Impact of benzodiazepines administration on selected biochemical parameters of albino Wistar rats (*Rattus rattus*)

Joyce N. Nzor, Augustin A. Uwakwe, Samuel C. Onuoha

Department of Biochemistry, University of Port Harcourt, Port Harcourt, Rivers, Nigeria

Correspondence to Joyce N. Nzor, MSc, Department of Biochemistry, Faculty of Science, University of Port Harcourt, Port Harcourt, Rivers, 500102, Nigeria.
e-mail: nzorj@yahoo.com

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Background
Considerable information has been reported on the adverse effect of benzodiazepines on the brain, with limited information of their effects on biochemical parameters.
Aim
This study aimed to investigate the effect of diazepam and bromazepam on selected biochemical parameters of albino Wistar rats.
Materials and methods
Diazepam and bromazepam at concentrations of 0.0046 mg/100 g body weight, 0.0036 mg/100 g body weight, 0.0026 mg/100 g body weight, and 0.0016 mg/100 g body weight were administered, respectively, to rats on a daily basis. At the end of each week, total serum protein, glucose, urea, and creatinine levels were evaluated. Histopathological examination of the liver was also performed for signs of possible damage.
Results
Reductions in serum glucose and creatinine levels were observed in the first, second, third, and fourth weeks in rats administered diazepam and bromazepam at various doses. Both drugs showed similar effects and the reductions were only significant ($P<0.05$) for creatinine, whereas total serum protein was elevated significantly ($P<0.05$). No effect was observed in urea levels throughout the study period. Histopathological examination of the liver showed pronounced morphological alterations in structure, indicative of hepatic damage.
Conclusion
Although hematological parameters such as glucose and urea were not affected significantly, the observed hepatotoxicity could be indicative of possible induction of hepatic damage with consequent metabolic aberrations.

Keywords:
bromazepam, creatinine, diazepam, glucose, hepatocytes, protein, urea

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Introduction

Anxiolytics are prescribed worldwide for the relief of pain, of which benzodiazepines are among the most frequently prescribed [1,2]. Benzodiazepines were introduced in the 1950s and are minor tranquilizers that act as central nervous system depressants through the facilitation of γ-aminobutyric acid binding at various γ-aminobutyric acid receptors throughout the central nervous system [3]. Hence, benzodiazepines are effective for the following therapeutic actions in short-term usage: anxiolytic (anxiety and panic disorder), myorelaxant (muscle spasms, spastic disorders), anticonvulsant (some forms of epilepsy, fits because of drug poisoning), hypnotic (insomnia), and amnesia (sedation for minor operations) [4]. Both diazepam and bromazepam belong to the 1,4-benzodiazepin-2-one class of benzodiazepines [5] and considerable information has been reported on the adverse effects of benzodiazepines on the brain [3,6], with limited information of its effect on biochemical parameters.

This study aimed to investigate the effects of diazepam and bromazepam on selected biochemical parameters of albino Wistar rats; the aim was to observe possible changes in serum total blood protein, serum glucose, serum creatinine, and serum urea, and also perform histological examination of the liver.

Materials and methods

Experimental animals

Approval was obtained from the ethics committee of University of Port Harcourt for the use of animals for experiments, after which 108 albino Wistar rats weighing between 100 and 110 g of both sexes were obtained from the animal house of the University of Port Harcourt, Choba, Rivers State, Nigeria; acclimatization

was allowed for 1 week before experimentation. Water and standard rat chow were provided *ad libitum* and the animals were kept under room temperature. At the end of each week, three rats were killed from each group and the animals were divided into nine groups as follows: group 1 (DZ1), which consisted of 12 rats administered 0.0046 mg/100 g body weight of diazepam orally, group 2 (DZ2), which consisted of 12 rats administered 0.0036 mg/100 g body weight of diazepam orally, group 3 (DZ3), which consisted of 12 rats administered 0.0026 mg/100 g body weight of diazepam orally, group 4 (DZ4), which consisted of 12 rats administered 0.0016 mg/100 g body weight of diazepam orally, group 5 (BZ1), which consisted of 12 rats administered 0.0046 mg/100 g body weight of bromazepam orally, group 6 (BZ2), which consisted of 12 rats administered 0.0036 mg/100 g body weight of bromazepam orally, group 7 (BZ3), which consisted of 12 rats administered 0.0026 mg/100 g body weight of bromazepam orally, group 8 (BZ4), which consisted of 12 rats administered 0.0016 mg/100 g body weight of bromazepam orally, and group 9 (control group), which consisted of 12 untreated normal rats.

Drugs and chemicals

Bromazepam (lexotan) tablet and diazepam (valium) used were manufacturred by F. Hoffmann-La Roche Ltd (Basel, Switzerland), the randox kit used was obtained from Randox chemical Laboratory (Crumlin, UK), and all other reagents used were of analytical grade.

Collection of blood and organ

The animals were killed under anesthesia (chloroform suffocation) and blood samples were collected into plain sample bottles. The liver was stored in plain bottles containing 75% formalin before embedding in parafin wax and further processed for examination [7]. Serum was separated at 3000 rpm for 10 min and stored in a refrigerator at −4°C for subsequent analysis.

Estimation of biochemical parameters

Serum creatinine was determined using the randox kit (Jaffe's reaction method) [8]. The glucose level was determined using the randox test kit (glucose oxidase PAP method) [9], whereas the serum urea level was determined using the diacetyl monoxime method and serum total protein was estimated using the biuret reagent method.

Analysis of results

Data obtained from the experimental design and set-up were subjected to statistical calculations using SPSS, version 18 (SPSS Inc., Chicago, IL, USA). The mean values±SD were calculated and the one-way analysis of variance test was performed. Significance level was calculated at a 95% confidence level ($P \leq 0.05$).

Results

The results obtained from the various analyses of the effect of diazepam and bromazepam of some biochemical parameters are presented in Tables 1–4. Nonsignificant reductiouns in serum glucose concentrations were observed at varied concentrations of the two drugs, whereas concentration-dependent increases in serum protein concentrations were observed in the presence of the drugs. No significant effect on the serum urea concentration was observed in the presence of the drugs at the concentrations investigated.

Histopathological examination of the liver indicated signs of inflammation, congested vessels, dilated sinusoids, and vacuolar degeneration. Damage to the liver was observed only at the third and fourth week,

Table 1 Effect of diazepam and bromazepam on serum glucose concentration

Drug concentrations (mg/l)	Serum glucose concentration (mmol/l)			
	Week 1	Week 2	Week 3	Week 4
Control	4.33±0.05	4.36±0.15	4.4±0.1	4.4±0.2
DZ1	3.6±0.2[a]	3.73±0.15	4.1±0.1	3.8±0.1
DZ2	4±0.36	3.7±0.1	4.3±0.2	4±0.1
DZ3	4.13 ±0.42	3.9±0.1	4.13 ±0.25	4.03±0.25
DZ4	4.5±0.1[b]	4.23±0.32	4.27±0.21	4±0.1
BZ1	3.87±0.25	3.8±0.1	4.13±0.15	4±0.2
BZ2	4.1±0.17	4±0.1	4.43±0.32	4±0.3
BZ3	4.1±0.3	4±0.2	4.23±0.35	3.97±0.12
BZ4	4.43±0.15[b]	4.23±0.15	4.33±0.25	4.03±0.15

The values are represented as mean±SD of triplicates. Note that DZ1, DZ2, DZ3, and DZ4 represent diazepam at concentrations of 0.0046 mg/100 g body weight, 0.0036 mg/100 g body weight, 0.0026 mg/100 g body weight, and 0.0016 mg/100 g body weight, respectively, whereas BZ1, BZ2, BZ3, and BZ4 represent bromazepam at concentrations of 0.0046 mg/100 g body weight, 0.0036 mg/100 g body weight, 0.0026 mg/100 g body weight, and 0.0016 mg/100 g body weight, respectively (at a drug concentration of 0.00 mg/100 g body weight). BZ1, group 5; BZ2, group 6; BZ3, group 7; BZ4, group 8; control, group 9; DZ1, group 1; DZ2, group 2; DZ3, group 3; DZ4, group 4. [a]Significant difference from the control ($P \leq 0.05$). [b]Significant difference from DZ1.

with higher doses causing more damage. Histopathology of the liver at the third and fourth week is shown in Plates 1–17.

Discussion

Effect of diazepam and bromazepam on serum glucose concentration

Data from this study indicated that the administration of diazepam (0.0046 mg/100 g body weight, 0.0036 mg/100 g body weight, 0.0026 mg/100 g body weight, and 0.0016 mg/100 g body weight), respectively, and bromazepam (0.0046 mg/100 g body weight, 0.0036 mg/100 g body weight, 0.0026 mg/100 g body weight, and 0.0016 mg/100 g body weight) produced a nonsignificant reduction in the serum glucose concentration throughout the duration of the experiment (Table 1). However, 0.0046 mg/100 g body weight of the drugs caused a significant

Table 2 Effects of diazepam and bromazepam on serum protein concentration

Drug concentrations (mg/l)	Serum protein concentration (g/l)			
	Week 1	Week 2	Week 3	Week 4
Control	66.33±2.08	66±2	66±1	66.33±2.08
DZ1	78±3[a]	74±1[a]	74±1[a]	76±1[a]
DZ2	76.33±3.06[a]	72±1	72±1.73	71.67±1.53
DZ3	73±4.36	69.67±1.53	71.67±2.08	70±2[c]
DZ4	70±1[b,c]	68±1[c]	68±1[c]	68±1[b,c]
BZ1	78.33±2.08[a]	75.67±2.52[a]	76±3[a]	77.67±1.53[a]
BZ2	77±1[a]	72.33±3.51	75±3[a]	71±1
BZ3	74±1	71±1	75±5[a]	70±2[c]
BZ4	72.33±1.53	69±1	70±1	69.67±0.58[c]

The values are represented as mean±SD of triplicates. BZ1, group 5; BZ2, group 6; BZ3, group 7; BZ4, group 8; control, group 9; DZ1, group 1; DZ2, group 2; DZ3, group 3; DZ4, group 4. [a]Significant difference from the control ($P \leq 0.05$). [b]Significant difference from DZ1. [c]Significant difference from BZ1.

Table 3 Effects of diazepam and bromazepam on serum urea nitrogen concentration

Drug concentrations (mg/l)	Serum urea concentration (mmol/l)			
	Week1	Week 2	Week 3	Week 4
Control	3.4±0.26	3.47±0.15	3.5±0.3	3.5±0.2
DZ1	3.5±0.1	3.57±0.15	3.5±0.1	3.53±0.12
DZ2	3.57±0.12	3.4±0.17	3.57±0.25	3.47±0.15
DZ3	3.4±0.1	3.3±0.1	3.6±0.2	3.5±0.2
DZ4	3.57±0.06	3.33±0.06	3.5±0.1	3.4±0.2
BZ1	3.3±0.1	3.5±0.1	3.5±0.2	3.53±0.21
BZ2	3.43±0.15	3.33±0.21	3.6±0.2	3.5±0.1
BZ3	3.5±0.1	3.47±0.15	3.4±0.1	3.5±0.17
BZ4	3.47±0.15	3.33±0.25	3.53±0.25	3.53±0.06

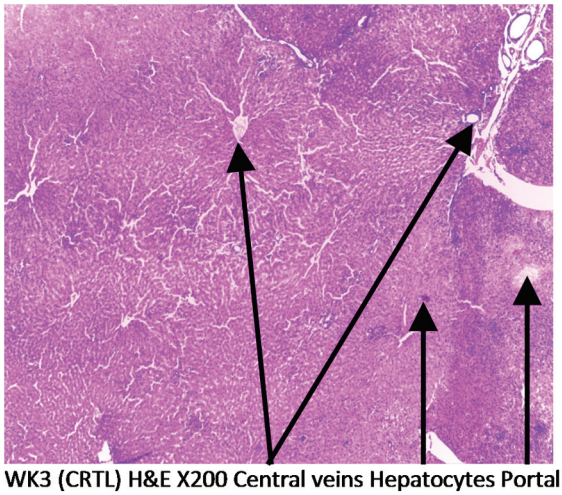
The values are represented as mean±SD of triplicates. BZ1, group 5; BZ2, group 6; BZ3, group 7; BZ4, group 8; control, group 9; DZ1, group 1; DZ2, group 2; DZ3, group 3; DZ4, group 4. The values obtained were not significantly different from each other ($P < 0.05$).

Table 4 Effects of diazepam and bromazepam on serum creatinine concentration

Drug concentrations (mg/l)	Serum creatinine concentration (μmol/l)			
	Week 1	Week 2	Week 3	Week 4
Control	85.67±3.79[a]	87±2[a]	88.33±3.06[a]	89.33±5.51[a]
DZ1	68.33±5.77[a]	68.67±1.53[a]	71.67±2.89[a]	71±1.73[a]
DZ2	73.33±3.06[a]	70±1.73[a]	74.67±3.06[a]	75.33±1.53[a]
DZ3	76±5.20[b,c]	70.67±2.31[a]	75.33±3.51[c]	78.67±1.15[b,c]
DZ4	82.67±4.73	76.67±3.79	80±2	84±1
BZ1	68.67±5.03[a]	71±1[a]	70.33±1.53[a]	73±1[a]
BZ2	73.33±2.52[a]	76±3[a]	76±2[a]	74±2[a]
BZ3	74.33±1.53[a]	76.33±3.51[a]	77.67±2.08[a]	77.33±2.52[a]
BZ4	76.33±1.53	80±2[b]	79±1	80±2

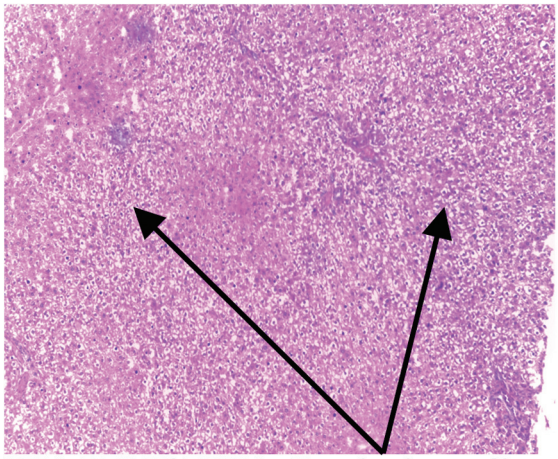
The values are represented as mean±SD of triplicates. BZ1, group 5; BZ2, group 6; BZ3, group 7; BZ4, group 8; control, group 9; DZ1, group 1; DZ2, group 2; DZ3, group 3; DZ4, group 4. [a]Significant difference from the control ($P \leq 0.05$). [b]Significant difference from DZ1. [c]Significant difference from BZ1.

Plate 1



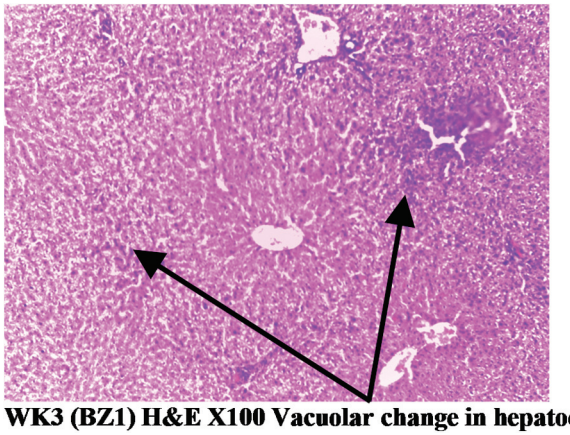
Control photomicrograph of liver at week 3 shows normal histology.

Plate 2



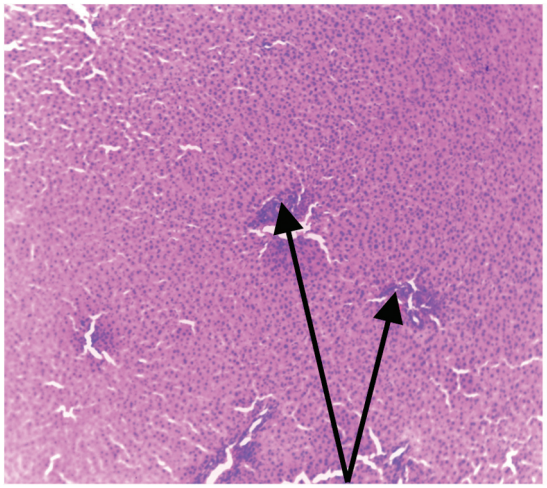
DZ1 photomicrograph of liv.er at week 3 shows vacuolar change in hepatocyte (reversible injury).

Plate 3



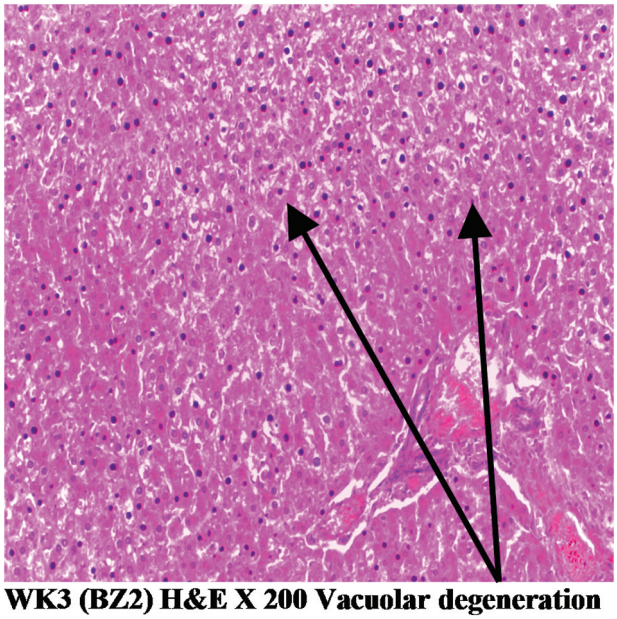
BZ1 photomicrograph of liver at week 3 shows vacuolar change in hepatocyte (reversible injury).

Plate 4



DZ2 photomicrograph of liver at week 3 shows mild periportal inflammation.

Plate 5

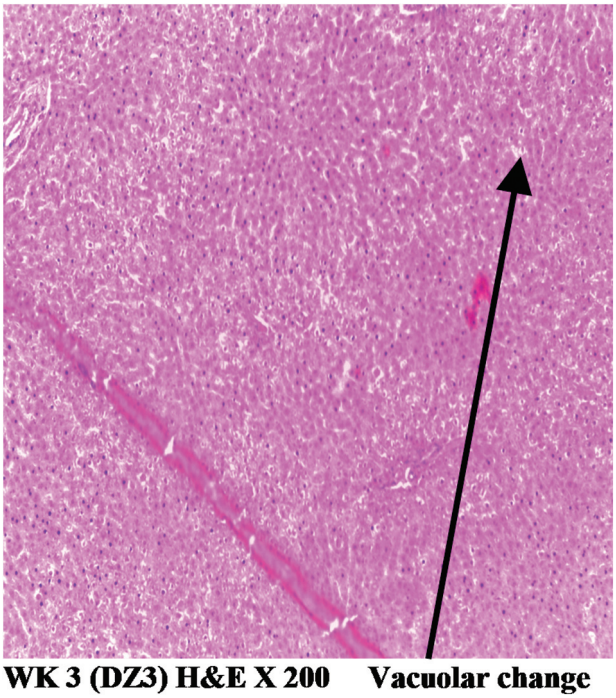


BZ2 photomicrograph of liver at week 3 shows vacuolar degeneration.

reduction ($P<0.05$) compared with the control as an initial effect.

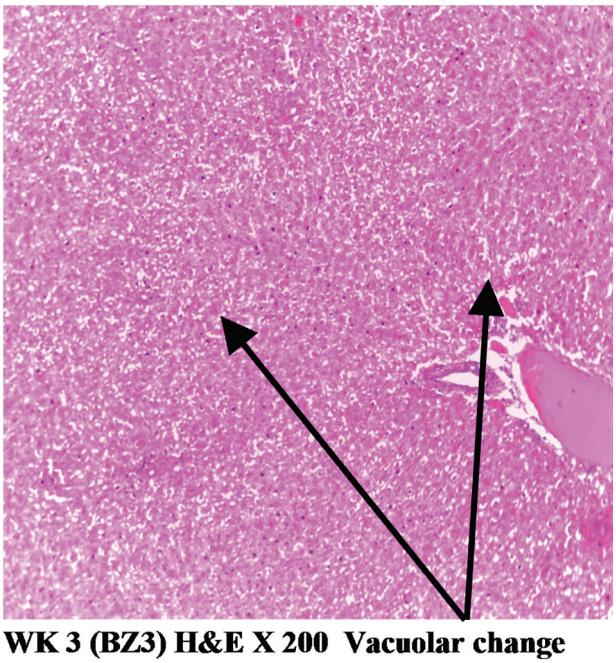
In this study, it was found that diazepam and bromazepam had no effect on serum glucose levels. This finding is in agreement with the previous research work [2,10], whose results showed no significant reduction in blood glucose levels of patients and rabbits when diazepam was administered; the current clinical study found decreases in glucose levels of benzodiazepine patients, although the reduction was significant [11]. On the basis of these

Plate 6



DZ3 photomicrograph of liver at week 3 shows vacuolar change.

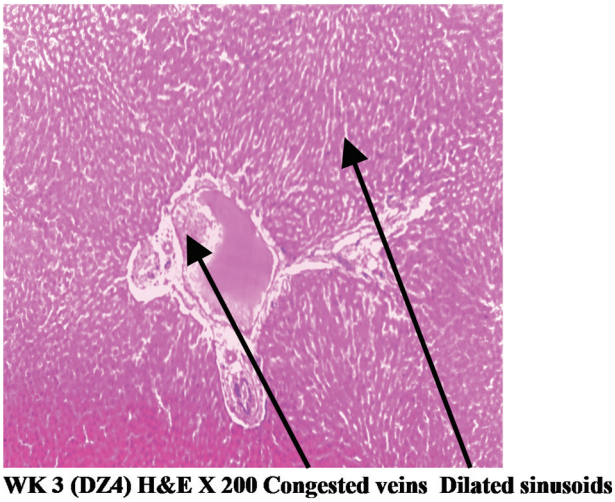
Plate 7



Liver photomicrograph of BZ3 at week 3 shows vacuolar change in the hepatocytes.

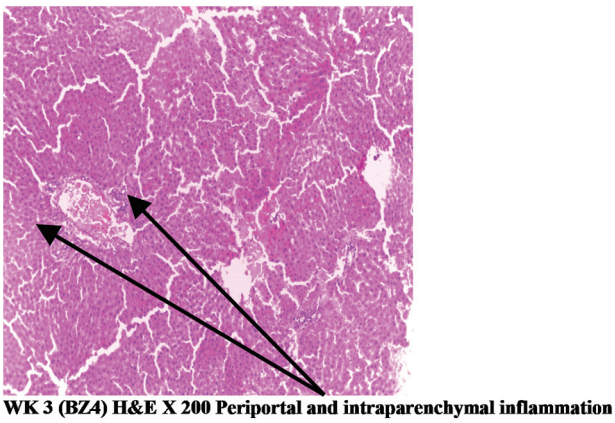
results, it is clear that diazepam and bromazepam can be administered safely for the short-term treatment of anxiety or as a sedative for both diabetic and nondiabetic patients without the fear of alteration of blood glucose at these concentrations of diazepam and bromazepam.

Plate 8



DZ4 photomicrograph of liver at week 3 shows congested veins and dilated sinusoids.

Plate 9



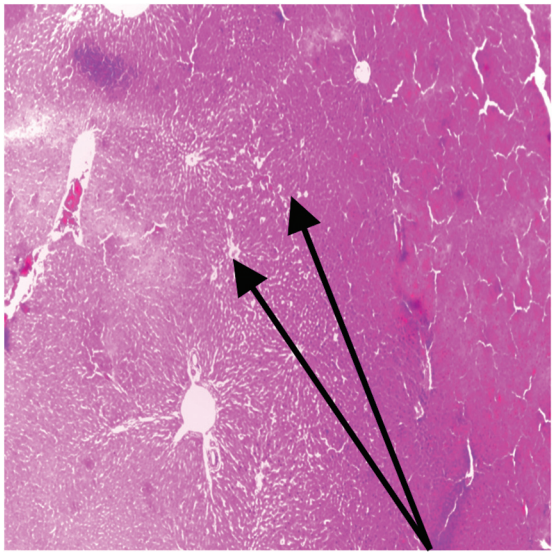
BZ4 photomicrograph of liver at week 3 shows periportal and intraparenchymal inflammation.

Short-term administration of benzodiazepines leads to reduction or suppression of cortisol and adrenalin production through the inhibition of the corticotrophin-releasing factor in the hypothalamus–pituitary axis [12]. However, with long-term administration, the receptors are no longer sensitive to the same concentration of benzodiazepine that initially produced that effect; thus, the cortisol and adrenalin levels may then increase.

Effect of diazepam and bromazepam on serum total protein concentration

Total serum protein increased significantly ($P \leq 0.05$) in a dose-dependent manner compared with the control; higher doses of both drugs led to higher concentrations of serum total proteins (Table 2). Subsequent administration showed a similar pattern, with no significant difference between both drugs at similar

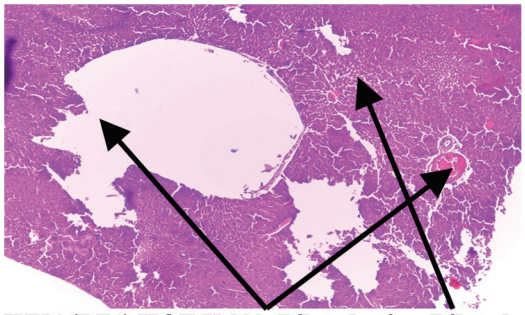
Plate 10



WK 4 (DZ1) H&E X 200 Mild vacuolar change

DZ1 photomicrograph of liver at week 4 shows mild vacuolar change.

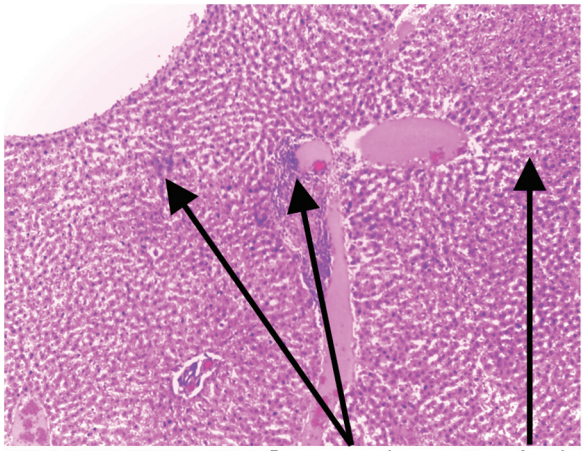
Plate 11



WK4 (BZ1) H&E X 200 Dilated veins Dilated sinusoids

BZ1 photomicrograph of liver at week 4 shows dilated veins and dilated sinusoids.

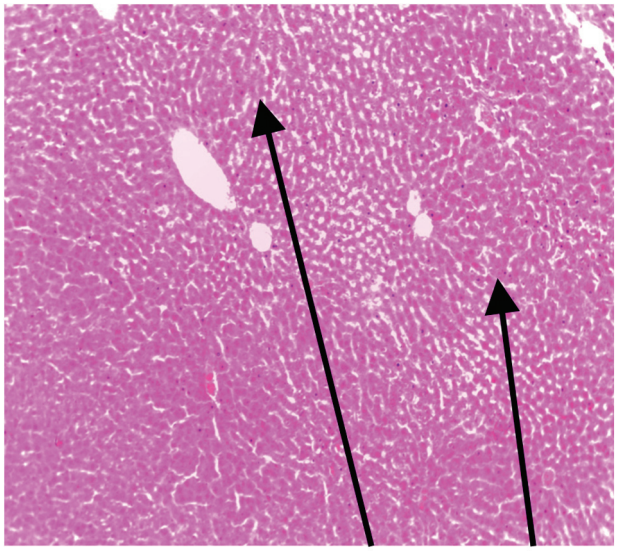
Plate 12



WK 4 (DZ2) H&E X 400 Inflammatory changes Vacuolar change

DZ2 photomicrograph of liver at week 4 shows inflammatory change and vacuolar change.

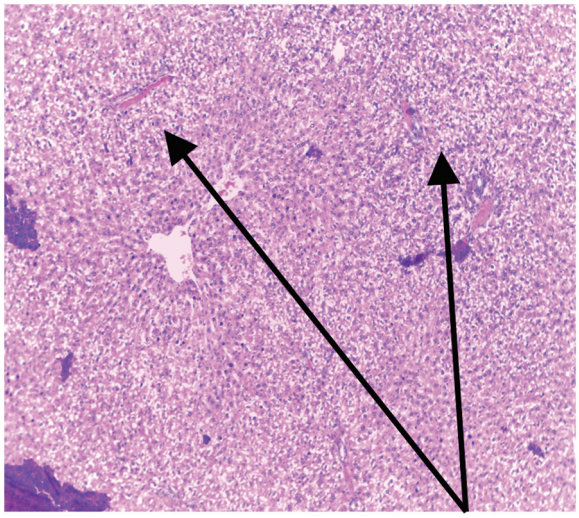
Plate 13



WK4 (BZ2) H&E X 200 Congested sinusoids Vacuolar change

BZ2 photomicrograph of liver at week 4 shows congested sinusoids and vacuolar change.

Plate 14



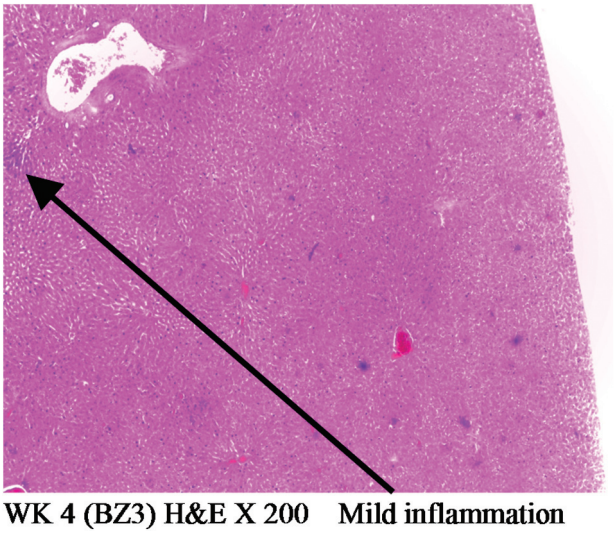
WK 4 (DZ3) H&E X 400 Marked vacuolar change

DZ3 photomicrograph of liver at week 4 shows marked vacuolar change.

concentrations; bromazepam led to a greater increase in protein level than diazepam.

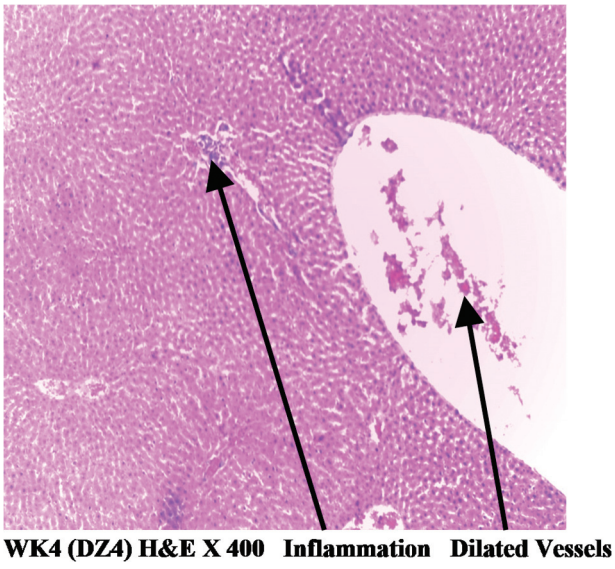
In a relaxed state/condition, the net synthesis of protein occurs as catabolic activities are reduced, protein synthesis has been linked to rapid eye movement sleep [13], and benzodiazepine serves as a hypnotic/sedative drug. Sleep recordings have shown that because of tolerance, the efficacy of the hypnotic effect declines, resulting in patterns of sleep suppressed originally by benzodiazepine returning to the same state before treatment started [13].

Plate 15



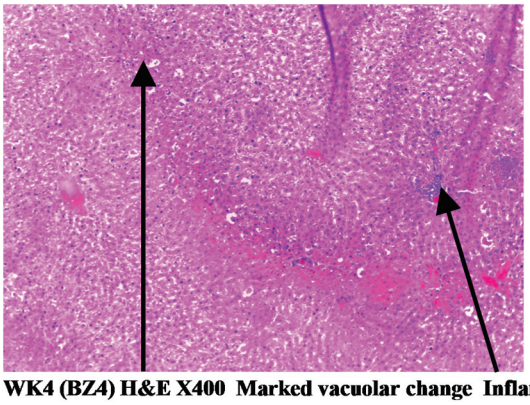
BZ3 photomicrograph of liver at week 4 shows mild inflammation.

Plate 16



DZ4 photomicrograph of liver at week 4 shows inflammation and dilated vessels.

Plate 17



BZ4 photomicrograph of liver at week 4 shows marked vacuolar change and inflammation.

Effect of diazepam and bromazepam on kidney function markers (serum creatinine and serum urea)

The use of blood urea nitrogen as a marker for renal damage by diazepam and bromazepam showed no effect; the alteration in serum urea nitrogen throughout the duration of the experiment was minimal as shown in Table 3. However, an alteration in the serum creatinine concentration was caused by the drugs; diazepam and bromazepam significantly reduced the serum creatinine level in a dose-dependent manner ($P<0.05$) (Table 4).

Urea concentration was minimally affected by diazepam and bromazepam, indicating proper functioning of the kidney, but the creatinine concentration was reduced significantly. This condition (reduction in the creatinine concentration) only occurs when there is muscle wasting or overhydration and this was not the reason for the reduction in the creatinine concentration in this study as there was no weight loss in the experimental animals. However, the reduction in the creatinine concentration could be a result of a reduced metabolic rate leading to creatinine generation or a consequence of possibly increased renal clearance of this metabolite as suggested earlier by Tawfiq and colleagues [14,15].

Effect of diazepam and bromazepam on liver histology

Benzodiazepine liver injury has been classified as an unpredictable or an idiosyncratic hepatotoxic reaction [16], but this study showed that hepatotoxicity of benzodiazepine can also be studied in experimental animals. The liver indicated signs of inflammation, supporting the possibility of hepatotoxicity as reported in cholestasis liver injury cases [14,16].

Conclusion

The findings from this study suggest that diazepam and bromazepam may exert deleterious effects on the hepatocytes, with possible metabolic implications in the long run. Further research is required to confirm whether what was observed in the experimental animals can be replicated in humans. This would be necessary to establish the safety or otherwise of the administration of these drugs to those with an established history of hepatic damage or dysfunction. Moreover, studies on the possible effect of long-time administration of these drugs are also needed to fully establish their safety or otherwise in key metabolic systems.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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