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Hematological, Biochemical, and Histopathological Changes Resulting From Administration of Three Different Concentration of Diazepam in Mice

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***Abstract*---**Diazepam is a benzodiazepine derivative that is commonly used for its sedative and anxiolytic activities. The liver plays a major role in the metabolism of this drug. By using the experimental animals, female white mice aged 2-3 months were divided into four groups. Each group contains three mice. The first group (the control) was given distilled water for four weeks. While the second, third and fourth groups were given diazepam at concentrations (0.25 mg / ml, 0.50 mg / ml, 0.75 mg / ml), respectively, for four weeks, orally using stomach tubes. Blood was drawn, to obtain the blood for hematological tests and the serum for biochemical tests. Rats were dissected, and samples were taken to the laboratory for the purpose of analyzes and tissue segmentation of the liver, spleen. Liver and spleen tissues were obtained for the histopathology analysis and serum for the assay of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and Alkaline phosphatase (ALP).

***Keywords*---**concentration, diazepam, female mice, histopathology, liver enzymes.

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Introduction

Diazepam is a drug that has been used since the last century. It is the most well-known and commonly used of the class of medications known as benzodiazepines. These medications aid in the relief of tension and anxiousness, as well as the relaxation of muscles and the induction of sleep, it is widely used to treat anxiety and insomnia when it using for a short period, this drug is given to treat alcoholism and to reduce the frequency of epileptic seizures. It is also used intravenously to calm patients before major surgeries. (Calcaterra et al., 2014). The serious point about Diazepam is that its overdose can lead to death (Rahimi et al., 2017).

Relaxing, hypnosis, reduced anxiety, stress relief, forgetfulness, and anticonvulsant effect are all pharmacological actions of benzodiazepines due to their interaction with the central nervous system (CNS). When administered intravenously in high quantities, benzodiazepines can cause cardiac vasodilation and neuromuscular inhibition. Stimulation of the receptors of gamma-aminobutyric acid A (GABA-A) and modification of their inhibition of neurotransmission are thought to be involved in the CNS effects of this drugs. Diazepam, sometimes known as valium, is a benzodiazepine medication that has calming effects. It's frequently used to treat various ailments, such as alcohol withdrawal syndrome, disorder of sleeping, and restless the syndrome of legs (Cicero and Colletti, 2021). It can be swallowed, injected into the rectum as suppositories, or injected intramuscular or intravenously, when it given by vein, effects appear through 5minutes and last over to one hour when taken the drug via the mouth its effects may take 40mins to appear. Sleepiness and coordination problems are two common side effects. Serious negative effects are uncommon (Calcaterra and Barrow 2014).

Receptor complex called γ- aminobutyric acid it's the first effector of Diazepam which is locate in the central nervous system. In parallel to the central benzodiazepine receptors disclosed, peripheral-type binding sites in endocrine steroidogenic tissues have been discovered, also in immune cells and liver cells (Berger et al., 2021). Because of its better safety profile, decreased side effects, and the availability of the antagonist flumazenil to reverse excessive sedation and benzodiazepine intoxication, benzodiazepines have essentially supplanted barbiturates in the treatment of anxiety and sleep problems. (Weintraub, 2017). Study amid to investigate the effects of different doses of diazepam on liver function enzymes, and hematological variables, also to know Histopathological changes of liver, and spleen.

Methodology

Animals and experimental design

Albino female rates average weight (25- 34gm) were used in this experiment, the number of animals were 12 which were distributed in to 4 groups each of it contain three animals. The first Group is (Control) was given the water and food as a normal condition. The Second, third, and fourth group was treated with diazepam (0.25 mg/ml, 0.50 mg/ml, and 0.75 mg/ml, respectively. These doses

were administrated orally with the drinking water for a period of four weeks by stomach tubes period of four weeks by stomach tubes daily. The animals of all the groups were anaesthetized with diethyl ether and the samples of blood were collected from the animals through cardiac puncture, then transferred into centrifuge tubes and allowed to clot for 30minutes. The samples were centrifuged to separate the serum from the which were then aspirated into smaller labeled tubes and stored in the freezer at -15 0C until the time of using extracted organs from the animal (liver and, spleen) were put in formalin to prepare the histological study.

Diazepam stock solution

The drug (diazepam) stock solution was obtained by dissolving 0.25 mg, 0.50 mg, and 0.75 of it in 10 ml of distilled water. The dose to be given was estimated using the average body weight of the mice in each group as a starting point. 1 ml each stock solution was given to each group

Biochemical examination

Biochemical marker were prepared according to the kit's instructions, using colorimetric absorption principles, the study marker are: (ALT, AST ,ALP), and total protein.

Determination of hematological parameters

Hemoglobin concentration (Hb), and packed cell Volume (PCV) prepared according to Sahli method.

Histological examinations

The samples for histological study were prepared according to method of Luna-1969.

Statistical analysis

The program “SPSS” was using to analyzer the results statistically, the mean and standard error were calculated, and the data were analyzed using the independent t-test sample.

Discussion

Biochemical variables

Biochemical variables such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total protein were included in the current investigation. To compare the concentrations of these variables in the treatment and control groups

Table 1
Serum liver enzymes in control and treatment groups

Variable	Group 1 (control)	Group2 (0.25mg/ml)	Group 3 (0.50mg/ml)	Group 4 (0.75mg/kg
ALT (GOT) (U/L)	46.23±8.365	69.58±6.32*	66.847±7.58*	88.41± 5.368**
AST (U/L)	33.32± 2.58	35.25±2.58	48.21±2.89*	49.29±3.56*
ALP (U/L)	85.58± 4.35	120.67±5.98*	110.35±4.36*	122.58±7.98*

Significant differences at (p≤0.05)
** Significant differences at (p≤0.01)

The results in table (1) showed significant increase (p≤0.05) in mean level of the liver enzymes ALT, AST, and Alp enzymes in the second and third group, and there was highly significantly increased in fourth group (p≤0.05) compare to control group. The increase in liver enzyme could be related to tissue damage caused by oxidative stress in the liver as a result of repeated administration of diazepam, or it could be due to the negative effects of its active metabolites (desmethyldiazepam), The increase in these enzyme levels was mostly attributable to the leaking of these enzymes from the cytoplasm of the liver into the bloodstream as a result of tissue injury. (Sadar et al., 2021).

Previous studies showed that diazepam caused an increase in liver enzymes activity, mention that the repetiting use of diazepam drug terminates in a complex liver damage diazepam-administered rats, there was an increase in lipid peroxides activity, as well as a decrease in glutathione concentration and superoxide dismutase activity. Liver enzymes "ALT, AST, ALP" increase linked to liver disease and injury, and reduced antioxidant defenses are linke to elevate GGT, particularly reduced glutathione levels. Last study by (Eger et al., 2016) noted that Diazepam-treated rats had altered oxidative stress enzyme activity in the brain, including catalase, glutathione peroxidase, and superoxide dismutase, as well as lower glutathione levels and superoxide dismutase activity in the liver, also, (Md et al., 2022) mentioned that in the liver tissues, diazepam administration changed glutathione homeostasis and enzymatic activity of glutathione peroxidase, glutathione reductase, superoxide dismutase, and glutathione-S-transferases.

Hematological variables

The current study in table (2) showed there are non-significant differences in Hb and pcv concentration among the control group and diazepam treatment group except the fourth group which was administrated high dose in this study (p≤0.05), since there was anemic symptoms of animals. Long-term diazepam treatment resulted in a considerable in Hb and PCV levels reduction. Low Hb and PCV levels may cause anemia in the animals, which is defined by a low number of red blood cells. This research is comparable to that of (Eman et al., 2022), who discovered that when Wistar rats were treated with a chemical (Taurine) for a long period, their PCV values fell. In a study (Evdokia et al., 2021), it was observed that diazepam has an influence on the cardiovascular makeup of rats by inhibiting blood flow. This is consistent with the findings of this study, which showed that

low Hb and PCV levels influence circulating red blood cells and may contribute to heart disease.

Table 2
The concentration of Hb and PCV in control and treatment groups

Variables	Group 1(control)	Group2 (0.25mg/ml)	Group 3(0.50mg/ml)	Group 4(0.75mg/ml)
Haemoglobin (g/dl)	11.6	11.6	11	9.6.3*
PCV%	35	36	34	28*

Significant differences at ($p \leq 0.05$).

Histological study
The liver

- Control group"

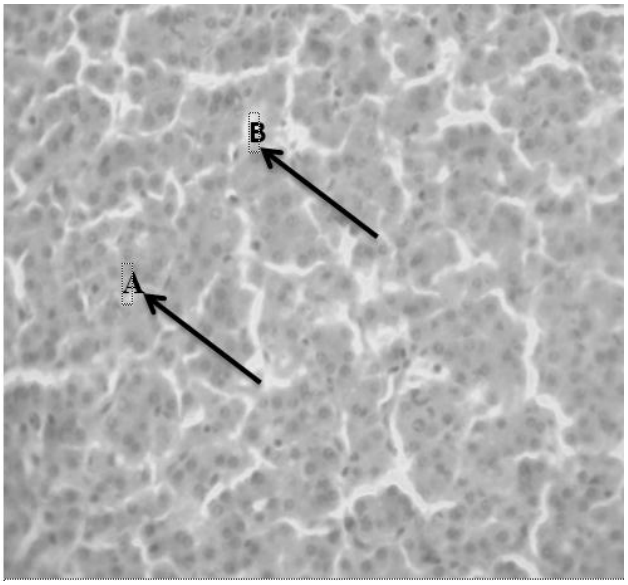

Figure 1. Liver paranchyma: groups of liver cells (A) with multinucleas nucli , blood sinusoids (B). (H&E, X40)

The parenchyma of the liver had groups of liver cell with its multinucleated cytoplasm which appeared pinkish color and the nuclei were spherical shape. The blood sinusoids were network of narrow channels.

- First treatment group

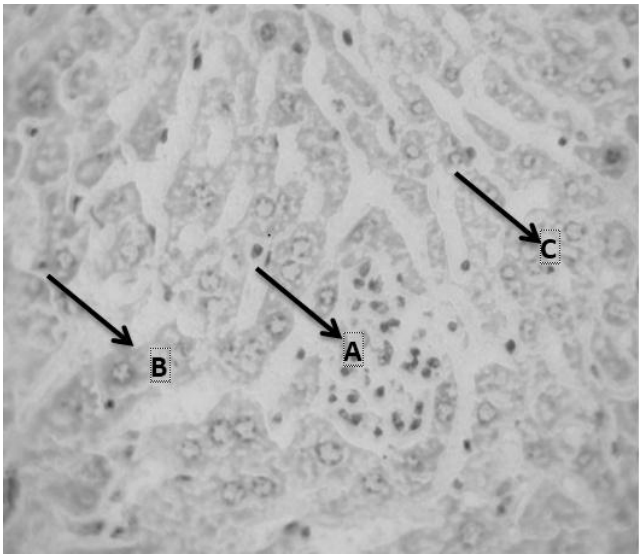

Figure 2. Liver parenchyma and lymph nodule (a) vacuolated cytoplasm and of liver cells (b) blood sinusoid with kupffer cells (c) (H&E, x40)

The parenchyma of liver tissue was containing aggregation of lymphatic nodule which was surrounded by blood sinusoid with Kupffer cells and the columns of vacuolated liver cells.

- Second treatment group

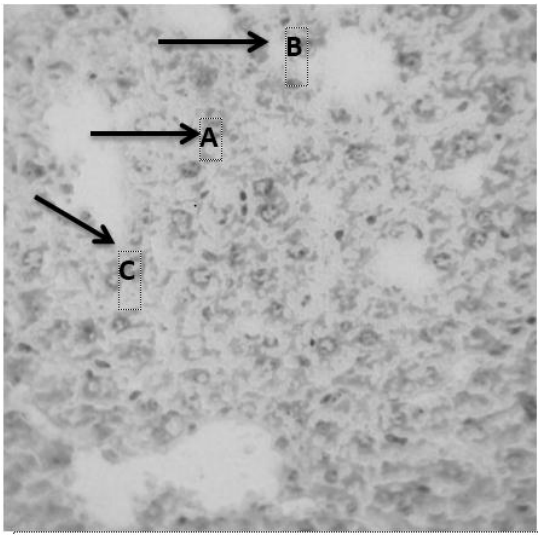

Figure 3. Degenerated and necrotic hepatocytes (A) WBC cells (b), sinusoid with Kupffer cells (C) (H&E, x40)

The liver tissue was containing degenerated and necrotic hepatocytes, associated with blood hemorrhage , WBC were recognized with pyknotic nuclei of certain liver cell, the blood sinusoid were containing RBC.

- Third treatment group

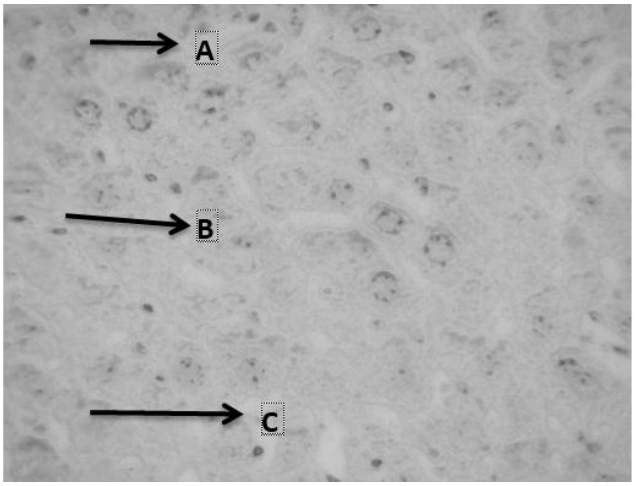

Figure 4. Degenerated liver cells (A) necrotic cells (B), blood sinusoid with Kupffer cells (C) (H&E, x40)

The spleen

- Control group

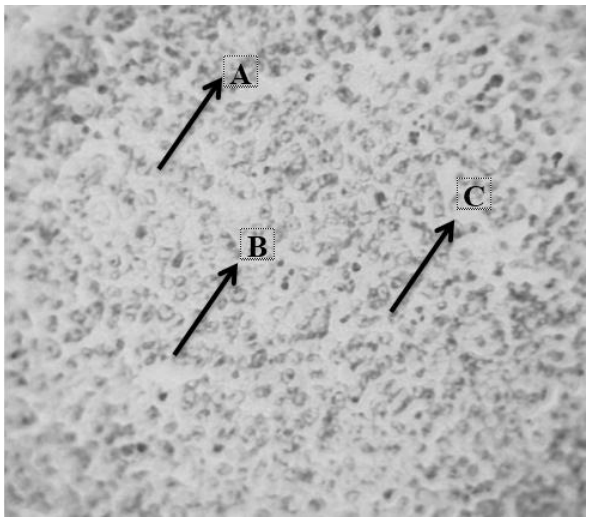

Figure 5. Blood sinuses of spleen with RBC, (A), lymphocytes (B), Purkanje cells (C). (H&E, x40)

The red pulp was formed by blood sinuses of splenic venous filled with RBC, lymphocyte and giant cells of Purkinije.

- First treatment group

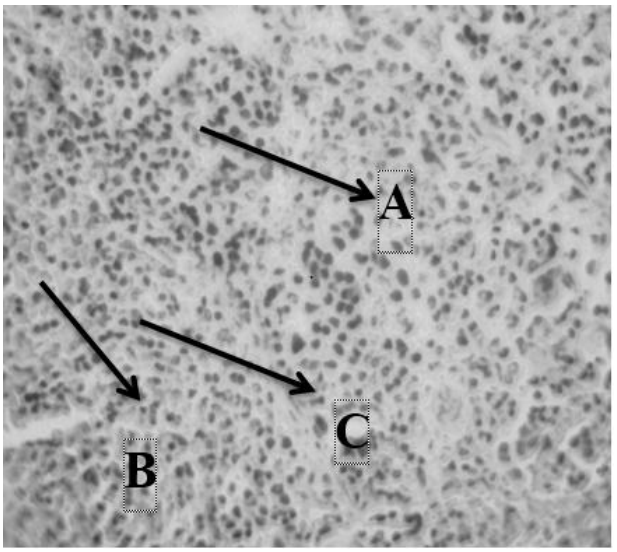

Figure 6. Parenchyma of spleen RBC, (A), lymphocytes (B), Purkanje cells (C). (H&E, x40)

- Second treatment group

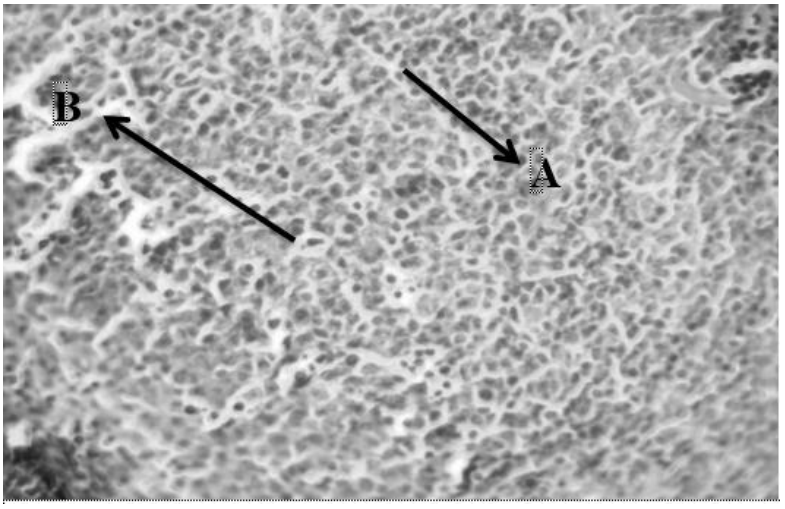

Figure 7. Spleen germinal center (A) white pulp with lymphoblast's (B), cracks inside the white pulp cells. (H&E, x40)

- Third treatment group

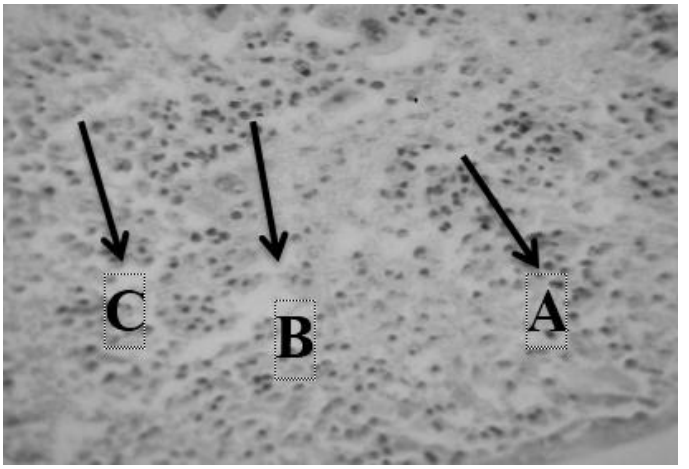

Figure 8. Parenchyma of capsule (A) parenchyma empty of blood, lymphocytes, plasma cells and Purknji cells (B),. (H&E, x40)

Regarding the histopathological examination of the liver; the present study showed an increase in the infiltration of inflammatory cells inside the sinusoids and with increasing the dose of diazepam; there was a degeneration and necrosis of hepatocytes and congestion of the central vein, these results were in agreement with that noticed who noticed a prenecrotic and necrotic changes in the liver in the high - dose group of diazepam treatment. The present histopathological results could be attributed to the toxic effect of the drug and its metabolites on the liver. Oxidative stress disrupts lipids, proteins and DNA, induces necrosis and apoptosis of hepatocytes and amplifies the inflammatory response and it stimulate the production of profibrogenic mediators from Kupffer cells and circulating inflammatory cells resulting in the initiation of fibrosis (Li et al., 2021).

Conclusion

The current findings, which corroborated the biochemical findings and were supported by histopathological changes in the liver of the treated rat groups, indicated that diazepam administration in large doses; for long periods could cause hepatic and splenic tissue damage. As a result, the drug should only be taken on a doctor's prescription, and the amount should be lowered, especially if the patient has liver illness. Future research should also look into the hazardous effects of long-term usage of this drug on other organs such as the brain, heart, and kidney.

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