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Comparative Study on the Protective Effect of Zinc Nanoparticle and Zinc Supplement on Carbamazepine Induced Reproductive Damage in Male Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author ABB designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AMH performed the statistical analysis, and author ML managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study is to compare the protective effect of zinc nanoparticle and zinc supplement against carbamazepine induced reproductive changes in male Albino rats.
Study Design: In this experiment, 60 Male albino rats were used which are divided into six groups of 10 rats each. The first group was used as the control for the experiment and they were given distilled water. The second Group, Group 2 were administered with 20 mg/kg body weight of carbamazepine, group 3 were administered with 20 mg/kg of carbamazepine plus 10 mg/kg of zinc nanoparticle and Group 4 were administered with 20 mg/kg of carbamazepine plus 10 mg/kg of zinc supplement while group 5 were administered with 10 mg/kg of zinc nanoparticle and also group 6 were administered with 10 mg/kg of zinc supplement only.

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Place and Duration of Study: Department of Biochemistry, Bells University of technology, Ota, Ogun State Nigeria, the research was carried out from February to June, 2018.
Methodology: Zinc nanoparticle extraction was carried out to obtained Zinc nanoparticle. A 60 male albino rats with weight ranging from 140 g - 230 g were used. They were fed with normal rat chow and were allowed to acclimatize for a period of two weeks. They were then divided into six groups according to their body weight which contained the test groups and the control group. The rats were sacrificed after two weeks of test administration. They were allowed an overnight fast (24 hours). The cervical dislocation was done, and the blood was collected from the heart, in to a lithium heparinized bottle. The testes of the rats were also collected and stored in a sample bottle containing buffer and stored, the liver, kidney and the brain were collected too. The rats liver and testes were weight and macerated in 5 times the volume of the actual organ weight using homogenate buffer (phosphate buffer). The resulting homogenate was centrifuge at 10000 rmp speed for 15 mins then it was removed from the centrifuge and the supernatant was decanted and stored below 4°C.
Result: The group administered with carbamazepine only show significant decreased in the level of follicle stimulating hormone, luteinizing hormone and testosterone when compared with control group, followed by the group administered with zinc nanoparticle only. However, group 6 and group 4 that were administered with zinc supplement and zinc supplement plus carbamazepine showed a significant increase in the level of these hormones when compared with control group, while group 6 which were administered with carbamazepine and zinc nanoparticle showed no significant different when compared with control group. In addition carbamazepine alone significantly increased the level of alanine transaminase (ALT) in the liver and also the group administered with zinc nanoparticle alone significantly increased the level of aspartate transaminase (AST) with decrease in the level of ALT. However, the groups administered with zinc supplement alone and in combination with carbamazepine reduced ALT and AST levels in the liver.
Conclusion: Therefore, this study suggest that, carbamazepine induced toxicity by affecting the level of sex hormones and activities of the kidney in the male albino rats, and also zinc nanoparticle have more protective effect than zinc supplement against carbamazepine toxicity.

Keywords: Carbamazepine; zinc supplement; zinc nanoparticle; reproductive damage in Albino rats.

1. INRODUCTION

Zinc plays an essential role in the regulation of gene expression through metal-binding transcription factors and metal response elements in the promoter regions of the regulated genes. Zinc also plays a significant role in zinc-finger motifs. Zinc fingers typically have 4 cysteines within the protein that permit zinc to be bound in a tetrahedral complex.

In general, Zinc Nanoparticles can be defined as particles with size range of 1–100 nm in one dimension. Nanoparticles have two specific properties including their large surface area that dominates contributions of the material small bulks and their quantum effects.

ZnO nanoparticles have a large specific surface area and small size effect, when compared with ordinary ZnO powder and show wide application potential in microbial inhibition and mildew removal [1]. Insufficiency of trace elements affects almost all physiological processes like growth, reproduction, milk production, immunity and other processes of animals. Histological sections of organs also revealed their ultrastructural changes due to the elemental deficiencies [2]. Maternal Zinc insufficiency may compromise infant development and lead to poor birth outcomes.

Carbamazepine is a mood-stabilizing and anticonvulsant drug used mainly in the control of epilepsy, schizophrenia, bipolar disorder (trigeminal neuralgia), attention-deficit hyperactivity disorder (ADHD), phantom limb syndrome, neuromyotonia, complex regional pain syndrome, paroxysmal extreme pain disorder, disorder, borderline, and post-traumatic stress disorder (such as postcerbervascular accident thalamic pain). Among commonly prescribed anticonvulsants, carbamazepine appears to have the strongest aggravating potential in patients with juvenile myoclonic epilepsy, so it is significant to uncover any history of jerking, principally in the morning, prior to starting the drug. In highly strung cells which is includes neurons sodium channels are responsible for the mounting part of exploit potentials. In addition to

diminution associated with high occurrence rhythmic release of action potentials, carbamazepine also decreases excitatory neurotransmitter glutamate (Glue) and increases the inhibitory neurotransmitter GABA (gamma amino butyric acid).

Liver disease, particularly alcoholic liver disease (ALD), has been associated with zinc deficiency and hypozincemia for more than half a century [3,4]. These early alcoholic liver disease observations were confirmed by different investigators, and tissue concentrations of zinc have been proven to be decreased in alcoholic cirrhosis as well as animal models of liver disease [5,6].

A long-term oral zinc supplementation (200 mg tid for 2–3 months) in cirrhotic patients, including alcoholics, produced beneficial effects on both nutrition parameters and liver metabolic function [7]. Likewise, the Child-Pugh score, an overall clinical estimation of hepatocellular failure, was enhanced by zinc supplementation on average by greater than 1 point. Zinc supplementation also considerably improved nutrition parameters, such as retinol-binding protein, serum prealbumin, and insulin-like growth factor 1 (IGF-1).

Therefore, the present study was designed to investigate the effects and compare the protective activity of zinc Nano particle and zinc supplement on carbamazepine induced reproductive changes in male albino rats.

2. MARERIALS AND METHODS

2.1 Zinc Nanoparticle Extraction Procedure

The plant was shade dried and grinded to a fine powder. The dried powdered sample (4 grams) was dissolved in to 100 ml of distilled water, the solution was then filtered to remove the insoluble part of the leaf. 0.863 grams of zinc chloride ($ZnCl_2$) was measure and dissolved in to 100 ml of distilled water. 90 ml of this ZnCl solution is mixed with 10 ml of plant extract. The mixture was centrifuged at 3000 rpm for 5 minutes. The supernatant was removed by further filtration and the pellet was carried for the confirmatory test.

2.2 Experimental Animals

60 male albino rats with weight ranging from 140 g - 230 g were used. The rats were obtained from Ibadan State. The rats were kept in the animal house of Bells University of technology, Ota, Ogun State Nigeria where they were fed with normal rat chow and were allowed to acclimatize for a period of two weeks. They were then divided into six groups according to their body weight which contained the test groups and the control group.

2.3 Experimental Design

The rats were randomly distributed into 6 groups of 10 rats each and the animals were treated daily for the period of three weeks. The Zinc nanoparticle and Zinc supplement were given orally. Groups 1 and 2 were normal and untreated (20 mg/kg b.w of carbamazepine) reproductive damage control groups respectively and received a vehicle throughout the study. Groups 3 and 4 were treated with 10 mg/kg b.w of zinc nanoparticle + 20 mg/kg b.w of carbamazepine and 10 mg/kg b.w of zinc supplement + 20 mg/kg b.w of carbamazepine respectively. Group 5 was treated with only 10 mg/kg b.w of zinc nanoparticle and Group 6 was treated with only 10 mg/kg b.w of zinc supplement.

2.4 Sample Collection

The rats were sacrificed after two weeks of test administration. They were allowed an overnight fast (24 hours). The cervical dislocation was done, and the blood was collected from the heart, in to a lithium heparinized bottle. The testes of the rats were also collected and stored in a sample bottle containing buffer and stored, the liver, kidney and the brain were collected too.

2.5 Homogenization of Sample

The rats liver and testes were weight and macerated in 5 times the volume of the actual organ weight using homogenate buffer (phosphate buffer). The resulting homogenate was centrifuge at 10000 rmp speed for 15 mins then it was removed from the centrifuge and the supernatant was decanted and stored below 4°C.

2.6 Biochemical analysis

The serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) were assayed using wet reagent kits according to the guidelines. The Luteinizing Hormone (LH),

Follicle Stimulating Hormone (FSH), Testosterone level and Malonidialdehyde (MDA) enzyme activity were assayed from homogenates of liver and testes in rats.

2.7 Statistical Analysis

Data were presented as mean and standard error of the mean. They were analyzed by one-way ANOVA using the 17th version of SPSS. While difference between test and control group where examine using Duncan multiple range test $p<0.05$ was considered to be the level of significance.

3. RESULTS AND DISCUSSION

Follicle stimulating hormone, Luteinizing hormone and testosterone are important hormone in the diagnosis of testes damage caused by infection or toxicity. In this research, the testes damages were understood when the level of these hormone from each group were compared to their level in the control group. In this experiment, male albino rats were divided into six groups of 10 rats each. The first group was used as the control for the experiment and they were given distilled water. The second Group, Group 2 were administered with 20 mg/kg body weight of carbamazepine, group 3 were administered with 20 mg/kg of carbamazepine plus 10 mg/kg of zinc nanoparticle and Group 4 were administered with 20 mg/kg of carbamazepine plus 10 mg/kg of zinc supplement while group 5 were administered with 10mg/kg of zinc nanoparticle and also group 6 were administered with 10 mg/kg of zinc supplement only. From the Fig. 1 the group administered with carbamazepine only showed significant decreased in the level of follicle stimulating hormone, luteinizing hormone and testosterone when compared with control group, followed by the group administered with zinc nanoparticle only. However, group 3 which were administered with carbamazepine and zinc nanoparticle showed no significant different when compared with control group, while group 4 that were administered with zinc supplement plus carbamazepine showed a significant increase in the level of these hormones when compared with control group, while group 5 which were administered with only zinc nanoparticle showed significant increase when compared with control group and group 6 which were administered with zinc supplement showed significant increase when compared with control group. This result agree with the finding that revealed lower serum testosterone concentration in the CBZ group may be linked to the inhibitory effect of CBZ on the secretion of pituitary gonadotropins (FSH and LH), which aid in testosterone biosynthesis [8]. Similarly, decrease in testosterone concentration seen in the CBZ-treated group may occur due to direct destruction to Leydig cells [9]. Therefore this suggested that the zinc supplement has more protective effect than the zinc nanoparticle on carbamazepine induced reproductive changes of male albino rats.

ALT and AST are very important enzymes in the in the diagnosis of the heart and liver damage caused by liver attack, infection or during toxicity. After liver attack, a variety of enzymes including these aminotransferase leak from the injured liver in to the blood stream. The levels of serum AST, ALT, total and direct bilirubin and urea and creatinine were significantly ($p< 0.05$) increased in acute hepatotoxicity when compared to the normal control group due to Diabetic diseases or any other metabolic disorder [10].

From the Fig. 2 the result obtained in this study showed statistically significant ($p< 0.05$) increased in the serum levels of alanine aminotransterases (ALT) and significant ($p< 0.05$) decreased in serum levels of aspartate aminotransterases (AST) in the test group 2 treated with 20 mg/kg b.w of carbamazepine when compared to the control group treated with water only. The improvement seen in the test group 3 treated with 20 mg/kg b.w of carbamazepine plus 10 mg/kg b.w of zinc nanoparticle showed no significant ($p> 0.05$) change in alanine aminotransterases (ALT) level and significant ($p< 0.05$) decreased in aspartate aminotransterases (AST) level, than the test group 4 treated with 20 mg/kg b.w of carbamazepine plus 10 mg/kg b.w of zinc supplement that showed slightly increased in alanine aminotransterases (ALT) levels and significant ($p< 0.05$) decreased in aspartate aminotransterases (AST) levels when compared to the control group. Therefore this suggested that the zinc nanoparticle has protective effect than the zinc supplement on carbamazepine.

From the Fig. 3 The MDA level was observed to be high in the group that were administered with carbamazepine, which suggest the effect of carbamazepine on the MDA level in both testes and liver of the rats. However the groups administered with carbamazepine plus zinc nanoparticle showed the effect of zinc nanoparticle by reducing the level of MDA when compared with control group and group

administered with Carbamazepine. And also the group that ware administered with carbamazepine plus zinc supplement showed a relatively increased in the level of MDA when compared with control group. Therefore this study suggest that, zinc nanoparticle have more protective effect on carbamazepine that caused the elevation of MDA level in rats testes and liver when compared with groups that were administered with carbamazepine plus zinc supplement.

From the Fig. 4 the histological examination of the testes of the test group 2 treated with 20 mg/kg b.w of carbamazepine showed that there are numerous seminiferous tubules with regular outlines and moderate amounts of spermatocytes in the germinal epithelium. No visible lesion, when compared with the control group treated with normal water showed that there are seminiferous tubules with severely depleted spermatocytes and necrotic spermatogonia. The numerous seminiferous tubules with regular outlines and moderate amounts of spermatocytes in the germinal epithelium in the carbamazepine treated group in this study may due to the cytotoxic effect of carbamazepine. This agrees with the findings of [11], who reported that CBZ depresses spermatogenesis, which results in the death of immature germ cells present in the seminiferous epithelium. The Improvements in the histopathological pictures were observed more in the test group 3 treated with 20 mg/kg b.w of carbamazepine plus 10 mg/kg b.w of zinc nanoparticle showed that the seminiferous tubules are severely depleted of spermatogenic cells and show irregular outlines than the test group 4 treated with 20 mg/kg b.w of carbamazepine plus 10 mg/kg b.w of zinc supplement showed that the testis section is underdeveloped with irregular non patent seminiferous tubules and increased amounts of inter tubular connective tissue when compared to the control group. Therefore this result suggested that the zinc nanoparticle has more protective effect than the zinc supplement on carbamazepine.

From the Fig. 5, Histological examination of the liver of the test group two treated with 20 mg/kg b.w of carbamazepine showed that hepatic plates are closely packed. There are scant foci of random single cell hepatocellular necrosis, when compared to the control group treated with normal water showed that there are multiple foci of moderate thinning of hepatic cords with resultant dilation of hepatic sinusoids, there are mild aggregates of mononuclear cells in the portal tracts. The hepatic plates are closely packed; there are scant foci of random single cell hepatocellular necrosis in the carbamazepine treated group in this study may due to the cytotoxic effect of carbamazepine. The Improvements in the histopathological pictures were observed more in the test group three treated with 20 mg/kg b.w of carbamazepine plus 10 mg/kg b.w of zinc nanoparticle showed that

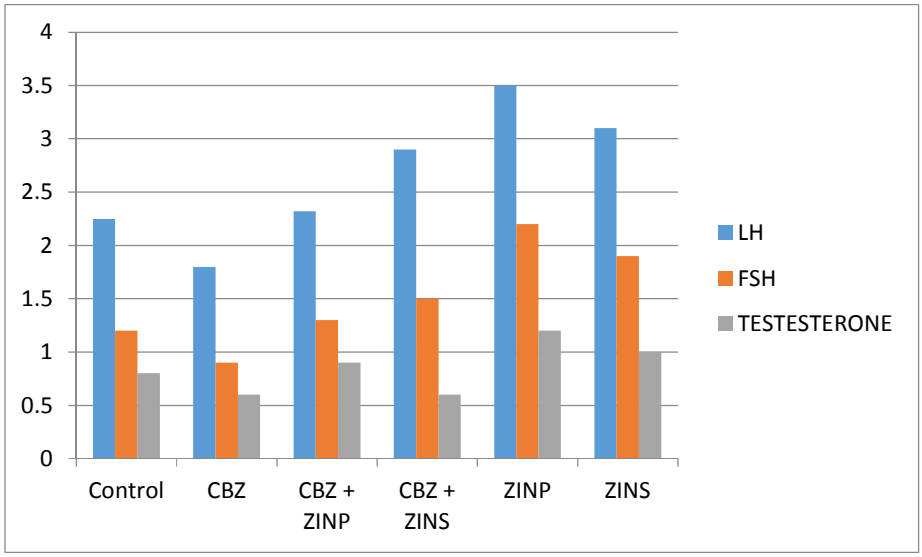

Fig. 1. LH, FSH and Testosterone level in the testes of Rats

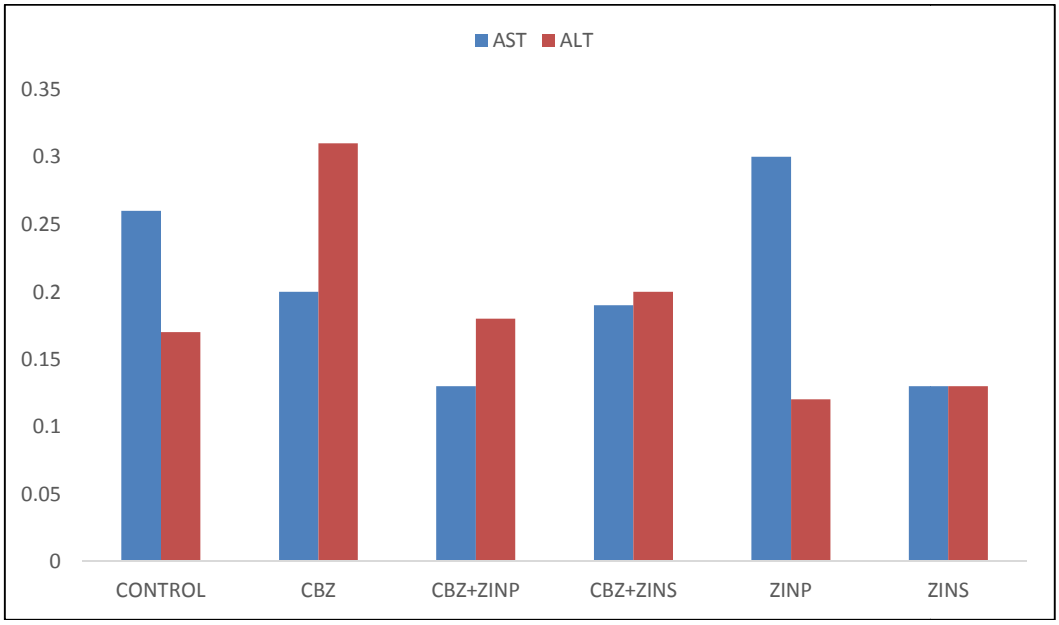

Fig. 2. ALT and AST activity in the serum of Rats

Hepatic plates are closely packed, than the test group 4 treated with 20 mg/kg b.w of carbamazepine plus 10 mg/kg b.w of zinc supplement showed that hepatic plates are closely packed, there are a few foci of single cell hepatocellular necrosis. There are dense aggregates of inflammatory mononuclear cells in the portal tract when compared to the control group. Therefore this result suggested that the zinc nanoparticle has protective effect than the zinc supplement on carbamazepine.

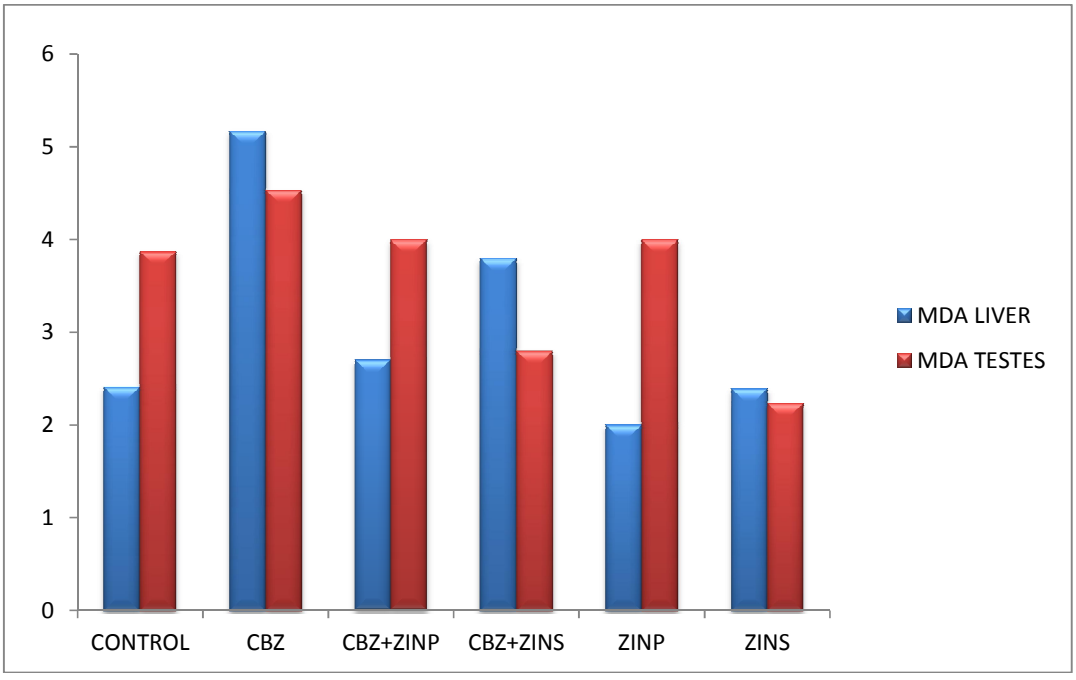

Fig. 3. MDA activity in Rat liver and testes

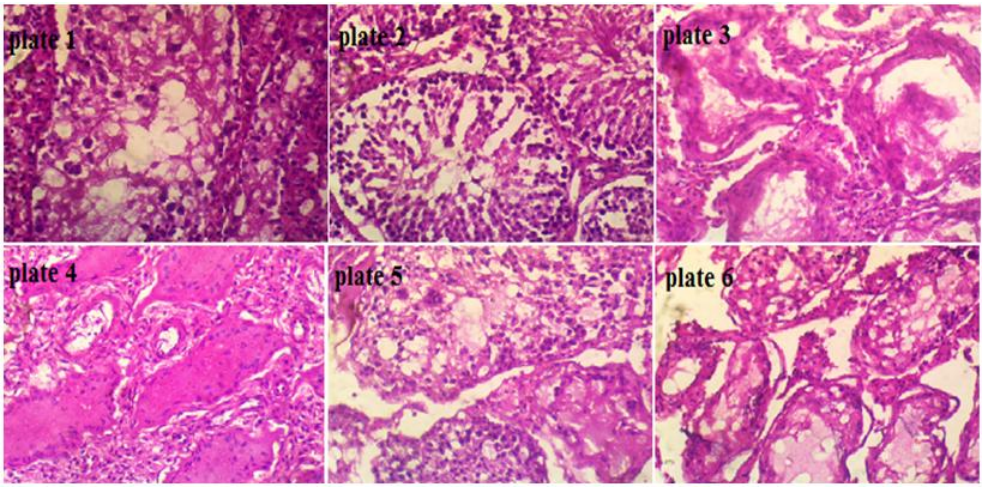

Fig. 4. (Plates 1-6) Histopathology results of the Testes

Plate 1 (Grp1): There are seminiferous tubules with severely depleted spermatocytes and necrotic spermatogonia

Plate 2 (Grp2): There are numerous seminiferous tubules with regular outlines and moderate amounts of spermatocytes in the germinal epithelium. No visible lesion

Plate 3 (Grp3): The seminiferous tubules are severely depleted of spermatogenic cells and show irregular outlines

Plate 4 (Grp4): The testis section is underdeveloped with irregular, non-patent seminiferous tubules and increased amounts of inter-tubular connective tissue (star)

Plate 5 (Grp5): There are variably-sized seminiferous tubules with irregular outlines containing necrotic spermatogenic cells.

Plate 6 (Grp6): The seminiferous tubules are irregular, small and severely depleted of spermatogenic cells

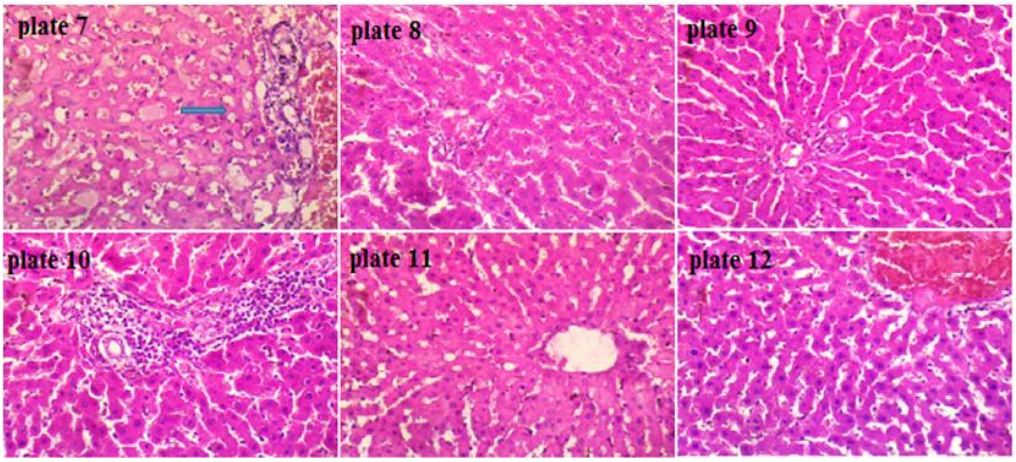

Fig. 5. (Plate 7-12) Histopathology Results of the Liver

Plate 7 (Grp1): There are multiple foci of moderate thinning of hepatic cords with resultant dilation of hepatic sinusoids. There are mild aggregates of mononuclear cells in the portal tracts.

Plate 8 (Grp2): Hepatic plates are closely-packed. There are scant foci of random single-cell hepatocellular necrosis.

Plate 9 (Grp3): Hepatic plates are closely-packed. No visible lesion

Plate 10 (Grp4): Hepatic plates are closely-packed. There are a few foci of single-cell hepatocellular necrosis. There are dense aggregates of inflammatory mononuclear cells in the portal tract.

Plate 11 (Grp5): Hepatic plates are closely-packed. Hepatocytes appear fairly normal.

Plate 12 (Grp6): There are multiple foci of mild thinning of hepatic cords with resultant dilated sinusoids. The hepatic veins are markedly congested

4. CONCLUSION

The series of test carried out in this research has shown that carbamazepine treatment induced reproductive changes in male albino rats. And also this study has evaluated that, treatment with zinc nanoparticle has more protective effect than zinc supplement against carbamazepine induced reproductive changes of male albino rats. It is advised that this work study should be carried out with aid of human subjects so as to substantiate this fact and help show how much would be taken and other precaution to be taking.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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