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Hepatorenal Features following oral administration of Primidone in Adult Wistar Rats

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Abstract

Primidone is an anticonvulsant used for the treatment of seizures. This study was undertaken to evaluate the effect on the histomorphology of the liver and kidneys of adult Wistar rats. Fifteen (15) adult Wistar rats were randomly assigned into three groups of five (5) rats each. Group A was the Control given free access to standard rat chow and water, treatment groups B and C received 75 mg/kg and 150 mg/kg per body weight of primidone representing low (Group B) and high dose (Group C) regimen respectively for four weeks. At the end of the treatment period, the rats were euthanized using chloroform and blood sample were obtained from the inferior vena cava for biochemical assessment of hepatic and renal function parameters. The liver and kidneys were harvested concurrently and processed following standard histological techniques for microscopic assessment. The histoarchitecture of the liver and kidneys of rats treated with primidone had features consistent with normal morphology, there were alterations observed. Biochemical assessment of liver function parameters shows significant ($p<0.05$) increase in Aspartate aminotransferase (AST) Alanine transaminase (ALT), Alkaline phosphatase (ALP), Total protein, Direct Bilirubin and Indirect Bilirubin levels. Multiple comparison between groups using least square difference showed statistically significant dose dependent increase ($p<0.05$) in the mean serum AST, ALT and ALP levels. Renal function tests showed no significant change in mean serum creatinine level, however mean serum level of electrolytes (sodium, chlorine and potassium) showed a significantly increased ($p<0.05$) across treatment groups when compared to control. There was no pathological changes in the liver and kidneys following oral treatment with primidone, however, there was increased level of liver enzymes, and alterations in renal function parameters as evident in elevated electrolyte levels

Keywords: Primidone, Liver, Kidney, Rat

Introduction

Primidone is a first-generation barbiturate-type antiepileptic drug used to treat partial and generalized seizures. It could also be used to treat tremors, as it has been considered a viable alternative to propranolol in the treatment of essential tremors (Bentué-Ferrer *et al.,* 2012). It is produced in tablets of 50 and 250 mg in various generic formulations and its indications include the treatment of grand mal and psychomotor epilepsy (Hedera *et al*., 2013). The efficacy of primidone in these forms has also been clinically recorded in cases of resistance to conventional medications, idiopathic and post-traumatic forms, linked with symptoms of brain injury. This antiepileptic medication can also be used to treat focal or Jacksonian, myoclonic and akinetic seizures (Verrier *et al.,* 2020).

Primidone treatment could result in elevated gamma glutamyl transpeptidase (GGT) levels. However, high concentration of alkaline phosphatase may be attributed to the enzyme isoforms. Because of its similarity in structure to phenytoin and phenobarbital (aromatic anticonvulsant), it has been suspected to cause anticonvulsant hypersensitivity syndrome, but

there seems to be no published convincing case study (El-Masri and Portier, 1998). It produces side effects such as drowsiness, dizziness, vertigo, epigastric pain, anaemia, edema of the face, rashes skin on skin and hypersensitivity (Micromedex, 2013).

The liver and kidneys play a vital role in metabolism, homeostasis as well as conducting several important biological functions notably detoxification and protein synthesis. Other roles in metabolism include glycogen storage management, red blood cell breakdown, and hormone generation (Abdel and Bloomston, 2010). A study by Banagar *et al.* (2020) evaluated the effect of primidone on liver enzymes levels in rats and showed a significant increase in AST, ALT, ALP, GGT serum levels in treated rats with evidence of tissues necrosis. However, long-term compensation for the absence of liver function is unknown (Zakim and Boyer, 2002). The kidneys main functions are filtration and excretion of waste and also regulation of body electrolytes, fluids, and blood pressure (Arkill *et al.,* 2014)

Materials and Methods

Ethical Consideration

Ethical clearance for this study was obtained from the Department of Human Anatomy and Cell Biology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka with Ethical Number DELSU/CHS/ANA/2022/26.

Experimental Animals

Fifteen (15) adult Wistar rats bred in the animal house of the Department of Anatomy and Cell Biology, Delta State University, Nigeria were included in this study. The rats were kept inside an iron cage with compartments and were allowed to acclimatize for two weeks. The cages were well ventilated and kept under controlled environmental conditions of temperature (25±5°C), relative humidity (50±5°C) and 12-hour light / dark cycle. They were fed ad libitum with standard grower's mash (feed) and had free access to water. Experimental animals were handled according to protocols approved by the institutional animal ethics committee (IAEC) as adopted by the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria.

Experimental Design

The animals were weighed and divided into three groups: A, B, and C, each consisting of five rats respectively. Group A- was the Control rats given free access to standard rat chow and water. The treatment groups B and C were orally administered 75 mg/kg and 150 mg/kg per body weight of primidone daily for twenty-eight (28) days.

Animal sacrifice and sample collection

At the end of the treatment period, the rats were sacrificed via cervical dislocation, blood samples were retrieved from the inferior vena cava for assay of liver and kidney function test. However, the liver and kidneys were harvested fixed and in 10% formal saline for histological study.

Preparation of Tissues for Microscopy

Hepatic and renal tissues excised were fixed in 10% formal saline and processed following standard histological protocols and routine haematoxylin and eosin technique as recommended by Drury and Wallington (1980). Photomicrographs of tissue images were captured using Digital Microscope Eyepiece SCOPETEK DCM 500, 5.0 megapixels.

Biochemical Analysis of Liver and Kidney Function

Blood sample was collected from the inferior vena cava, centrifuged and the serum used for the analysis of liver and renal function parameters. Data obtained was subjected to statistical analysis using one-way analysis of variance (ANOVA) and least square difference. Results were expressed as Mean ± Standard Error of Mean (SEM). P-value less than 0.05 were considered to be statistically significant.

Results

Effects of Primidone on the Liver and kidney functions of Adult Wistar rat

Figure 1 shows comparison of mean serum level of Liver function parameters in adult rat treated with Primidone at graded doses of 75 mg/kg and 150 mg/kg per body weight (group C high dose). The results showed a significant increase

(p<0.05) in Aspartate aminotransferase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) levels across treatment groups when compared to controls. Multiple comparison between groups using least square difference showed statistically significant dose dependent increase (p<0.05) in the mean serum AST, ALT and ALP levels at low and high dose groups compared to control. There was also a significant increase in mean serum level of total protein, direct and indirect bilirubin across treatment groups compared to control. Multiple comparison between groups using least square difference showed statistically significant dose dependent increase (p<0.05) more evident at high dose in the total protein, direct and indirect bilirubin level when compared to control.

Figure 2 shows the comparison between the mean serum level of the renal function parameters in adult rat which were treated with primidone at graded doses of 75 mg/kg and 150 mg/kg per body weight. The results showed that there was a significant increase (p<0.05) in urea level that is more evident at high dose when compared to that of the control. Multiple comparison between groups using least square difference showed statistically significant increase in the mean serum urea level in high dose group compared to control. However, at the low dose group there was no significant change. There was no significant change in mean serum creatinine level (p<0.05) across the treatment groups compared to that of the control group. Mean serum level of electrolytes (sodium, chlorine and potassium) significantly increased (p<0.05) across treatment groups when compared to control. Multiple comparison between groups using least square difference showed statistically significant dose dependent increase in the mean serum electrolyte levels observed at high doses indicating a dose dependent response in renal parameters.

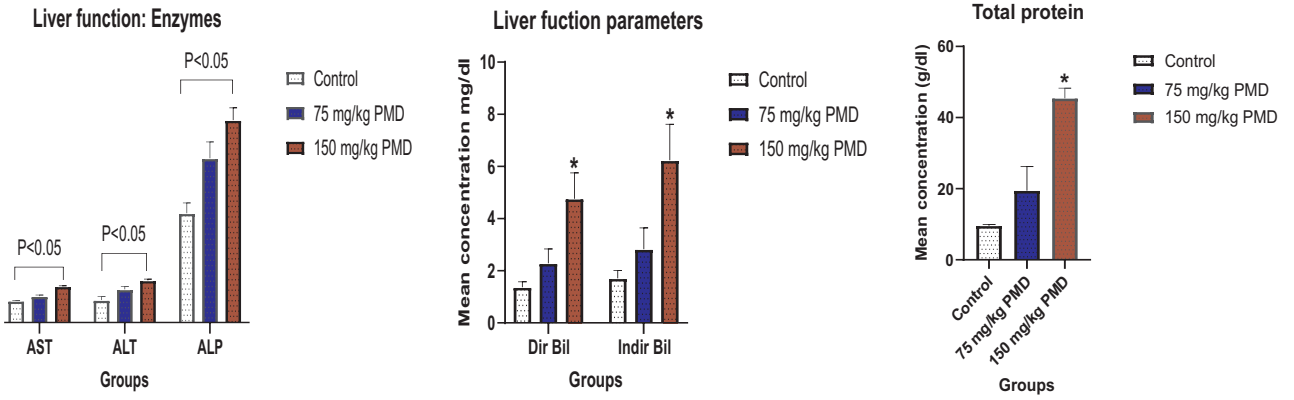

Figure 1: Effects of Primidone on liver function of rats. Values are presented as mean ± SEM for each group; n=5/group, * p<0.05 indicates significant difference compared to control

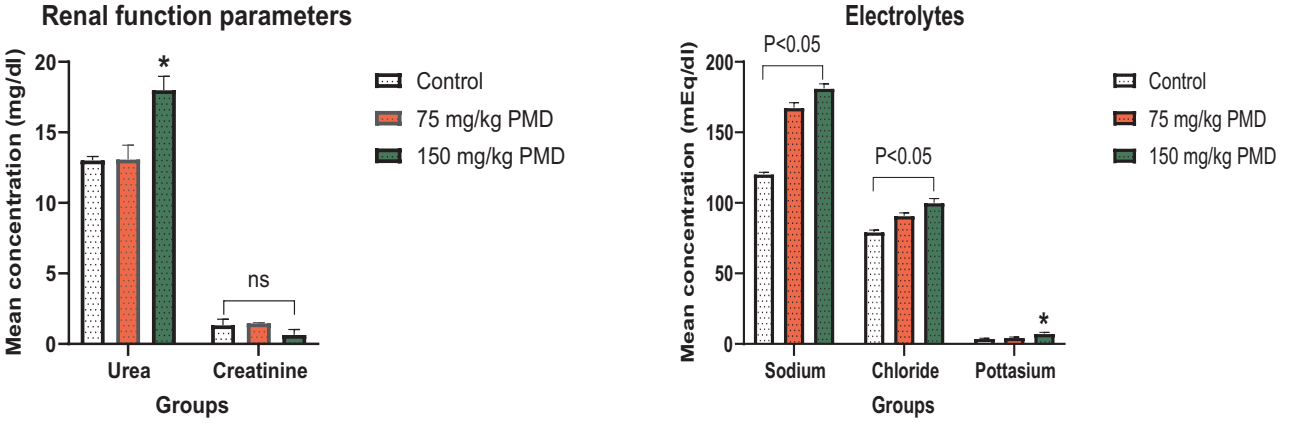

Figure 2: Effects of primidone on renal parameters of rats. Values are presented as mean ± SEM for each group; n=5/group, * p<0.05 indicates significant difference compared to control.

Effect of Primidone on the Histomorphology of the Liver of Wistar Rat

The control group (group A) photomicrograph showed that the liver tissue composed predominantly of the hepatic parenchyma and portal regions. The hepatocytes (H) appear polygonal and are disposed in sheet with a well outlined nucleus (N) the hepatocytes are separated by the sinusoids (S) with thin endothelial lining. The Portal Region (Circle), composed of branches of the Hepatic portal vessels (HPV), and bile duct (BD) appear normal (see plate 1a).

In group B, which is treated with low dose of primidone, the photomicrograph shows the liver tissue composed predominantly of the hepatic parenchymal and portal regions. The hepatocytes (H) appear polygonal and are disposed in sheet with a well outlined nucleus. The hepatocytes are separated by the sinusoids (S) with thin endothelial lining. The Portal Region (Circle), composed of branches of Congested (star), marginating leucocytes (arrow ahead) Hepatic portal vessels (HPV) and bile duct (BD) (see plate 1b).

In group C, which is treated with high dose of primidone, the photomicrograph showed that the liver tissue composed predominantly of the hepatic parenchymal and portal regions. The hepatocytes (H) appear polygonal and are disposed in sheet with a well outlined nucleus (N). The hepatocytes are separated by the sinusoids (S) with thin endothelial lining, free from collections and inflammatory cells. The Portal Region (Circle) composed of branches of the hepatic portal vessels (PV), Bile duct (BD) appeared normal (see plate 1c).

Effect of Primidone on the Histomorphology of Wistar Rat Kidneys

The renal tissue of rats in the control group showed several segments of renal tissue consisting of renal corpuscle (arrow) and the renal tubules. The renal corpuscle was made up of the glomerulus surrounded by podocytes separated by the defined Bowman's space. The renal tubules (proximal and distal) are lined with columnar-cubiodal epithelium with the proximal having densely packed microvilli which form a brush boarder. Section microanatomy appears normal and unremarkable (plate 2a).

The rats in the low dose group showed several segments of renal tissue consisting of renal corpuscle (arrow) and the renal tubules. The renal corpuscle was made up of the Glomerulus which is surrounded by the podocytes and separated by a defined Bowman's space (BS). The renal tubules (proximal and distal) are lined by columnar-cubiodal epithelium with the Proximal having densely packed microvilli forming a brush boarder. Section microanatomy appears normal and unremarkable (see plate 2b).
The rats in the high dose group presented showed several segments of renal tissue consisting of renal corpuscle (arrow) and the renal tubules. The renal corpuscle was made up of the glomerulus which is surrounded by the podocytes and separated by a defined Bowman's space. The renal tubules (proximal and distal) are lined by columnar-cubiodal epithelium with the proximal having densely packed microvilli forming a brush boarder. Section microanatomy appears normal and unremarkable (see plate 2c).

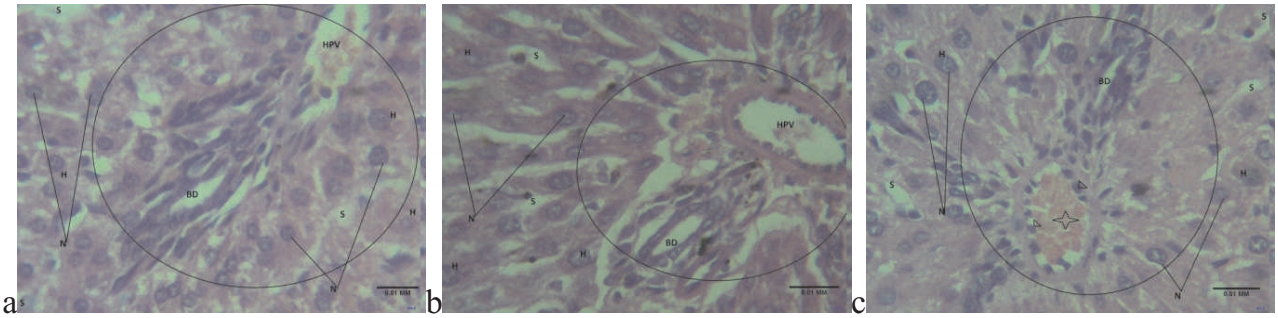

Plate 1: Photomicrograph of the Liver of Adult Wistar Rat in Control Group (H &E X 400)

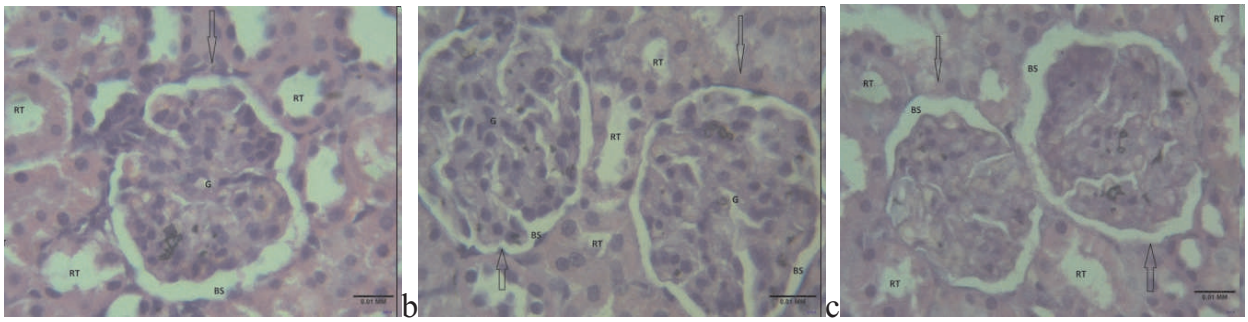

Plate 2: Photomicrograph of Kidney of Adult Wistar Rat in Control Group (H& E X 400)

Discussion

Histomorphological analysis revealed that the liver tissue of control rats composed predominantly of the hepatic parenchyma and portal regions. The hepatocytes appear polygonal and are disposed in sheets with a well outlined nucleus. The hepatocytes are separated by the sinusoids with thin endothelial lining. The Portal region, composed of branches of the Hepatic portal vessels and bile duct appear normal.

The rats treated with 75 mg/kg body weight of primidone showed similar features with the control, liver tissue composed predominantly of the hepatic parenchymal and portal regions. The hepatocytes appear polygonal and are disposed in sheet with a well outlined nucleus. The hepatocytes are separated by the sinusoids with thin endothelial lining. However, there was a difference in the portal region which was congested with marginating leucocytes, hepatic portal vessels and bile duct.

Similarly, the liver of rats treated with high dose of primidone showed a similar result of hepatic parenchymal though the portal region composing of branches of the hepatic portal vessels, bile duct appears normal. There was no difference in the histoarchitecture of the liver of rats administered with primidone (across the different level of dosage. This suggests the non-toxic potential of primidone on the liver though there is a dearth of literatures concerning the effect of primidone on the liver of normal rats more so, report from Ferranti *et al.,* (2001) on the effects of encapsulation of primidone on its oxidative metabolism in rats deduced that no difference was observed in the primidone administered rats an effect which is consistent with the finding in this study. However, the report of Banagar *et al.* (2020) contradicts the current findings as its study showed evidence of tissue necrosis. More so, rats that received 75 mg/kg and 150 mg/kg body weight of Primidone showed features consistent with normal renal morphology when compared to the control group, the renal tissue consisted of the glomerulus surrounded by the podocytes and separated by well defined Bowman's space. The renal tubules were lined by columnar-cuboidal epithelium with the proximal convoluted tubule possessing densely packed microvilli forming a brush border.

Liver function test evaluates the amount of several enzymes, proteins, and other chemicals produced by the liver. In the present study, AST, ALT, ALP, total protein, direct and indirect bilirubin was examined. The evaluation of mean serum level of liver function parameters in adult rats treated with Primidone at graded dosages of 75 mg/kg and 150 mg/kg per body weight was determined. When compared to the control, there was a substantial increase in Aspartate aminotransferase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) levels across treatment groups. Multiple group comparisons using least square difference revealed a statistically significant dose - dependent increase in mean serum AST, ALT, and ALP levels at low and high dose groups compared to control. This finding is in tandem with the study of Banagar *et al.* (2020) on the effect of primidone on liver enzymes level and hepatic tissue changes in adult male rat, it was opined that primidone administration increased serum level of AST, ALT, ALP and GGT. A similar report of Lisa *et al.* (2022) also confirms that the effect of primidone on hepatic function.

There was a significant increase in mean serum levels of total protein, direct bilirubin and indirect bilirubin across treatment groups compared to control. Multiple comparison between groups using least square difference showed statistically significant dose dependent increase more evident at high dose in the total protein, direct and indirect bilirubin when compared to control. This finding is in line with the study of Ali (2014) on primidone and liver abnormalities.

Drugs affect the kidneys, and these effects may be evident in alteration of some renal functional markers notably electrolytes (sodium, potassium and chloride), urea and creatinine balance or exert effects outside the renal system such as secondary action of nephrotoxic substances resulting from muscle breakdown (Marisa and Lauren, 2022). At lower dose, Primidone did not alter mean serum urea and creatinine level but showed significant increase in the mean serum electrolyte (sodium, chlorine and potassium). However, at 150 mg/kg per body weight, there was significant increase in mean serum urea level, with no effect on serum creatinine and electrolyte (sodium, chlorine and potassium) levels. An increase in mean serum urea levels, also known as uremia, can lead to renal disorders and may elicit moderate to severe life-threatening effects. An increase in mean serum electrolyte levels disrupts the electrolyte balance and this may affect normal body function. This dose dependent alteration of urea and electrolyte levels might increase the risk of renal disorders.

Conclusion

There was a substantial rise in Aspartate aminotransferase (AST), Alanine transaminase (ALT), and Alkaline phosphatase (ALP), Total protein, Direct Bilirubin and Indirect Bilirubin levels with slight increase in urea level at high dose treatment groups. No pathological changes were seen in the histoarchitecture of the liver and kidneys.

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Nil.

Conflicts of Interest

None to declare.

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