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(RESEARCH ARTICLE)

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The effect of methanol extract of *Piper guineense* on paracetamol induced toxicity

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Abstract

Methanol extract of *Piper guineense* seed was administered to Swiss albino rats to asses the effect on paracetamol induced toxicity. Thirty-six albino rats were divided into six groups of six animals per group. Four groups were administered in increasing doses of the extract 50, 100, 150, 200 mg/body weight where as the fifth group was administered 500kg/kg of paracetamol as the positive control, while the sixth group was used as normal control. The duration of the experiment was two weeks. This study revealed that the paracetamol increased the biochemical parameters. This was attenuated by the co-administration of *Piper guineense* extract by oral gavage (50, 100, 150, 200mg/kg b.w). The biochemical parameter studied showed consistent changes. The chronic administration of paracetamol 500 kg/b,w showed an elevation in ALT, AST, MDA, total protein, total bilirublin, total protein, globulin And a depletion of SOD(superoxide dismutase), catalase, ALP, Albumin and Albumin/ globulin GSH (reduced glutathione),levels. Administration of methanol extract of *Piper guineense* attenuated the levels ALT, AST, GSH(reduced glutathione), SOD(superoxide dismutase),total protein, total bilirublin, total protein, globulin levels. *Piper guineense* ameliorated MDA, catalase, urea, ALP, Albumin and Albumin/ globulin levels restoring it significantly. The histopathology results of the effect methanol extract of *Piper guineense* on paracetamol induced toxicity. It Concurs with the result of the biochemical parameters. From this study it can be concluded that methanol extract of *Piper guineense* possess some potent antioxidants which can ameliorate damage associated with chronic exposure to paracetamol exposure in rat models.

Keywords: Paracetamol Toxicity; *Piper guineense*; Methanol Extract; Albino rats

1. Introduction

Piper guineense is an African spice plant of the Piperaceae family and the genus piper. It is a perennial climbing plant whose main commercial output is its fruit, while traditional medicine also uses its roots, seeds, stem bark, and leaves. [1] According to [2] It is a West African spice that goes by the names Ashanti pepper, Iyere in Yoruba, and Uziza in Igbo. Other frequent names are false cubeb, Guinea pepper, and Benin pepper [2]. It serves as a preservative and as a spice. It is utilized in both the creation of herbal medicines and insecticides. [3]According [4], the seeds—the component of the plant that has traditionally been used—are abundant in a variety of natural compounds, including volatile oils, lignans, amides, alkaloids, flavonoids, and polyphenols.

Acetaminophen poisoning, also known as paracetamol poisoning, has been linked to overusing paracetamol (acetaminophen) [5].One of the most common causes of poisoning worldwide has been linked to paracetamol toxicity. When given in therapeutic doses, paracetamol is said to be mostly harmless, but it is known to be toxic when taken in a single or repeated high dose. Hepatic toxicity, which is the main cause of acute liver failure (ALF), can be caused by frequent overuse, unintentional abuse, and purposeful intake[6]. More than 80% of the health needs of the rural population in Nigeria and other developing nations are met by traditional medicine [7]. One of the most common causes

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of poisoning in the globe is paracetamol toxicity [6]. According to [4], *Piper guineense* (Ashanti pepper) is high in antioxidants and may have an impact on paracetamol-induced toxicity. Therefore, the purpose of this study is to assess the impact of *Piper guineense* methanol extract on toxicity caused by paracetamol.

2. Materials and Methods

2.1. Identification and collection of samples

2.1.1. Plant Identification

The seeds of *piper guineense* (African black pepper) was purchased from Ogbete main market Enugu and authenticated by the Biological Sciences department of Godfrey Okoye University Enugu. The voucher number of the plant is 39050

2.1.2. Plant Extraction Process

The seeds of *Piper guineense* were bought in large quantities washed using water and air dried. The air dried seeds was pulverized using a blender.

Six hundred grams (600g) of the crushed seeds were macerated in a mixture of 1,800 ml of methanol for 72hours. The extract was filtered using whatman no 4 filter paper. The filtrate was concentrated using a water bath at 65°C . The extract was stored at -4°C in the refrigerator until required. The percentage yield of the extract was determined.

2.2. Experimental Animals

36 male Swiss Albino Rats were purchased from the animal house of the Faculty of Biological Sciences, Michael Okpara University of Agriculture Abia State Nigeria and transported to Federal University of Technology (FUTO); where the research work was carried out. The Rats were acclimatized for 2 weeks, after which they were grouped into four according to their body weights, for drug administration.

2.3. Administration of Extracts

Thirty six Wister Albino Rats were divided into six groups of six animals per group according to their body weights. The extract was dissolved in normal saline according to their specified weight doses and the experimental groups were administered increasing doses of 50, 100, 150 and 200mg/kg body weight respectively of the extract, daily for 14days. The animals in the control group did not receive any extract but were administered only normal saline, while the animals in the positive control group was administered 500mg/kg of paracetamol. The extract administration was performed via oral gavage once daily for two weeks and observed for one week before the termination of the experiment.

At the end of the experiment, all animals were sacrificed according to their groups. The blood samples were taken with sterile syringes and needles. It was then emptied into different labeled test bottles by means of sterile syringes and needles. The sample was used for the assay of biochemical parameters.

2.4. Biochemical parameters

Assays were carried out to estimate the liver functions using practical biochemical markers. The sera samples collected from the whole blood were analyzed for alkaline phosphatase activity (ALP), transferase serum aspartate amino transferase activity (AST), serum alanine amino transferase activity (ALT), albumin concentration, bilirubin concentration were quantified spectrophotometrically using Randox assay kit, Protein Estimation in serum was carried out using Biuret Methodas described by [8] Bilirubin was carried by colometric method as described by [9] The level of SOD activity was determined by the method of [10] the level of reduced GSH in the supernatant fraction were estimated using [11].

2.5. Histological Studies of the Animals Organs

Histological study was carried out on the organs of each rat in each group. The animal's liver tissues were studied. This was carried out to cross check the results that were obtained from the biochemical assays.

2.6. Statistical Analysis

The statistical analysis of the data obtained from this research work were analyzed using one way Analysis of variance (ANOVA) with the Tukey Post-Hoc test with the aid of GraphPad Prism Version 5.3 and Version 9 (GraphPad, USA). Values for $p<0.05$ were considered statistically significant.

3. Results

The methanol extract of the *Piper guineense* did not show any sign or symptom of toxicity and no mortality was recorded during the study. Administration of the 500kg/b.w elevated the levels of ALT, AST, Bilirubin, Globulin and serum protein (figure 3 to 9) and significant decrease in the Albumin and Albumin/ globulin ration as shown in figure (10 and 11). This was attenuated by the administration of *Piper guineense.* There was a rise in MDA Concentration, when compared to the normal control group (group A) figure 2, this is associated with a decline in SOD, catalase and reduced glutathione. There was a decrease in a dose dependent manner with administration of *Piper guinneese* at different doses. (figure 9 to 12).

3.1. Biochemical Parameter Assay

The biochemical parameters showed some consistent changes. There a significant increase ($p<0.05$) increase in serum transaminases activities (AST, ALT) MDA, Potassium, Bilirubin, creatinine, Total protein, urea and globulin levels when compared to the control. Co-administration of methanol extract of *Piper guineense* at an increasing dose level significantly ($p<0.05$) decreased the elevated levels of serum marker enzymes, while creatinine, globulin, total protein was decreased at a dose of 100 and 200mg/kg b.w of *Piper guineense* respectively.

The GSH, SOD, ALP, albumin and albumin\ globulin levels catalase were reduced when compared to the control. Co-administration of methanol extract of *Piper guineense* at an increasing dose level significantly increased the reduced levels of the biochemical parameters when compared to the control groups. While albumin\globulin, catalase, and ALP increased at 100 and 200mg\kg b.w. of *Piper guineense.*

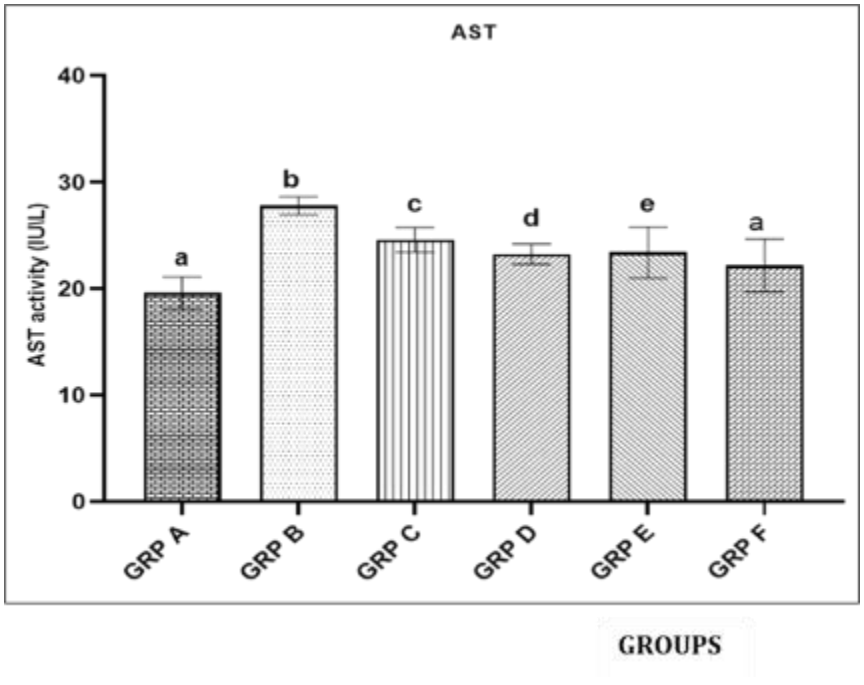

Figure 1 Effect of *Piper guineense* (Ashanti pepper) on AST activity of albino rats exposed to paracetamol-induced toxicity

Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant ($p<0.05$). GRP A, Normal control group; GRP B, Positive Control (500 mg/kg paracetamol) group; GRP C, 500 mg/kg paracetamol + 50 ml/kg Extract group; GRP D, 500 mg/kg paracetamol + 100 ml/kg Extract group; GRP E, 500 mg/kg paracetamol + 150 ml/kg Extract group; GRP F, 500 mg/kg paracetamol + 200 ml/kg Extract group

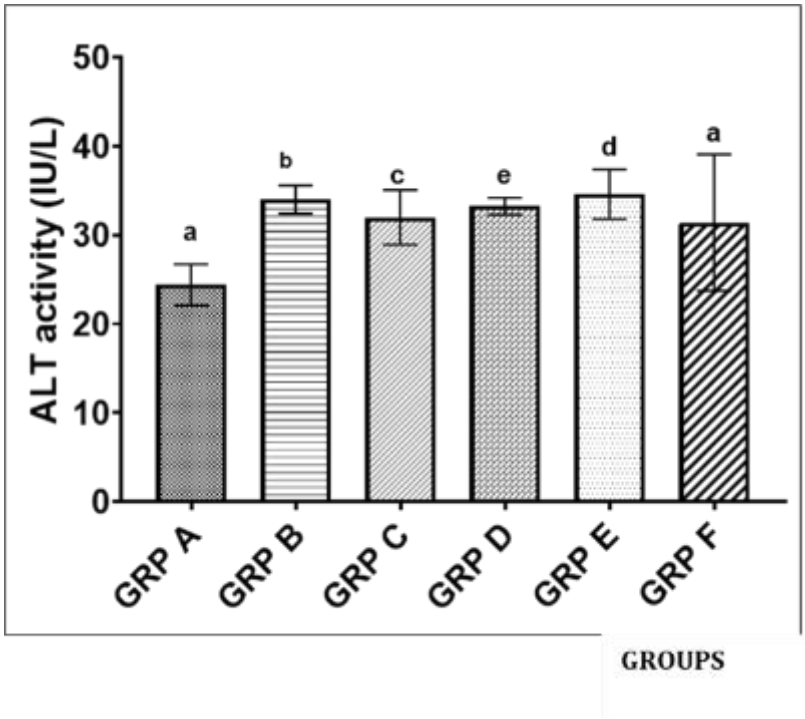

Figure 2 Effect of *Piper guineense* (Ashanti pepper) on ALT activity of albino rats exposed to paracetamol-induced toxicity

Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant ($p<0.05$). GRP A, Normal control group; GRP B, Positive Control (500 mg/kg paracetamol) group; GRP C, 500 mg/kg paracetamol + 50 ml/kg Extract group; GRP D, 500 mg/kg paracetamol + 100 ml/kg Extract group; GRP E, 500 mg/kg paracetamol + 150 ml/kg Extract group; GRP F, 500 mg/kg paracetamol + 200 ml/kg Extract group.

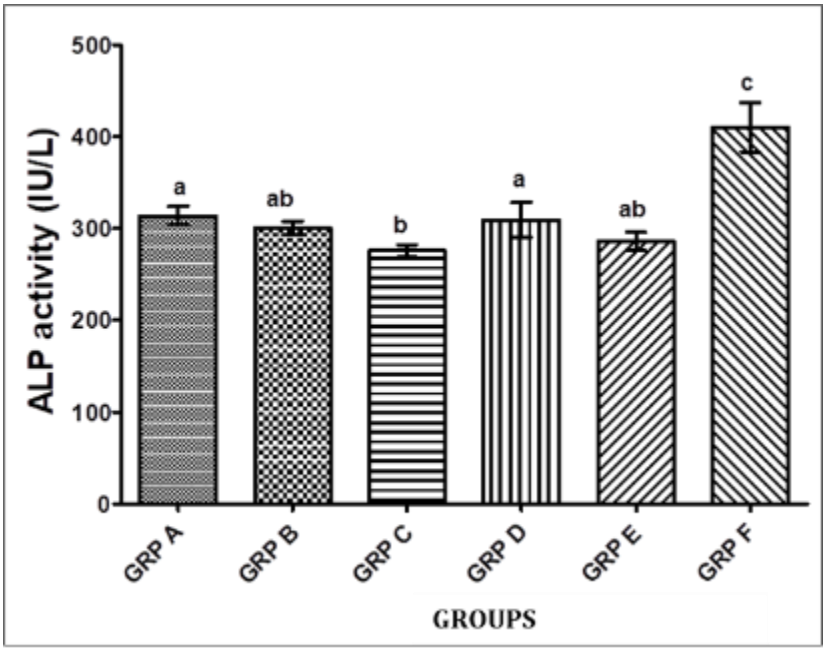

Figure 3 Effect of *Piper guineense* (Ashanti pepper) on ALP activity of albino rats exposed to paracetamol-induced toxicity

Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant ($p<0.05$). GRP A, Normal control group; GRP B, Positive Control (500 mg/kg paracetamol) group; GRP C, 500 mg/kg paracetamol + 50

ml/kg Extract group; GRP D, 500 mg/kg paracetamol + 100 ml/kg Extract group; GRP E, 500 mg/kg paracetamol + 150 ml/kg Extract group; GRP F, 500 mg/kg paracetamol + 200 ml/kg Extract group.

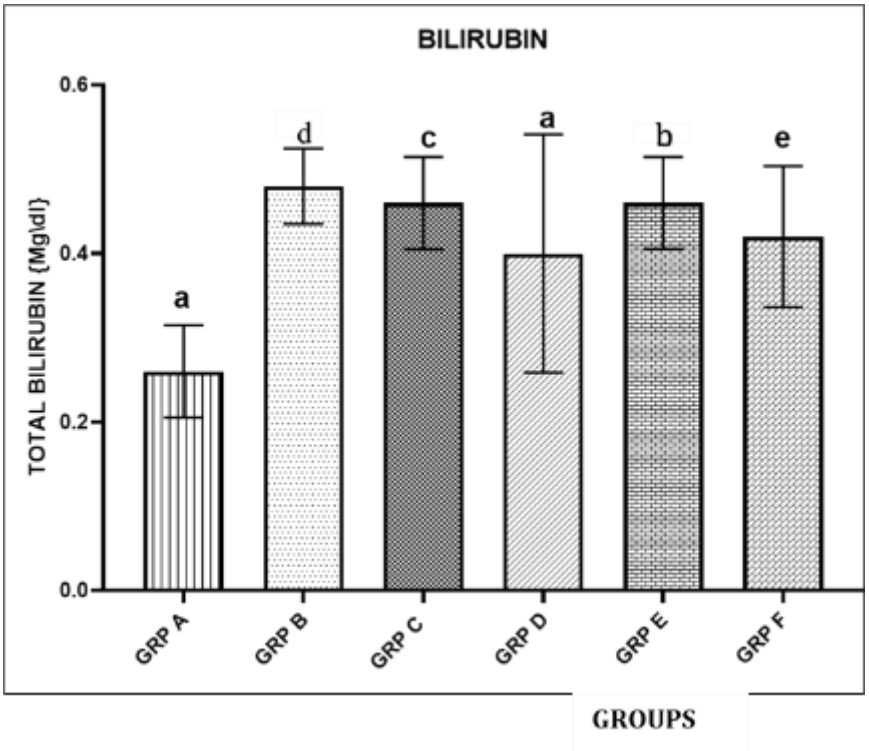

Figure 4 Effect of *Piper guineense* (Ashanti pepper) on Total Bilirubin of albino rats exposed to paracetamol-induced toxicity

Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant ($p<0.05$). GRP A, Normal control group; GRP B, Positive Control (500 mg/kg paracetamol) group; GRP C, 500 mg/kg paracetamol + 50 ml/kg Extract group; GRP D, 500 mg/kg paracetamol + 100 ml/kg Extract group; GRP E, 500 mg/kg paracetamol + 150 ml/kg Extract group; GRP F, 500 mg/kg paracetamol + 200 ml/kg Extract group.

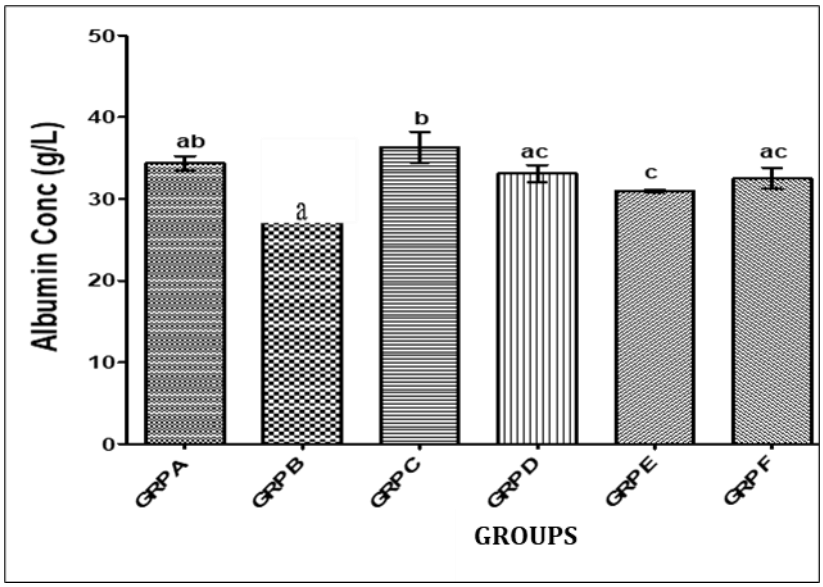

Figure 5 Effect of *Piper guineense* (Ashanti pepper) on Albumin Concentration of albino rats exposed to paracetamol-induced toxicity

Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant ($p<0.05$). GRP A, Normal control group; GRP B, Positive Control (500 mg/kg paracetamol) group; GRP C, 500 mg/kg paracetamol + 50 ml/kg Extract group; GRP D, 500 mg/kg paracetamol + 100 ml/kg Extract group; GRP E, 500 mg/kg paracetamol + 150 ml/kg Extract group; GRP F, 500 mg/kg paracetamol + 200 ml/kg Extract group.

3.2. Serum Total Protein

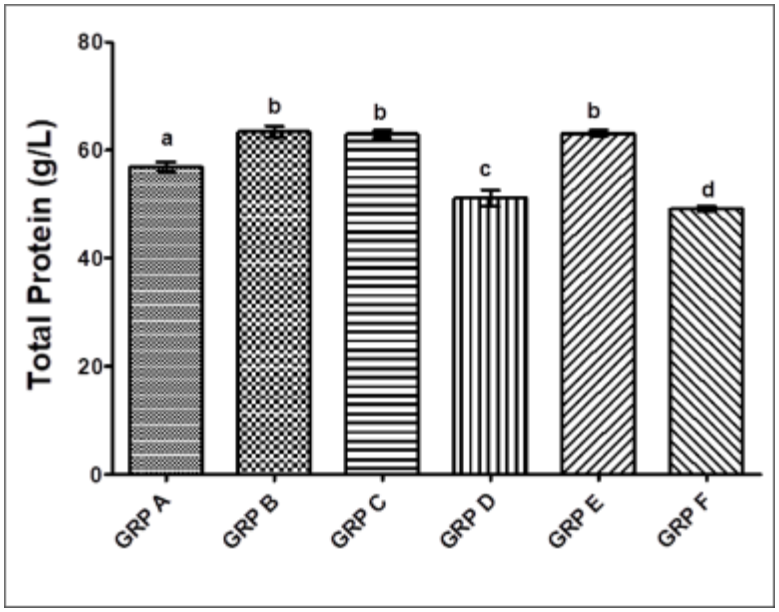

Figure 6 : Effect of *Piper guineense* (Ashanti pepper) on Total Protein of albino rats exposed to paracetamol-induced toxicity

Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant ($p<0.05$). GRP A, Normal control group; GRP B, Positive Control (500 mg/kg paracetamol) group; GRP C, 500 mg/kg paracetamol + 50 ml/kg Extract group; GRP D, 500 mg/kg paracetamol + 100 ml/kg Extract group; GRP E, 500 mg/kg paracetamol + 150 ml/kg Extract group; GRP F, 500 mg/kg paracetamol + 200 ml/kg Extract group.

3.3. Serum Globulin

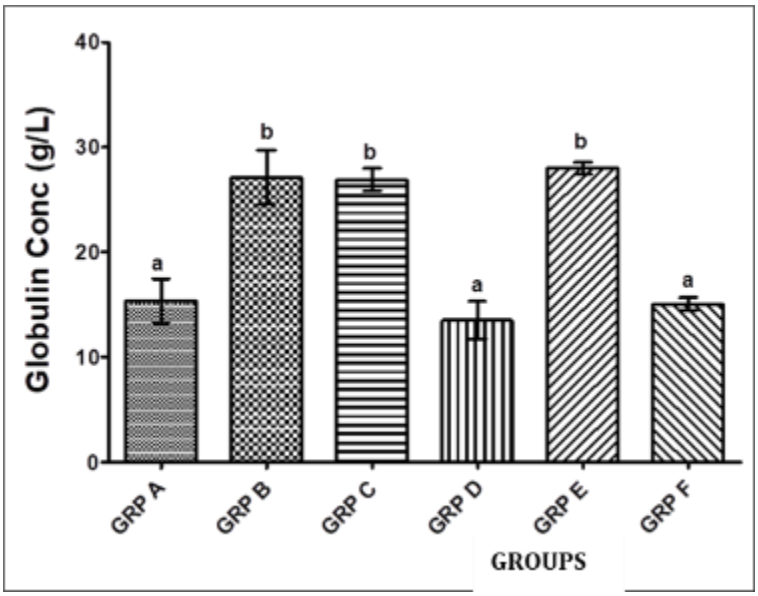

Figure 7 Effect of *Piper guineense* (Ashanti pepper) on Globulin Concentration of albino rats exposed to paracetamol-induced toxicity

Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant (p<0.05). GRP A, Normal control group; GRP B, Positive Control (500 mg/kg paracetamol) group; GRP C, 500 mg/kg paracetamol + 50 ml/kg Extract group; GRP D, 500 mg/kg paracetamol + 100 ml/kg Extract group; GRP E, 500 mg/kg paracetamol + 150 ml/kg Extract group; GRP F, 500 mg/kg paracetamol + 200 ml/kg Extract group.

3.4. Serum Albumin/ Globulin

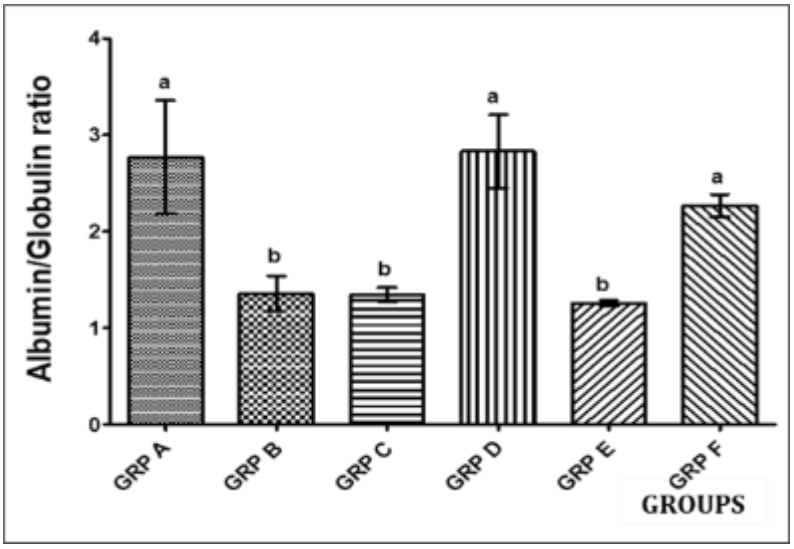

Figure 8 Effect of *Piper guineense* (Ashanti pepper) on Albumin/ Globulin ratio of albino rats exposed to paracetamol-induced toxicity

Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant (p<0.05). GRP A, Normal control group; GRP B, Positive Control (500 mg/kg paracetamol) group; GRP C, 500 mg/kg paracetamol + 50 ml/kg Extract group; GRP D, 500 mg/kg paracetamol + 100 ml/kg Extract group; GRP E, 500 mg/kg paracetamol + 150 ml/kg Extract group; GRP F, 500 mg/kg paracetamol + 200 ml/kg Extract group.

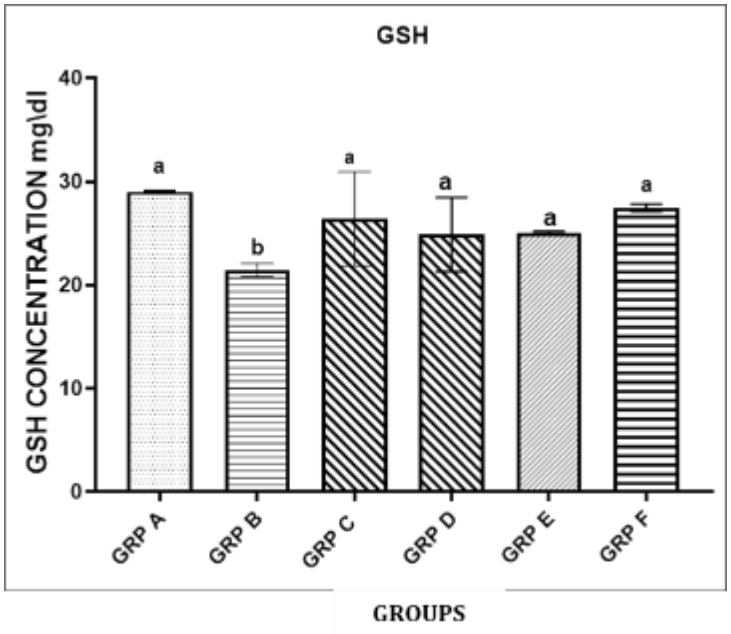

Figure 9 Effect of *Piper guineense* (Ashanti pepper) on GSH Concentration of albino rats exposed to paracetamol-induced toxicity

Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant (p<0.05). GRP A, Normal control group; GRP B, Positive Control (500 mg/kg paracetamol) group; GRP C, 500 mg/kg paracetamol + 50 ml/kg Extract group; GRP D, 500 mg/kg paracetamol + 100 ml/kg Extract group; GRP E, 500 mg/kg paracetamol + 150 ml/kg Extract group; GRP F, 500 mg/kg paracetamol + 200 ml/kg Extract group.

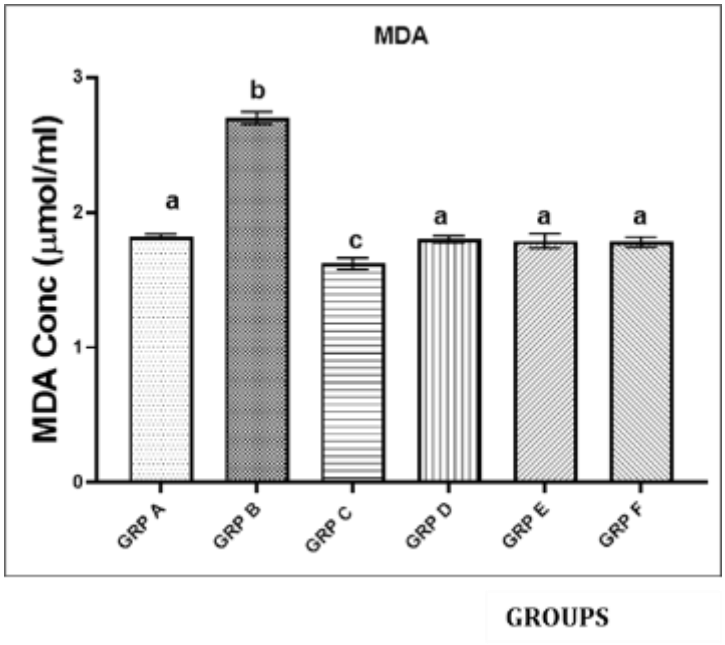

Figure 10 Effect of *Piper guinneense* (Ashanti pepper) on MDA Concentration of albino rats exposed to paracetamol-induced toxicity

Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant (p<0.05). GRP A, Normal control group; GRP B, Positive Control (500 mg/kg paracetamol) group; GRP C, 500 mg/kg paracetamol + 50 ml/kg Extract group; GRP D, 500 mg/kg paracetamol + 100 ml/kg Extract group; GRP E, 500 mg/kg paracetamol + 150 ml/kg Extract group; GRP F, 500 mg/kg paracetamol + 200 ml/kg Extract group.

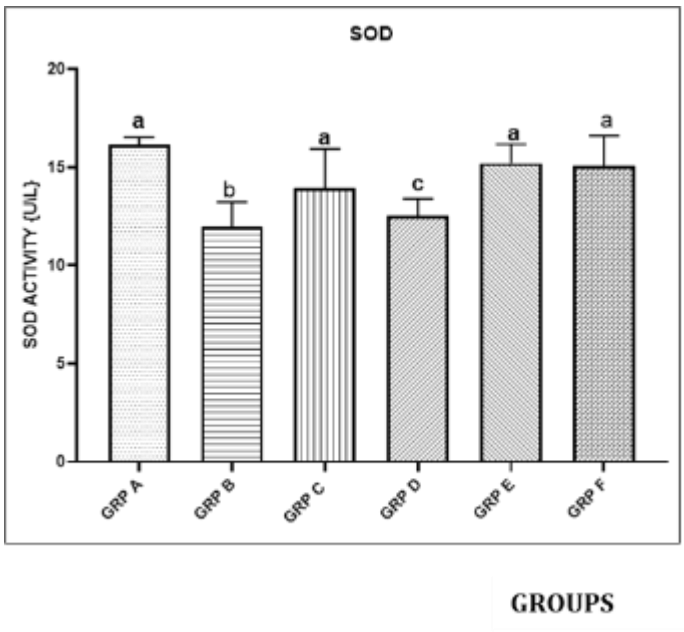

Figure 11 Effect of *Piper guineense* (Ashanti pepper) on SOD Activity of albino rats exposed to paracetamol-induced toxicity

Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant (p<0.05). GRP A, Normal control group; GRP B, Positive Control (500 mg/kg paracetamol) group; GRP C, 500 mg/kg paracetamol + 50 ml/kg Extract group; GRP D, 500 mg/kg paracetamol + 100 ml/kg Extract group; GRP E, 500 mg/kg paracetamol + 150 ml/kg Extract group; GRP F, 500 mg/kg paracetamol + 200 ml/kg Extract group.

3.5. Catalase Concentration

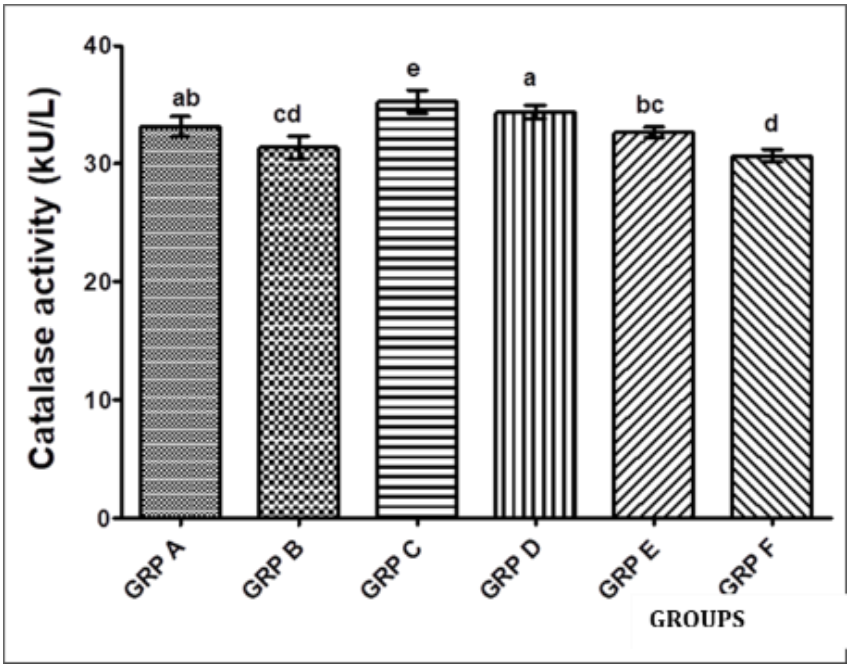

Figure 12 Effect of *Piper guineense* (Ashanti pepper) on Catalase activity of albino rats exposed to paracetamol-induced toxicity

Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant (p<0.05). GRP A, Normal control group; GRP B, Positive Control (500 mg/kg paracetamol) group; GRP C, 500 mg/kg paracetamol + 50 ml/kg Extract group; GRP D, 500 mg/kg paracetamol + 100 ml/kg Extract group; GRP E, 500 mg/kg paracetamol + 150 ml/kg Extract group; GRP F, 500 mg/kg paracetamol + 200 ml/kg Extract group.

3.6. Histopathological Studies Organs

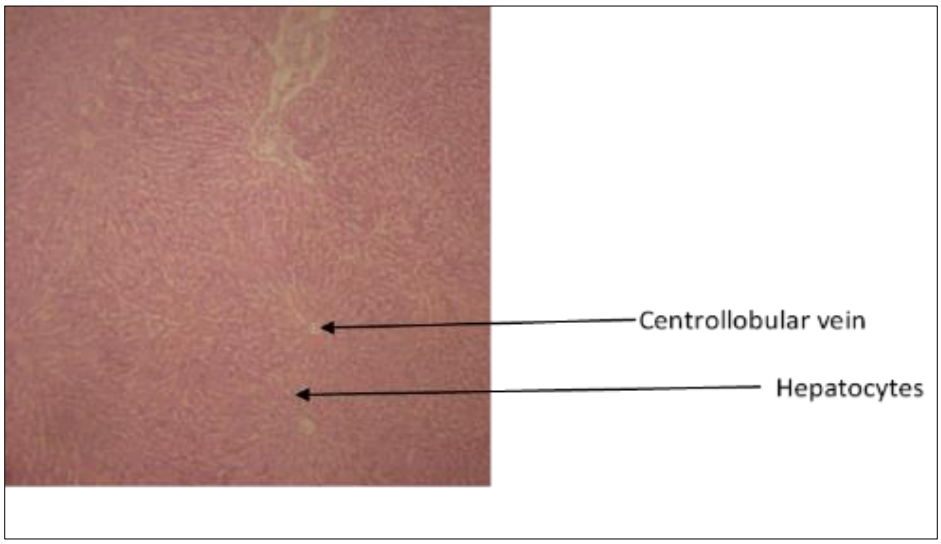

Figure 13 Histopathology of liver of rats in the Control Group Showing No Distortion

Macro and Microscopic examination of the liver showed extensive congested branches of portal vein congested central vein associated with cellular infiltration. hepatocytes with microvesicles and hepatocytes with pyknotic nuclei. The normal control group showed normal hepatic structure with no lessions or abnormalities. The positive control group showed extensive congested branches of portal vein, congested central vein associated with cellular infiltration. hepatocytes with microvesicles and hepatocytes with pyknotic nuclei(figures 1 to 2)

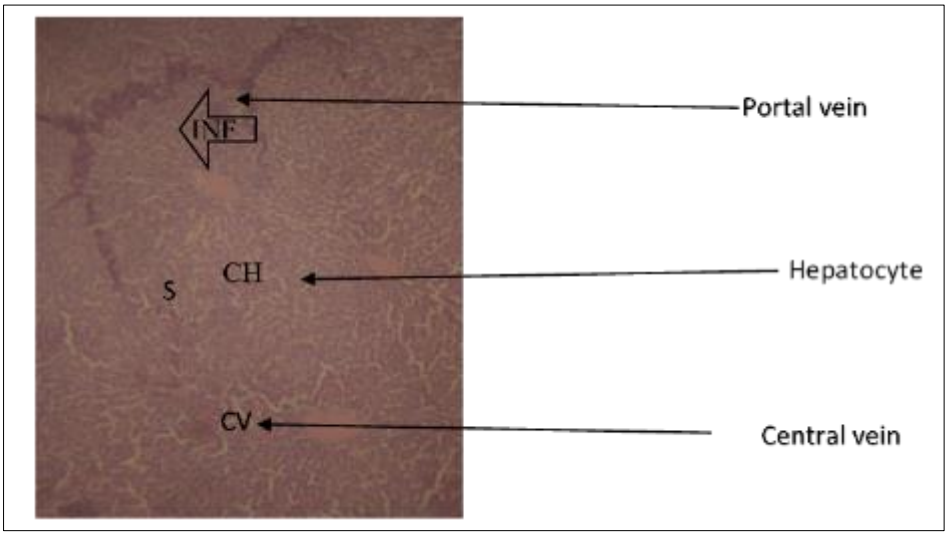

Figure 14 Histopathology of liver of Rats Administered with 500mg\kg paracetamol (Positive Control Group) X400 Stain H and E

INF-Inflammation H- Hepatocyte S = Sinusiods CV = Central vein CH = Enlarged central vein

In group C it showed a degeneration periportal necrosis, group D and group E showed mid necrosis while mild periportal hepatic necrosis was seen in group F (Figures 3 to 6).

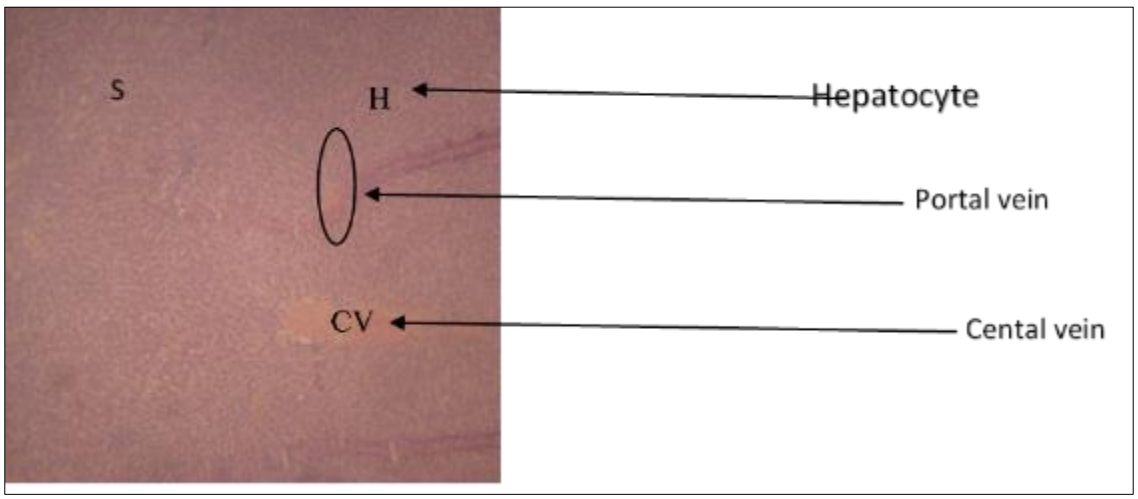

Figure 15 Histopathology of liver of Rats Administered with 500mg\kg paracetamol (Positive Control Group) (x400), Stain: H and E.

The photomicrograph of this section of the liver shows a central vein (centrolobular vein) in the center with arrays of binucleated hepatocytes which are uniformly distributed throughout the cytoplasmic matrix. The hepatocytes in the liver lobule are radially disposed and are arranged like the bricks of a wall. These cellular plates are directed from the periphery of the lobule to its center and anastomose freely, forming a labyrinthine and sponge like structure. The nuclei appeared rounded and are eccentrically positioned. The sinusoids are intact and no pathological lesion seen. Morphological features are in line with that of a normal liver.

The photomicrograph of this section shows a dilated central vein (centrolobular vein), necrotic or constricted hepatocytes adjacent the central vein. There is distortion of the sinusoid and enlargement of distant hepatocytes.

The photomicrograph of this section shows a dilated central vein(centrolobular vein), necrotic or constricted hepatocytes adjacent the central vein. There is distortion of the sinusoid and enlargement of distant hepatocytes.

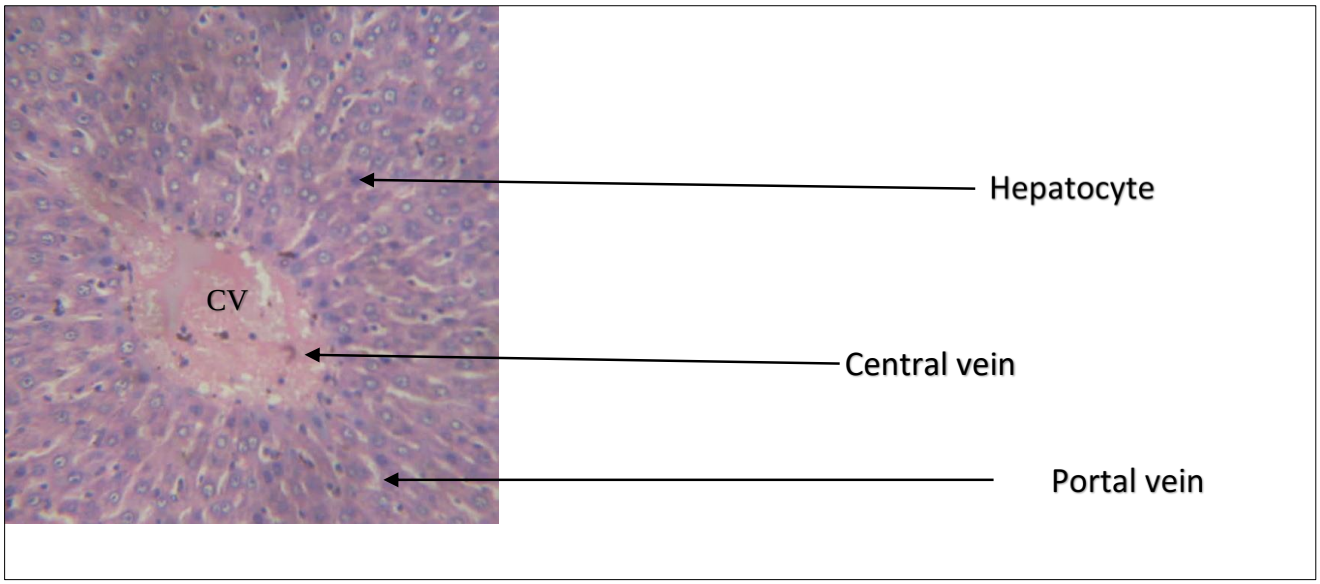

Figure 16 Histopathology of liver of Rats Administered with 500mg\kg paracetamol + 100mg\kg b.w. of methanol extract of *Piper guineense* (x400), Stain: H and E.

The photomicrograph of this section shows a central vein (centrolobular vein) without enlargement. There are few population of necrotic hepatocytes.

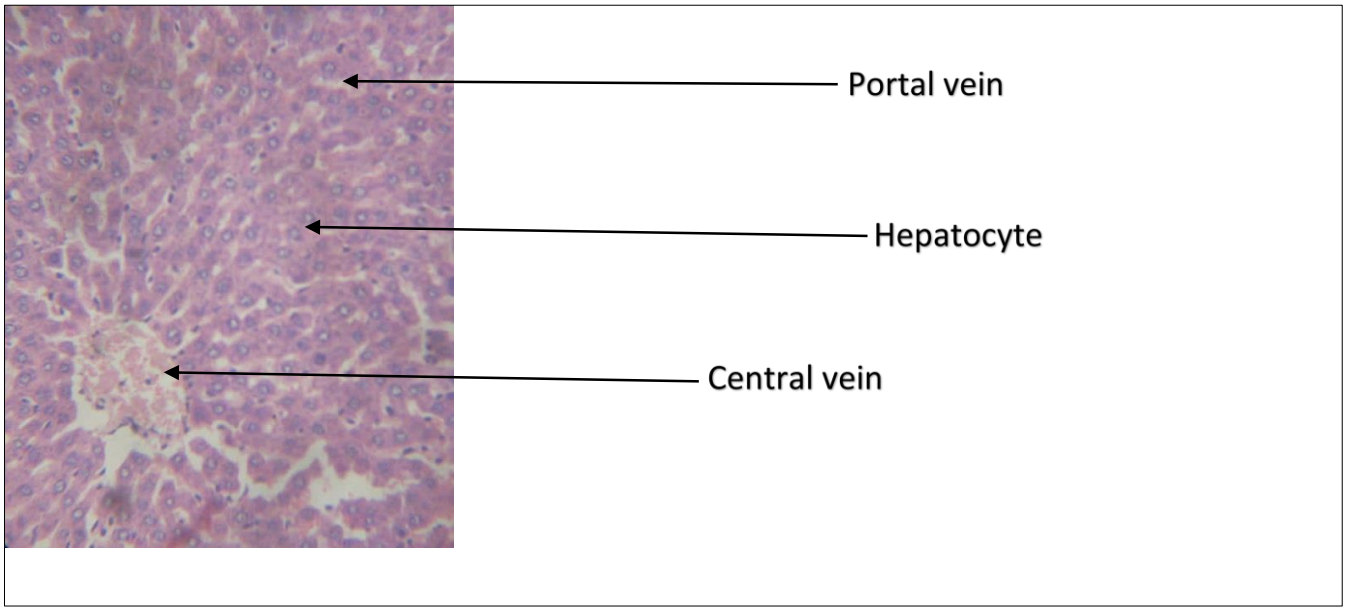

Figure 17 Histopathology of liver of Rats Administered with 500mg\kg paracetamol + 150mg\kg b.w. of methanol extract of *Piper guineense* (x400), Stain: H and E.

The photomicrograph of this section shows a central vein (centrolobular vein) without enlargement. There are few population of necrotic hepatocytes.

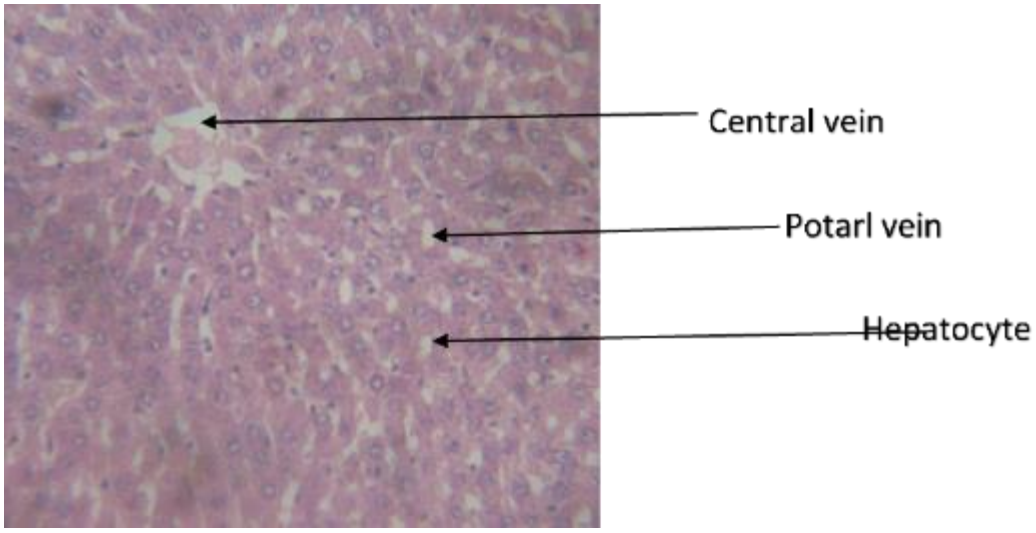

Figure 18 Histopathology of liver of Rats Administered with 500mg\kg paracetamol + 200mg\kg b.w. of methanol extract of *P.guineense* (x400), Stain: H and E.

The photomicrograph of this section shows a central vein (centrolobular vein) without enlargement. There are few population of necrotic hepatocytes.

4. Discussion

Non-steroidal anti-inflammatory drugs, such as paracetamol, are frequently used to treat pain, headaches, fever, and other symptoms; however, because these medications are considered over-the-counter and may be purchased without a prescription, there is a substantial risk of toxicity and overdose [12].The aminotransferases (ALT, AST), ALP, and bilirubin are blood indicators of liver function; an increase in these markers denotes hepatic injury. However, when there is hepatic injury, total protein and albumin levels are lowered [13]. It has been demonstrated that tissue leaking causes an increase in these enzymes. Which caused the blood to carry this enzyme. [14]. If acute renal injury, coagulopathy, hyperbilirubinemia, and acidosis develop, alanine aminotransferase (ALT) increase will likely continue. [15]. The treatment of the extract decreased the levels of AST, ALT, and bilirubin while increasing the levels of total protein and albumin. This demonstrates that the seed extracts might have chemical components with potential hepato-protective properties. [16]

Globulin levels were elevated in the positive control group, this was attenuated by the extracts showing that the extracts encourage protein biosynthesis hence enhancing liver function. [16]

The depletion of glutathione, an essential intracellular and extracellular tripeptide antioxidant, was the predominant adverse impact of paracetamol poisoning. [12]. This study's results for the paracetamol-induced toxicity similarly followed a similar pattern. When 500b.w/kg of paracetamol was administered, the glutathione level decreased, and this is because the tissue-damaging effects of oxidative stress caused by paracetamol-induced toxicity are exerted. When methanol extract of *Piper guineense* was administered, there was a dose-dependent rise in glutathione levels. demonstrating the possibility of lipid peroxidation protection provided by *Piper guineense*. MAPK activation, which is a critical component of the intracellular signalling cascade of paracetamol-induced hepatotoxicity, can also be brought on by oxidative stress. Cell death and the MAPK family are connected and is responsible for the production of ROS and proinflammatory cytokines. Reduced glutathione (GSH) and scavenging enzymes like CAT and SOD are recruited by the cell as first-line cellular defense in response to oxidative challenges to safeguard cellular integrity from oxidative damage. [17]. Increased MDA levels indicate tissue injury.[18], The concurrent drop of one or more antioxidants (such as catalase, superoxide dismutase, and reduced glutathione) and an increase in MDA level are indicators of toxicity [19]. Administration of the varying doses of the plant extract resulted in about increase in the activities of GSH and SOD; the increase observed could be as a result of bioactive constituents of the extracts mainly alkaloids and flavonoids. [20].

The histopathological findings from the analysis of the effect of on methanol extract of *piper guineense* on paracetamol-induced toxicity are consistent with the biochemical measurements. NAPQI, a very hazardous metabolite of paracetamol, is produced in greater quantities when paracetamol is taken [21]. These findings imply that renal damage is more likely to develop in paracetamol chronic users who take large dosages (500 mg/kg). When high doses of this

extract were given along with paracetamol to the test animals, lesions were seen in the liver with *Piper guineense* attenuating it.

5. Conclusion

In conclusion, the results of this study have demonstrated that overdose of paracetamol at a dose of 500 mg / kg b.w. could be dangerous to the liver. From this findings, the methanol extract of *Piper guineese* was able prevent liver damage in the paracetamol-intoxicated rats; thereby, enhancing the synthesis of antioxidant, reduce lipid peroxidation, prevent leakage of liver enzymes into system, hence its used as antioxidants, hepato-protective agents may have scientific bases. The present study also highlights that the extract possesses a high antioxidant activity which can enhance the body defense mechanism in conditions of oxidative stress and as a potent therapeutic agent in the management of liver disorders.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study

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