THE HAEMATOTOXICITY AND NEPHROTOXICITY EFFECTS OF DIAZEPAM ADMINISTRATION IN MALE ALBINO RATS

Original Research Article

ABSTRACT

Objectives: This study aimed to assess the haematological and nephrotoxic effects of diazepam at varying doses in male albino rats.

Materials and Methods: Rats were administered therapeutic (0.062 mg/kg/day), high (0.33 mg/kg/day), and extremely high (0.661 mg/kg/day) doses of diazepam orally for 14 and 28 days. Blood and kidney samples were collected for haematological and biochemical analysis.

Results: Diazepam administration significantly reduced red blood cell count (RBC), packed cell volume (PCV), and platelet count (PLT), while other haematological indices were not notably affected. Plasma creatinine and urea levels increased, and total protein decreased across all doses. The treatment also elevated renal thiobarbituric acid reactive substances (TBARS) levels, while reducing antioxidant enzyme activity superoxide dismutase (SOD), catalase (CAT), and reduced glutathione(GSH).

Conclusions: Diazepam poses significant risks of oxidative stress, haematotoxicity, and nephrotoxicity. While effective in managing anxiety, caution is necessary due to its potentially harmful effects on blood and renal function.

Keywords: Diazepam; haematotoxicity; oxidative stress; nephrotoxicity

1. INTRODUCTION

“Anxiety disorders are among the most prevalent mental health issues. As of 2005 over 40 million Americans experience conditions such as panic disorder, agoraphobia, PTSD, OCD, panic disorder, or generalized anxiety disorder (GAD)”[1]. “The widespread occurrence and lack of adequate treatment for anxiety disorders are significant issues in both industrialized and developing countries, though global prevalence estimates vary”[2].

“Historically, early pharmacological treatments for anxiety included general depressants and sedatives like alcohol, opiates, lithium bromide, and chloral hydrate”[3]. “These were largely replaced by carbamates (e.g., meprobamate) and barbiturates (e.g., phenobarbital) by the mid-20th century”[4]. “Benzodiazepines emerged as a promising class of potent anxiolytics with fewer fatal adverse events in 1960 and by 1963, diazepam (Valium) was introduced, offering even greater potency and a superior safety profile”[5].

“Benzodiazepines, such as diazepam, function as positive allosteric modulators by binding to a specific site at the alpha-gamma subunit interface of the GABAA (γ-aminobutyric acid type A) receptor complex” [2].“Instead of causing a functional response independently, they amplify the response of the endogenous ligand. Diazepam enhances CNS depression by increasing neuronal chloride-ion influx when GABA binds to the receptor, resulting in hyperpolarized postsynaptic membranes”[5]. “This potentiation of GABA's effects occurs in the limbic system, thalamus, hypothalamus, and cerebral cortex, leading to calming effects on neuronal processes in these regions, which in turn produces its anxiolytic and antiepileptic effects”[6].

“Globally, there is stringent control on the usage of Diazepam due to the risk associated with its usage. For instance, the United Kingdom Committee on the Review of Medicine (1980) recommended the use of Diazepam for 2-4 weeks at a low dosage”[7].“Furthermore, statistics have shown that globally, the use of benzodiazepines (diazepam) for non-medical purposes is on the increase” [8].“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 diazepam on body tissues”[9]. Despite the increasing use, limited information, however, exists on the effect of diazepam onhaematological and biochemical parameters which could reveal the toxicity of diazepam on specific tissues. Therefore, this study evaluated the effects of diazepam administration onhaematological and renal biochemical parameters todetermine diazepam toxicity potentials.

2.MATERIALS AND METHODS

2.1 Materials

Centrifuge machine, human automated haematology system analyzer (ERMA PCE 210, ERMA, Japan), weighing balance, dissecting sets, cuvette, spectrophotometer, pH meter, refrigerator, homogenizer, razor blade, 1 ml syringes, 2 ml syringes, and 5 ml syringes, surgical gloves, cotton wool, measuring cylinder, test tubes, beaker, spatula, plastic cages, EDTA bottles, plain sample bottles.

2.2 Reagents

Thiobarbituric acid (TBA), nicotinamide adenine dinucleotide reduced (NADH) and Codeine were obtained from Sigma–Aldrich Chemical Co. Ltd. (England). Nitrobluetetrazolium (NBT), 5,5′- Dithiobis (2-nitrobenzoic acid) (DTNB) are the product of Fluka (Buchs, Switzerland). All other chemicals used were analytical grade.

2.3 Animals

Thirty-five (35) male Wistar rats with an average weight of 170-200 g were used for the experiments. They were housed in the Ladoke Akintola University of Technology, (LAUTECH) animal house. They were allowed fourteen (14) days to acclimatize before the commencement of drug administration. The animals were maintained on a standard pellet diet throughout the acclimatisation and administration period. The animal experimental procedures were conducted in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023) revised in 2002 and approved by the institutional research committee.

2.4 Experimental Design

Thirty-five (35) male Wistar strain albino rats were divided into seven groups of five rats each according to their weight. Group I labelled control received saline solution for28 days through the oral route. Groups II, III and IVreceived a therapeutic dose of diazepam (0.062 mg/kg/day body weight of rats), a high dose of diazepam (0.33 mg/kg/day body weight of rats) and an extremely high dose of diazepam (0.66 mg/kg/day body weight of rats)respectively for 14 days.Similarly, groups V, VI and VII received a therapeutic dose of diazepam (0.062 mg/kg/day body weight of rats), a high dose of diazepam (0.33

mg/kg/day body weight of rats) and an extremely high dose of diazepam (0.66 mg/kg/day body weight of rats) respectively for 28 days. Diazepam was constituted in saline solution and administered through the oral route. During the experiment, the animals were allowed free access to food and distilled water. After 14 days and 28 days of diazepam treatment and after overnight fasting, animals were sacrificed by cardiac puncture under light ether anaesthesia. Blood and kidney samples were removed from the animals and stored for biochemical analysis.

2.5 Haematological Study

Freshly collected blood samples in EDTA bottles were analysed for haematological assay using an automatic haematological assay analyser (ERMA PCE 210, ERMA, Japan). Different tested haematological parameters were as follows: White Blood Cell (WBC), Red Blood Cells (RBC), Haemoglobin (HGB), Red cells (RDW%), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Platelet (PLT), Mean Corpuscular Volume (MCV).

2.6 Determination of Blood Biochemical Parameters

Plasma concentrations of urea, creatinine, total protein andcreatine kinase were determined using enzymatic kits (CYPRESS® Diagnostics, Langdorp, Belgium) according to the manufacturer's instructions.

2.7 Preparation of Kidney Homogenates

Before biochemical analyses, the kidney samples were cut into small pieces and homogenized in Phosphate buffer saline (PBS) with a homogenizer to give a 10% (w/v) kidney homogenate. The homogenates were then centrifuged at 12,000 rpm for 15 min. The supernatant obtained was used for the assay of superoxide dismutase, catalase, reduced glutathione, and thiobarbituric acid reactive substances (TBARS) content.

2.8 Determination of Renal Antioxidant Enzyme Activities and MDA Levels

Renal superoxide dismutase (SOD) activities were assayed in the tissue homogenates by the method of [10]. at 560 nm. One unit of enzyme activity was defined as that amount of enzyme which caused 50% inhibition of nitrobluetetrazolium reduction/mg protein. Catalase (CAT) activity was determined at room temperature by using the method ofAebi [11] and the absorbance of the sample was measured at 240 nm in a UV spectrophotometer. The reduced glutathione (GSH) concentration in renal homogenates was measured, as described by Jollow et al. [12]. The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid-reactive product malondialdehyde (MDA), using the method of

Draper and Hadley [13].All of the enzyme activities were expressed as per mg of protein and the tissue protein was estimated according to the method of Lowry et al., [14], using bovine serum albumin (BSA) as a standard.

2.9 Statistical Analysis

Results are expressed as mean ±S.E.M. The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Turkey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and p values < 0.05 were considered statistically significant.

3. RESULTS

3.1 Effect of Diazepam Administration on Blood Biochemical Parameters

Administration of diazepamfor 14 days and 28 days at normal, high and extremely high doses significantly increased the creatinine and urea concentrationsin the plasma. Administration of diazepam for 14 days at normal, high and extremely high doses significantly increased creatinine concentrations by 60.55%, 67.89% and 61.89%respectivelywhen compared with the normal rats. Also, diazepam administration for 28 days significantly increased urea concentrations by 38.26%, 151.36% and 83.84% respectively when compared with the normal rats. However, total protein levelswere decreased bydiazepam at all doses after 14 days and 28 days of administration when compared with the normal rats (Table 1).

Table 1. Effect of diazepam administration on Creatinine, Urea and Total Protein parameters of rats. Values are mean ± SEM (n=5). * = significantly different from control ($p<0.05$)

Parameters	Control	Therapeutic dose 0.062 mg/kg (14days)	High dose 0.330 mg/kg(14days)	Extreme high dose 0.661 mg/kg (14days)	Therapeutic dose 0.062 mg/kg (28days)	High dose 0.33 mg/kg (28days)	Extreme high dose 0.661 mg/kg (28days)
Creatinine (m/dL)	**141.7 ± 4.25**	**227.5 ± 10.04***	**237.9 ± 6.75***	**229.4 ± 5.84**	**188.4 ± 5.74****	**236.2 ± 7.34***	**270.0 ± 2.39***
Urea (mg/dL)	**19.37 ± 3.37**	**29.57 ± 1.25**	**45.38 ± 0.83***	**35.66 ± 0.82***	**26.78 ± 1.53**	**48.69 ± 3.77***	**35.61 ± 0.75***
Total Protein (g/dL)	**13.32 ± 0.95**	**7.77 ± 0.32***	**7.45 ± 0.26***	**7.92 ± 0.16***	**6.85 ± 0.11***	**7.14 ± 0.25***	**7.31± 0.36***

3.2 Effect of Diazepam Administration on SOD Activity

Administration of diazepam for 14 daysat normal, high and extremely high doses significantly reduced renal SOD activityby 50%, 40%, and 46% respectively when compared with normal rats while 28 days administration of diazepam significantly reduced renal SOD activity by 30%, 60%, and 40% respectively when compared with normal rats (Fig. 1).

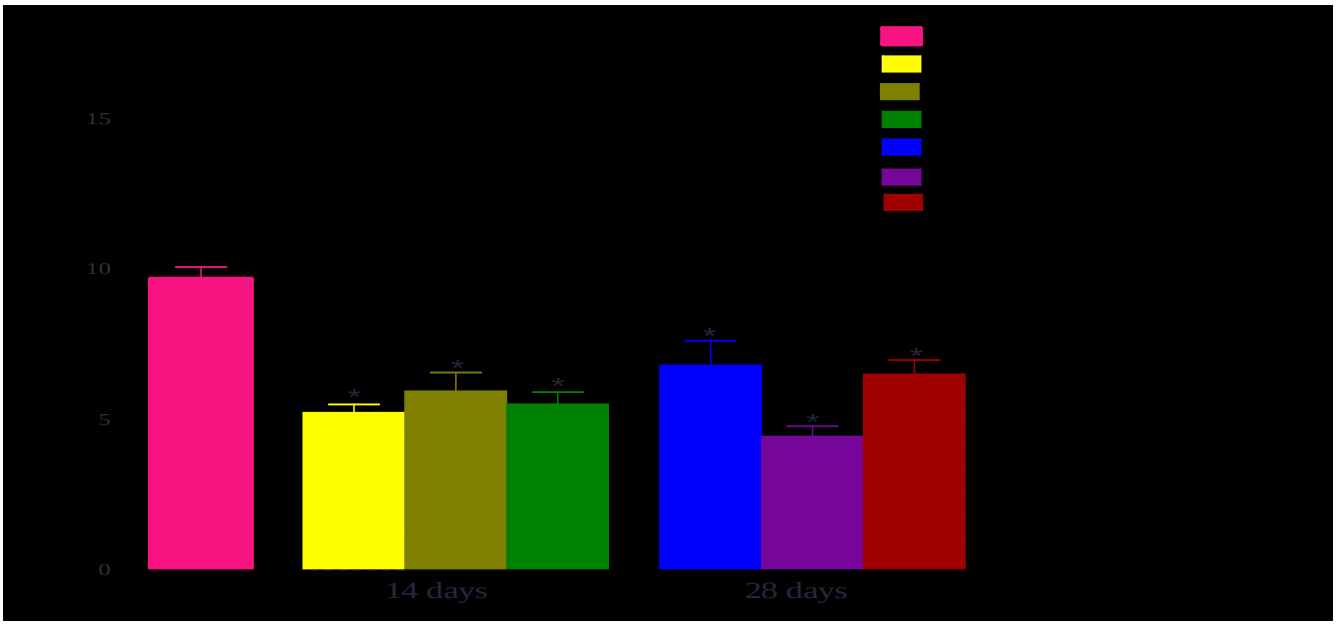

Fig 1. Effect of diazepam administration on renal SOD activity of rats. Values are mean ± SEM (n=5). * = significantly different from control ($p<0.05$)

3.3 Effect of Diazepam Administration on Catalase Activity

Diazepam administration for 14 days at normal, high and extremely high doses significantly reduced renal catalase activity by 53.13%, 34.38%, and 50% respectively when compared with normal rats while administration of diazepam at normal, high and extremely high doses for 28 days significantly reduced renal catalase activity by 46.88%, 68.75%, and 56.25% respectively when compared with normal rats (Fig. 2).

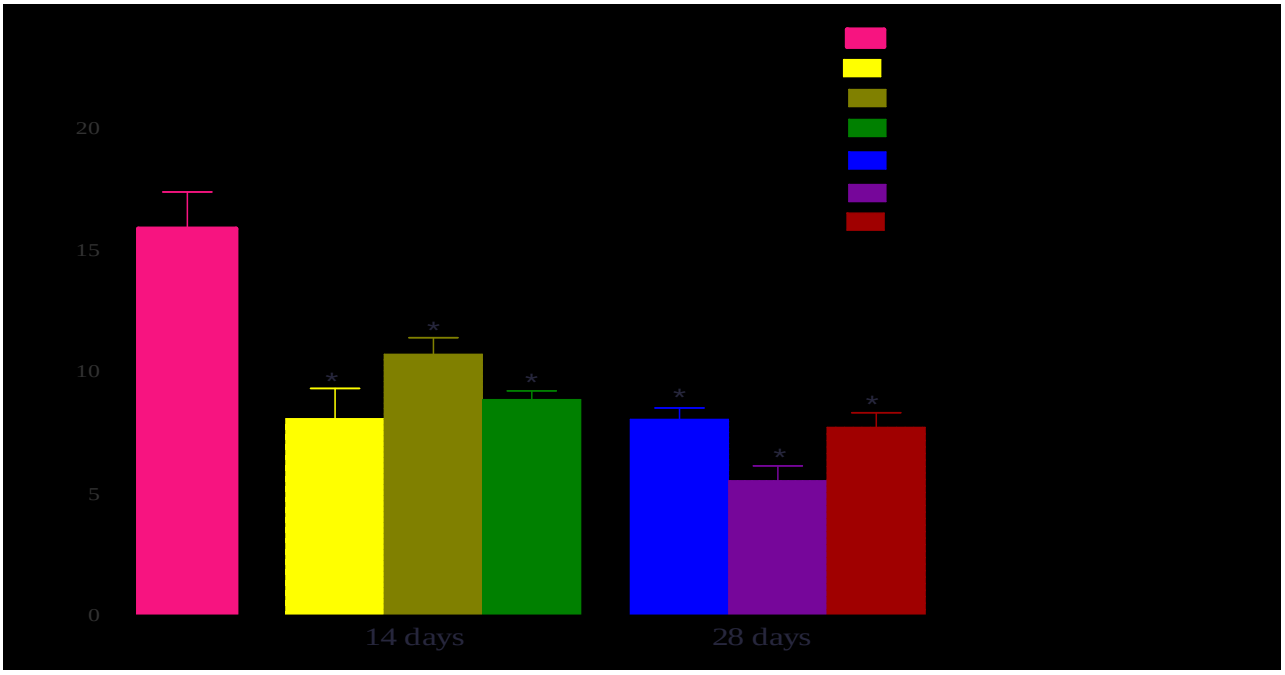

Fig 2. Effect of diazepam administration on renal catalase activity of rats. Values are mean ± SEM (n=5). * = significantly different from control ($p<0.05$)

3.4 Effect of Diazepam Administration on GSH Levels

Administration of diazepam for 14 days at normal, high and extremely high doses significantly reduced renal GSH levels by 70.59%, 64.71%, and 64.68% respectively when compared with normal rats while administration of diazepam for 28 days at normal, high and extremely high

doses significantly reduced renal GSH levels by 64.71%, 61.76%, and 64.71% respectively when compared with normal rats (Fig. 3).

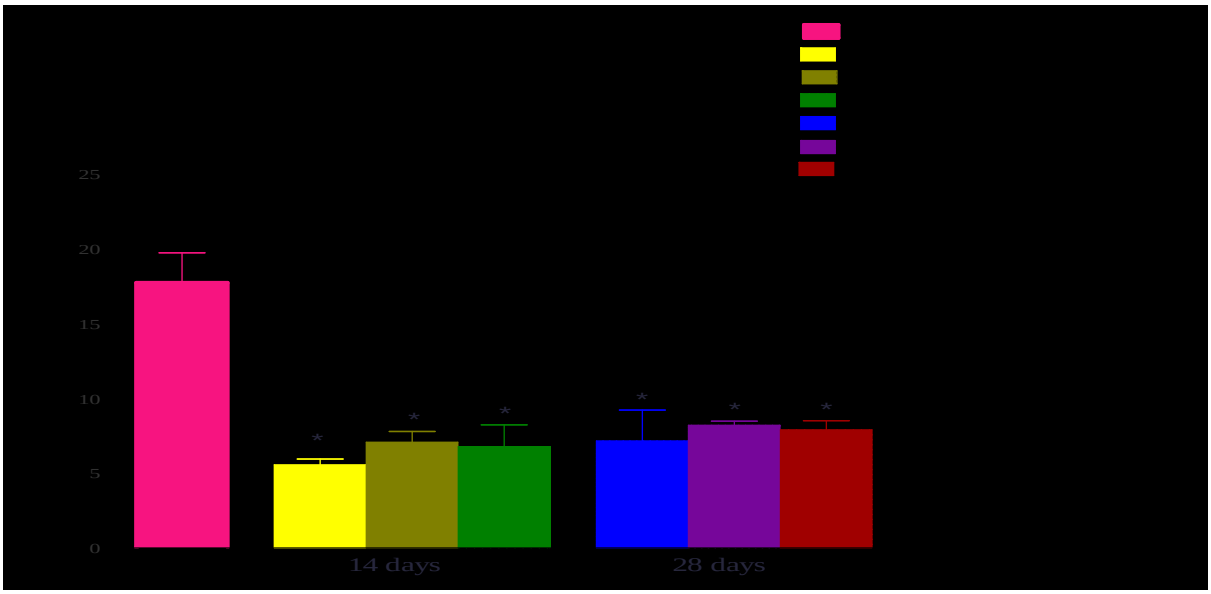

Fig 3. Effect of diazepam administration on renal GSH levels of rats. Values are mean ± SEM (n=5). * = significantly different from control ($p<0.05$)

3.5 Effect of Diazepam Administration on MDA Levels

Diazepam administration at all doses significantly increased MDA levels. Administration of diazepam for 14 days at normal, high and extremely high doses significantly increased renal

MDA levels by 1.75 fold, 4.50 fold, and 5.25 fold respectively when compared with normal rats while 28 days of diazepam administration increased renal MDA levels by 1.87 fold, 1.75 fold, and 1.62 fold respectively when compared with normal rats (Fig. 4).

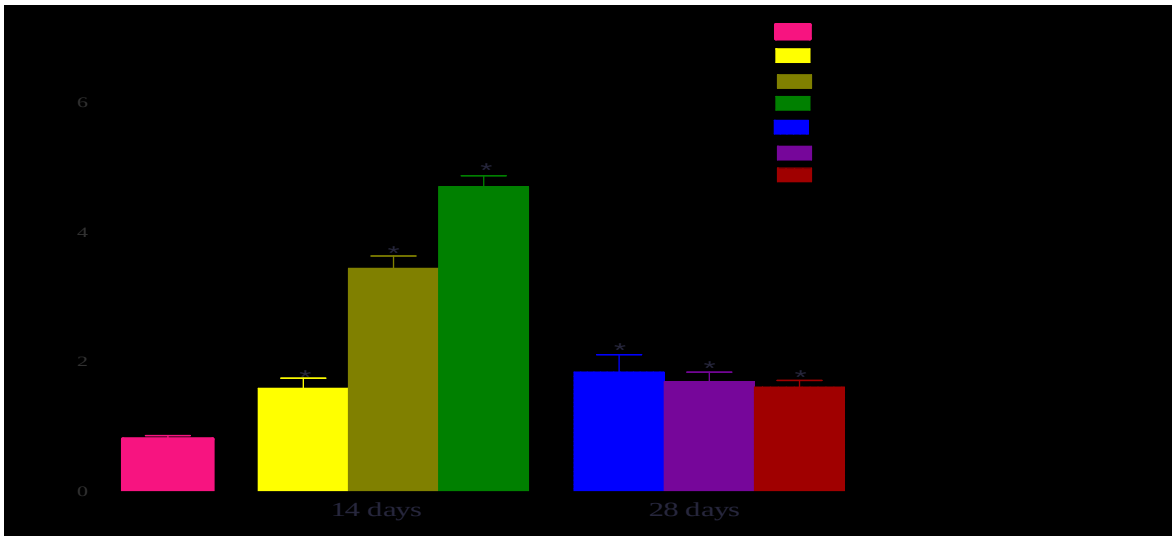

Fig 4. Effect of diazepam administration on renal MDA levels of rats. Values are mean ± SEM (n=5). * = significantly different from control ($p<0.05$)

3.6 Haematological Parameters

The effects of diazepam administration on haematological parametersare depicted in Table 1. No significant changes in the values of haemoglobin (HB), white blood cell (WBC) count, lymphocyte, mean corpuscular volume (MCV) and Mean Corpuscular Haemoglobin (MCH) when compared with control animals. However, administration of diazepam significantly lower ($p < 0.05$) packed cell volume (PCV), red blood cell (RBC) and platelet (PLT) when compared with control animals (Table 2).

Table 2. Effect of diazepam administration on haematological parameters of rats. Values are mean ± SEM (n=5). * = significantly different from control ($p<0.05$)

Parameters	Control	Therapeutic dose 0.062 mg/kg (14days)	High dose 0.330 mg/kg(14 days)	Extreme high dose 0.661 mg/kg (14days)	Therapeutic dose 0.062 mg/kg (28days)	High dose 0.33 mg/kg (28days)	Extreme high dose 0.661 mg/kg (28days)
PCV%	51.00 ± 1.00	37.00 ± 2.52*	41.25 ± 0.63	42.50 ± 2.22	51.50 ± 1.50	31.25 ± 2.83*	30.00 ± 2.28*
HB(g/dl)	11.95 ± 0.35	12.23 ± 0.43	12.78 ± 0.20	13.28 ± 0.68	14.55 ± 1.50	12.83 ± 1.86	10.46 ± 0.65
RBC($\times10^{12}$/L)	8.87 ± 1.38	6.65 ± 0.51*	6.60 ± 1.37*	7.53 ± 0.64	5.45 ± 1.80*	5.38 ± 0.45*	5.43 ± 0.42*
WBC($\times10^{9}$/L)	6.65 ± 0.45	6.65 ± 0.95	7.83± 0.73	6.90 ± 0.70	10.57 ± 1.37*	5.20 ± 0.70	8.80 ± 0.60
PLT ($\times10^{9}$/L)	666.5 ± 235.50	539.3 ± 83.32	536.8 ± 60.13	581.3 ± 56.78	594.0 ± 65.00	655.0 ± 23.79	425.7 ± 24.57
LYMPHOCYTE	88.33 ± 1.86	79.50 ± 3.50	81.60 ± 1.78	87.20 ± 1.11	86.00 ± 0.58	85.60 ± 1.08	84.40 ± 2.40
MCV (fL)	58.00 ± 2.04	61.00 ± 1.78	63.00 ± 1.10	61.60 ± 1.97	63.50 ± 4.21	60.80 ± 3.20	59.40 ± 1.29
MCH (pg)	18.25 ± 0.62	20.25 ± 0.75	19.60 ± 0.24	19.20 ± 0.37	18.25 ± 0.49	18.80 ± 0.58	19.40 ± 0.60
MCHC(g/L)	318.0 ± 0.91	328.0 ± 6.64	306.0 ± 4.60	309.2 ± 8.529	289.8 ± 19.29	307.0 ± 13.48	326.0 ± 14.54

4.0 DISCUSSION

Drugs are classified as psychoactive when they modify perception, mood, or awareness by interfering with the central nervous system's ability to operate[15].Worldwide, psychoactive drugs have shown promise in the treatment of a variety of illnesses, including mental problems. Caffeine, nicotine, and alcohol are the most commonly used drugs worldwide, and diazepam use is also on the rise [16].

Diazepam 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 in stimulating appetite [17]. Other therapeutic uses include relaxant and sedative effects [18]. This study evaluated the impact of administrations of therapeutic and high dosages of diazepam on the kidney and haematological parameters.

“The roleof kidneys in diazepam excretion predisposes them to toxic injury”[19]. “Renal functions are commonly determined by two parameters i.e. creatinine and urea. The level of plasma creatinine is used to determine the glomerular filtration rate while urea is used to determine the nephrotoxic profile of xenobiotics”[20].“In this study, impairment of the renal functions in diazepam-treated rats was indicated by a significant increase in urea and creatinine concentration in the plasma as compared to the control group. This observation was in support of previous studies and it is an indication of renal toxicity which causesa decrease in glomerular filtration rate leading to the buildup of creatinine and urea in blood” [21].

“Reactive oxygen species (ROS) are produced in many aerobic cellular metabolic processes, including superoxide and hydrogen peroxide, which react with various intracellular targets such as lipids, proteins, and DNA”[22].“Elevated levels of ROS present during oxidative stresscan lead to ROS-induced damage, including cell death, mutations, chromosomal aberrations, and carcinogenesis”[22].“The intracellular concentration of ROS depends on their production and/or removal by the antioxidant system. In this study, there is a significant decrease in kidney SOD levels in all groups treated with diazepam. This reduction could be linked to the exhaustion of these enzymes due to oxidative stress caused by diazepam administration, leading to a large population of unquenched free radicals and resulting in oxidative stress. This finding aligns with a previous study byEl Sokkary , which reported a significant decrease in SOD activity and GSH levels in the liver and kidneys of rats treated with diazepam” [23].

“Catalase activity is predominantly located in subcellular organelles known as peroxisomes. A decrease in catalase activity has been shown to correlate with the carcinogen-initiated emergence of the malignant phenotype in mouse keratinocytes”[24].“In this study, there was a significant decrease in kidney catalase levels in all groups treated with diazepam. These findings are consistent with a study bySuneetha, which reported a significant decrease in catalase activity in fish treated with diazepam, potentially due to the flux of superoxide radicals or activated metabolites generated by diazepam” [25].

“Reduced glutathione (GSH) is a major tissue antioxidant that neutralizes reactive oxygen species. As a result of donating an electron, GSH itself becomes reactive and will readily react with other reactive GSH molecules to form GSSG. Additionally, reduced GSH provides a reducing equivalent for the glutathione peroxidase (GPx) catalyzed reduction of lipid hydroperoxides to their corresponding alcohols and hydrogen peroxide to water. Overexpression

of this enzyme protects cells against oxidative damage, suppresses apoptosis induced by H_2O_2, and reverses the malignant phenotype in pancreatic cancer"[26-29]. In this study, there was a significant decrease in kidney GSH levels throughout the exposure period in all groups treated with diazepam. The decreased levels of GSH in the kidney indicated a reduced capacity to scavenge H_2O_2 and lipid hydroperoxides, as it reduced the GSH conversion to GSSG. These findings align with a study byEl-Sokkary[23], which reported a significant decrease in GSH levels in the liver and kidneys of rats treated with diazepam.

A large amount of polyunsaturated fatty acids found in all biological membranes is susceptible to peroxidation attacks by oxidants resulting in lipid peroxidation. So, lipid peroxidation production was used as a marker of oxidant-induced cell injury. In our study, we recorded a significant increase in renal malondialdehyde (MDA) levels in the diazepam-treated group when compared with the control. Our results are in support of earlier studies by Desuswoa[30], who reported an increase in the MDA level in diazepam-treated animals. Also, it has been reported that elevated MDA indicates an increase in free radical generation and is considered a useful measure of oxidative stress status [31].

"Reactive oxygen species and free radicals are also generated by chemicals and pollutants such as factory waste and toxic gases, which are known to disrupt haematological parameters in organisms"[32]. "Deviations from normal haematological parameter levels indicate the presence of toxicity or disease conditions"[33]. "In this study, diazepam administration caused a significant reduction in packed cell volume (PCV), red blood cell counts (RBC), and Platelet (PLT). The observed decrease in PCV suggests that diazepam administration resulted in anaemia, often occurring with haemorrhage, hemolysis, or failure in the erythroid lineage. The observed decrease in RBC suggested that diazepam administration resulted in blood loss due to serious gastrointestinal tract bleeding, red blood cell hemolysis, and poor iron absorption in the intestine"[34]. A decreased number of platelets (thrombocytopenia) by diazepam at extremely high dosage is indicative of significant danger that could be associated with indiscriminate use of diazepam.

"The toxic effect of diazepam administration leads to a large population of unquenched free radicals leading to the state of oxidative stress. Oxidative stress forms when there is an imbalance between free radical generating and scavenging systems has been implicated in the pathogenesis of a wide range of disorders, including neurodegenerative disorders, cardiovascular diseases, cancer, and ageing" [35].

5. CONCLUSION

The fast and efficient sedative effect of diazepam on muscle and nerve cells is the primary reason for its widespread therapeutic use in humans and animals. The dose and duration of diazepam therapy must be adjusted individually, as it affects the functioning of entire organs within an organism. In this study, impairment of the renal functions bydiazepam was demonstrated by the significant increase in urea and creatinine concentration in the plasma, reduction in endogenous

antioxidant SOD, CAT, and GSH and increased in malondialdehyde level (MDA). Administration of diazepam in this study also resulted in a reduction in the levels of Packed Cell Volume (PCV), Red Blood Cell (RBC), and Platelet levels in rats. The present study highlighted the toxicity effects of diazepam administration at therapeutic and high dosages on biochemical andhaematological indices in rats.

ETHICAL APPROVAL

As per international standards or university standards, ethical approval has been collected and preserved by the authors.

AUTHOR CONTRIBUTIONS

Kayode Abiodun Afolayan and Abiodun Olusoji Owoade:Conduction of practical parts, collection and/or assembly of data and interpretation, manuscript writing;Kayode Abiodun Afolayan: Haematological assessment and plant material collection; Abiodun Olusoji Owoade:conception, design, and final approval of manuscript. All the authors have read and approved the manuscript.

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