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EVALUATION OF THE INFLUENCE OF THERAPEUTIC, PROLONGED AND OVERDOSE INTAKE OF DIAZEPAM ON HAEMATOLOGICAL INDICES AND LIVER ENZYME MARKERS OF MALE WISTAR RATS

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ABSTRACT: The Influence of therapeutic, prolonged and overdose intake of diazepam on Haematological indices, and liver enzymes markers of male Wistar rats were evaluated. Twenty (20) Wistar rats were used for this study. The animals were grouped into 4 (5 rats each) and labelled groups A (Normal dose), B (Overdose), C (Prolonged), and D (Control). They were acclimatised for two weeks, food and water were given freely. Treatment was by oral therapy. Groups A, B and D were treated for Ten days, while group C (Prolonged) was treated for 21 days. At the end of treatment, the rats were sacrificed through cervical strangulation and blood samples collected for haematological and biochemical investigations. The result showed that the rats which received the normal therapeutic dose of Diazepam solution had a significant increase ($p<0.05$) in both Haemoglobin (Hb) and Packed cell volume (PCV) with mean values of 10.01+ 1.16 and 30.00 ± 2.16 respectively in comparison with the control (8.41 + 0.48 and 26.25 ± 0.96). Overdose (7.4 + 0.87 and 23.50 ± 2.52) and prolonged dose (7.13 + 0.51 and 21.50 ± 1.29) recorded a significant decrease when compared to the control. Also, a significant increase was observed in Total white blood cell count (TWBC) of the rats that received the normal dose of Diazepam when compared with the control. Treatment with Overdose and prolonged dose showed TWBC of 8.25 ± 0.37 and 8.08 ± 0.15.and are significantly ($p<0.05$) lower than the control

KEYWORDS: Diazepam, therapeutic, overdose, prolonged, hematology, liver, male Wistar rats

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1. INTRODUCTION

Many substances have been known to affect neurotransmitters of the central nervous system in organisms. Most of these substances also act in such a way that these neurotransmitters are made numb and temporarily unable to transmit signals to the brain. Such substances are generally called anaesthetics [1]. The benzodiazepines are a large class of medications that have multiple clinical uses including therapy of anxiety, insomnia, muscle spasm, alcohol withdrawal and seizures. As a class, the benzodiazepines do not cause significant serum enzyme elevations and have been linked to only very rare instances of acute, symptomatic liver disease.The pharmacological effects of the benzodiazepines are as a result of their interaction with the central nervous system (CNS), their effects being sedation, hypnosis, decreased anxiety, and muscle relaxation, amnesia and anticonvulsant activity. At high doses, when taken intravenously, the benzodiazepines may also cause coronary vasodilation and neuromuscular blockade. The CNS effects of benzodiazepines are believed to be mediated by activation of gamma-aminobutyric acid A (GABA-A) receptors and modulation of their inhibition of neurotransmission [2].Diazepam, first marketed as valium, is a medication of the benzodiazepine family that typically produces calming effects. It is commonly used to treat a range of conditions including anxiety, alcohol withdrawal syndrome, benzodiazepine withdrawal syndrome, muscle spasms, seizures, trouble sleeping and restless legs syndrome. It may also be used to cause memory loss during certain medical procedures [3]. It can be taken by mouth, inserted into the rectum, or injected into a muscle, or injected into a vein. When given into a vein, effects begin in one to 5minutes and last up to an hour. By mouth effects may take 40mins to begin. Common side effects include; Sleepiness and trouble with coordination. Serious side effect is rare [4]. They include suicide, decreased breathing, and an increased risk of seizures if used too frequently, in those with epilepsy. Occasionally excitement or agitation may occur. Long-term use can result in tolerance, dependence, and withdrawal symptoms on dose reduction. Abrupt stopping after long-term use can be potentially dangerous [4]. After stopping, cognitive problems may persist for six months or longer [5]. It is not recommended during pregnancy or breastfeeding [6]. Its mechanism of action is by increasing the effect of the neurotransmitter gamma-Amino-butyric acid (GABA) [5]. Biochemical parameters could be defined as any biochemical compound such as an antigen, antibody, abnormal enzyme, or hormone that is sufficiently altered in a disease to serve as an aid in diagnosing or in predicting susceptibility to the disease [7]. Linked with hematologic and urinalysis the biochemical profile forms the database for most analytical study. Many biochemical parameters tend to have specificity for an organ and/or a limited range of pathological processes. Investigative biochemical profiles are designed to provide all the data necessary for a broad investigation of internal disease. Profiles with limited data are best used for monitoring an

established diagnosis for which the results of a more wide-ranging profile have already been obtained. Individual biochemical evaluations may be used, for example, for therapeutic drug testing, assessing vitamin status and monitoring liver function (bile acids) and diabetic control [8]. Some biochemical parameters include aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, bilirubin level, blood glucose, cholesterol level, electrolytes (such as phosphorus, calcium, sodium, etc.). In this study, the focus was on the evaluation of the Influence of therapeutic, prolonged and overdose intake of diazepam on some haematological indices and liver function parameters of male Wistar rats.

2. MATERIALS AND METHODS

Reagents and chemicals

All chemicals used in this study were of analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstadt, Germany. Reagents used for the assays were products of Randox Commercial Kits, England.

Preparation of Diazepam stock

The stock solution of diazepam was prepared by dissolving 5 mg of the drug in 100 ml distilled water. The dose to be administered was calculated based on the average body weight of the rats per group.

Experimental design

A total number of 20 male rats (average weight of 160g) were purchased from Physiology animal house in Abuja campus, University of Port Harcourt. They were then grouped into four of 5 rats each

Group A; Normal dose: 0.012 mg /Kg body weight

Group B; Overdose: 0.036mg/kg body weight

Group C; Prolonged dose: 0.012mg/kg body weight ...treated for 21 days.

Group D; Control group: Received no treatment

Determination of haematological parameters

The determination of some haematological parameters (haemoglobin concentration Hb, packed cell volume PCV and total white blood cell count,) was carried out according to the method of [9].

Determination of liver enzymes

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP), were analysed using Randox (United Kingdom) test kits and values read with the aid of a double-beam spectrophotometer

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple range tests. Differences were considered to be significant at $p < 0.05$ against a control group. Data were presented as mean $\pm$ S/D

3. RESULTS AND DISCUSSION

Results showed a significant increase ($p<0.05$) in the Hb concentration (Figure 1) and PCV (Figure 2) of the rats that received the therapeutic dosage of diazepam when compared to the control. However, a significant decrease was observed in the group that received a prolonged dose of the drug. This indicates that long-term exposure of the rats to the drug, reduced the haemoglobin concentration and PCV. Equally the animals on overdose therapy were found to exhibit low haemoglobin and packed cell volume levels compared to those on the normal therapeutic regimen.

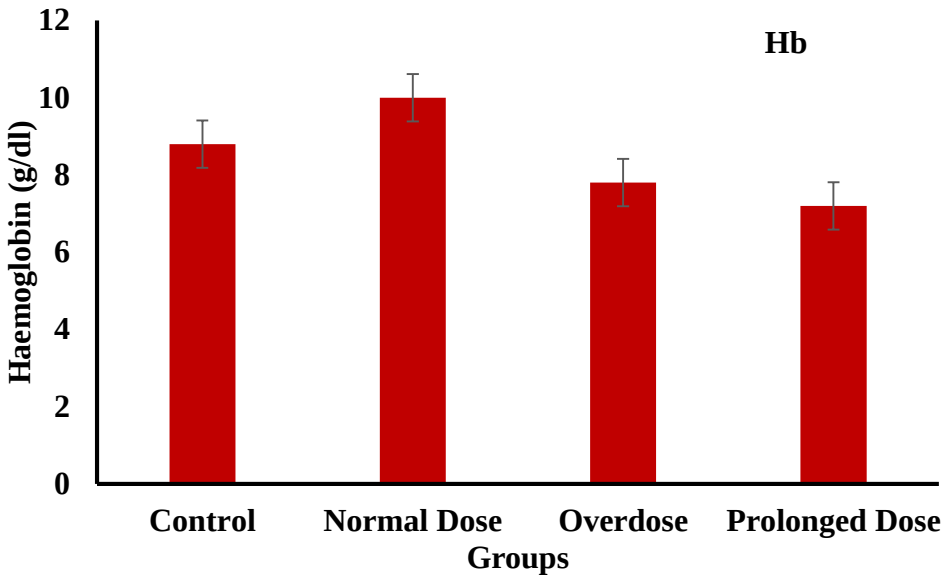

Figure 1: Haemoglobin (Hb) of Wistar rats that received a various dose of Diazepam

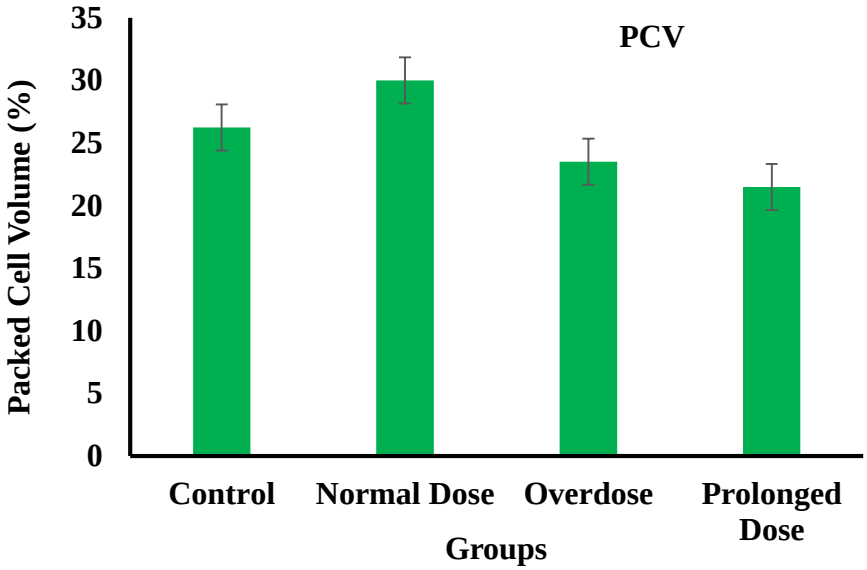

Figure 2: Packed cell volume (PCV) of Wistar rats that received a various dose of Diazepam.

Results for TWBC (Figure 3) showed a significant increase ($p<0.05$) in the groups that received the therapeutic dose when compared to the control group. On the other hand, the group that received a prolonged and overdose of diazepam recorded a significantly reduced ($p<0.05$) TWBC count when compared to the control group.

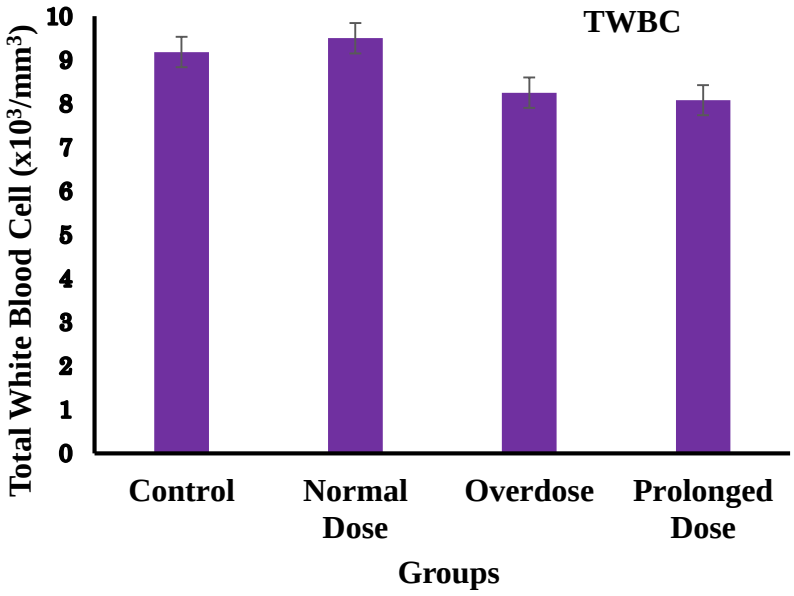

Figure 3: Total white blood cell count (TWBC) of Wistar rats that received various dose of Diazepam

The effect of diazepam on the AST level of male Wistar rats was also analysed. After treatment, it was observed that Diazepam had no significant effect ($p<0.05$) on AST level of the animals treated with normal and prolonged doses (Figure 5). However the animals that received the overdose of the drug had a significantly increased level ($p<0.05$) of AST activity when compared with the control.

The result of ALT activity in all the treatment groups was observed to be lower than the control group. Results of ALP activity for the overdose and prolonged therapy showed a significant increase at ($p<0.05$) compared to the normal dose treatment group, however, their value is lower than that of the control.

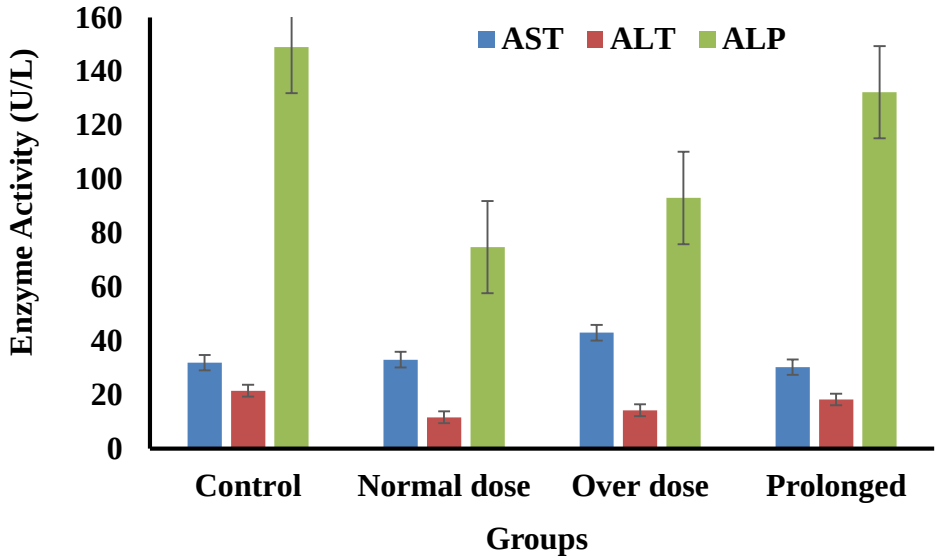

Figure 4: Liver enzyme activity of Wistar rats that received various doses of Diazepam

DISCUSSION

Toxicity studies of drug effects in animals are commonly used to calculate potential health risk in humans, caused by adverse effects of chemical compounds used in the manufacture of such drugs [10]. The deleterious effects of these substances may be accompanied or preceded by clinical signs of toxicity such as salivation, loss of hair, changes in animal eye colour, decreased the respiratory rate and motor activity. The haematological parameters investigated in this study are useful indices that can be employed to assess the toxic potentials of drugs to living systems. However, the significant increase of PCV in the treatment of the rats with normal diazepam shows that the drug help in the multiplying the red blood cells components of the rats. This agrees with the study by [11]. which showed increased haematological parameters with the rats exposed to a tranquillizing substance. However, the prolonged treatment with diazepam showed a significant decrease in Hb and PCV values. Low values in Hb and PCV may predispose the animals to anaemia characterised by a low number of red blood cells. This implies that the decrease is a factor of quantity and, as the overdose

group also showed reduced values of PCV. This is similar to the study carried out by [12], when it was discovered that PCV values in Wistar rats decreased when the rats were exposed to a substance (Taurine), for a long time. It was discovered in a research [13] that diazepam has effects on the cardiovascular makeup of the rats, in that it inhibits blood flow. This is similar to the results of this study showing that the reduced Hb and PCV values affects circulating red blood cells and may lead to a poor heart condition.Leukocytes (WBCs) are known for their capacity to fight disease in organisms. Exposure to certain drugs and substances can lead to the depletion of white blood cells in the body [10]. Results of the study showed increased values of TWBC counts in the group that received the therapeutic dose of diazepam as compared to the control. This indicates that diazepam may help to build up the immunity of the rats. However, the groups that received overdose and prolonged dose showed decreased values of TWBC which indicates that with a factor of time, increased dosage and exposure, the rats are more likely to have a reduced immune system, making them susceptible to diseases. A similar study carried out by [11], showed reduced levels of TWBCs of the Wistar rats when exposed to substances (ethanol) that have the same tranquilizing effects as diazepam. This shows that diazepam is an anti-immune drug as it depletes leukocytes in the blood. Hepatocellular damage with the subsequent disruption of the plasma membrane allows leakage of intracellular enzymes such as ALT or AST into the bloodstream. TB measurements are useful, but if the increase is due to liver toxicity it is normally accompanied by a much more rapid increase in ALT. If the cause of increased bilirubin is a biliary obstruction, then the increase in ALT is slower (days to weeks/months) and is accompanied by increased ALP and GGT, and there is much less risk of severe acute liver failure. If the cause of increased bilirubin is haemolysis, it has to be diagnosed by other means [14]. ALT is a more specific marker of hepatic injury than other parameters, because other parameters example AST elevation, can also be seen in cardiac tissue injury, haemolysis and muscle tissue [15], findings indicate that no damage was done to the hepatic cells of rats treated with a various dose of diazepam

4. CONCLUSION

Excessive and prolonged intake of diazepam showed a reduction in the Hb and PCV values as well as the TWBC counts of the selected Wistar rats. The decrease in Hb and PCV may lead to anaemia and cardiovascular damages. Reduced TWBC counts lead to reduced immunity against diseases. As such, normal dosage of this drug should be administered at all times and with the appropriate prescription. The result, for AST activity indicates that the drug may not cause any hepatic damage. At the ALT level, the result shows the effective functioning of the enzyme in the cell as its concentration is within normal range. Furthermore, ALP activity did not indicate any damage in the liver cells but may present a negative influence on the bile duct of the organism. Research findings

may indicate why diazepam is a restricted/ prescription drug as an unsupervised and abuse of it could lead to conditions which are deleterious to the kidneys, gall bladder, liver, spleen, red blood cells and gastrointestinal tracts. Diazepam usage should therefore be properly monitored so as to ensure the proper usage of the drug in its right dosage and for the right period of time so as to checkmate addiction and abuse of the drug e.g. the use of diazepam as a recreational drug without a prescription.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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