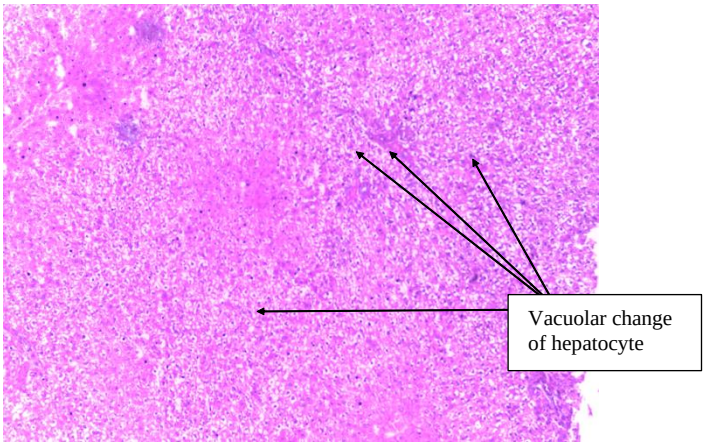

Plate 3: Photomicrograph of histology of liver of male wistar rat after week four (H&E mag. X 200). Result shows vacuolar change in hepatocyte (Reversible injury)

DISCUSSION

The study revealed significantly ($p<0.05$) raised serum activity of alanine aminotransferase in all test groups on daily administration of bromazepam when compared with the control group. This finding is in agreement with the study of Rasha *et al.* [14] and Mossallanejad *et al.* [15] whose results showed significant increase in the serum activity of ALT in rats and cats on the administration of bromazepam. Hepatocellular damage resulting to disruption of plasma

membrane allows leakage of ALT into the blood stream [16]. Due to its high concentration in the liver, serum ALT is one of the most universal markers for hepatic injury across species. However, Pappas [17] suggested that increased hepatic synthesis of ALT by some hepato-toxicants such as drugs is also a source of increase of serum activity as seen in this study. Also an elevated serum activity of ALT could also be attributed to the report that in rat cortisol enhances hepatic activities of ALT [3].

The AST activity was observed to significantly increase over control in a dose- dependent manner. That is higher doses having more serum AST activity than the lower doses. This finding also is in support of the findings of Rasha *et al*. [14] and Mossallanejad *et al*. [15] whose results indicated considerable increase in the serum activity of AST in rats and cats respectively in the administration of bromazepam. A high level of AST in the serum is an indicator of mitochondrial damage in mostly the centrilobular regions of the liver. This region is sensitive to toxic & hypoxic substances [18]. Although AST is also available in other tissues such as brain, kidney, heart and muscle, elevated AST and ALT are considered as important markers of hepatic injury.

ALP is mostly present in the cells which line the bile ducts in the liver and in lesser amount in other parts such as bone, placenta, kidney and intestine. This makes it primarily a biomarker of hepatobiliary effects [19]. Results obtained in this study agrees with the work of Mossallanejad *et al*. [15] whose results showed that continuous oral administration of bromazepam considerably elevated serum concentration of ALT, AST & ALP but differs from the works of Rasha *et al*. [14] whose results showed that chronic administration of bromazepam caused significant reduction in ALP activity. These differences could be attributed to the fact that in long term administration, the effect of bromazepam begins to wear off. The results obtained in the study supports the claim that elevated ALT, AST and modest increase in ALP is an indication of hepatocellular injury [19].

Evaluation of serum level of LDH is an essential tool in clinical analysis as it helps in detection of hepatocellular necrosis. Results obtained in this study showed that administration of bromazepam significantly ($p \leq 0.05$) increase serum LDH levels in all test groups when compared to control from week 1 to 4. This result supports the study carried out by Mossallanejad *et al*. [15] whose findings showed that repeated oral administration of bromazepam significantly elevated LDH. Although elevated LDH levels could also be as a result of its release from damaged cells from various part of the body as well as the liver due to exposure to drugs, elevated LDH levels together with higher activities of ALT, AST and ALP as an indication that the increase is of hepatic origin [19].

Creatine kinase (CK) is an important enzyme responsible for the generation of adenosine triphosphate (ATP) for high ATP requiring cells. The administration of bromazepam as shown from results in this study significantly elevated serum level of CK in all test groups when compared to the control. Known causes of elevated CK such as delirium, malignant neuroleptic trauma and intra muscular infections were not the case in this study. The increase in the serum activity of creatine kinase could be attributed to its net synthesis following exposure to these drugs. This work agrees with the work of Robert *et at*. [20] whose findings showed increased level of CK after post myocardial infarction following the administration of bromazepam. This increase in serum activity of CK should be considered when evaluating outcome of persons exposed to oral administration of bromazepam in order not to make an erroneous conclusion.

Prostate specific antigen (PSA) is a protein produced in the prostate. It is specific and sensitive for the diagnosis and management of prostatic carcinoma, a common male tumor. Prostate carcinoma is now most frequently diagnosed following the detection of elevated PSA. Therefore, factors that alter PSA level can potentially compromise the identification of prostate cancer. From this study, it was observed that the serum PSA level was not altered in all test groups when compared to control group from week 1 to week 4 following administration. Therefore, bromazepam administration does not compromise the role of PSA in early detection of prostate cancer and subsequent reduction in prostate cancer-specific mortality. It is therefore safe for normal and persons with prostate cancer.

Evaluation of the hepatic tissues of the control group and test groups at week one as well as the control group of weeks 2 to 4 showed no damage to hepatocyte following varying dose of bromazepam. Conversely, the histopathological investigation of the liver of the test groups from week 3 to week 4 showed reversible changes such as mild periportal inflammation, mild vascular change and congested central vein. The results obtained from the histopathological investigation of the liver revealed no serious histopathological change in the liver. All changes observed were mild and reversible. This implies that the increase in the serum levels of the metabolic enzymes (ALT, AST, ALP, and LDH) used in assessment of hepatic damage was not mainly due to membrane leakage caused by hepatic damage but as a result of increase in synthesis of these hepatic enzymes following the administration of bromazepam [3]. The result obtain in this study is in support of works of Aiges *et al*. [10] whose result showed increase in ALT and AST for more than twenty weeks in the absence of specific histopathology following the administration of

anticonvulsants in humans, works of Wall *et al*. [21] where administration of antiepileptic drugs to rats exhibited high levels of ALT, AST and GGT without any indication of hepatocellular injury and the research of Haldukewych and John [22] whose result showed ALT levels up to three times and AST levels up to two times following the administration of antiepileptic drugs without any significant changes in the histopathology of the liver. The increase in ALT and AST were attributed to enzyme induction by the drug.

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