



Antioxidant properties of *Psidium guajava* leaf extract on paraquat induced hepatotoxicity and oxidative stress

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Abstract

Thirty healthy male rats were used for the *in vivo* experiment. Animals received 3.5 mg/kg of Paraquat (PQ) intraperitoneally on the 15th day of experiment. Extracts were administered daily for thirty (30) days using normal saline. Liver parameters were analyzed to assess hepatoprotection. Malondialdehyde concentration was estimated to assess peroxidation. Glutathione (GSH) concentration was estimated to assess non-enzymatic antioxidant status. Activities of antioxidant enzymes were further used to assess antioxidant capacity of *Psidium guajava* (PG) against PQ-induced oxidative damage. Hematological indices were evaluated to assess the effect of PG on haemopoiesis. Histological study of the liver was also carried out to find out the level of effectiveness of PG. The antimicrobial activity of the extract was also determined. Results showed that there was a significant increase in ALT and AST activities, albumin/globulin ratio, malondialdehyde (MDA), following PQ exposure at ($p < 0.05$). There was also a significant decrease of total protein and globulin concentrations, Packed Cell Volume (PCV), Red Blood Cell (RBC) count, Superoxide Dismutase (SOD), and GSH after PQ exposure at ($p < 0.05$). The histological result showed that PG leaf extract significantly improved changes in oxidative stress parameters, blood parameters, and hepatotoxicity induced by PQ administration at $p < 0.05$. Thus, PG leaf ethanol extract may be recommended in hepatotoxicity.

Keywords: Antioxidant, Hepatotoxicity, Oxidative stress, Paraquat, *Psidium guajava*

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1. Introduction

1.1. *Psidium guajava*

Psidium guajava (PG) commonly known as Guava is widely cultivated in tropical and subtropical regions around the world. Native tribesmen have used *Psidium guajava* for a lot of medicinal purposes thousands of years before modern medicine documented the specific chemical compounds in the tropical fruit. Traditionally, the leaves were brewed to treat intestinal tract problems or ground into a poultice and applied to the skin to treat open wounds and rashes. Both the unripe and ripe fruits were consumed to suit stomach upset and when the flesh is ready to eat it acts as a mild laxative and the unripe fruit works as an anti-diarrheal (Joseph *et al.*, 2011). Some recent studies have shown that certain extracts from the leaves and bark can act as an anti-inflammatory, prevent bacterial growth, and in some cases inhibit the spread of cancer (Manosroi *et al.*, 2006).

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Guava is an excellent antioxidant and a good source of vitamin C. Guava leaf extracts and essential oil from the stem, bark have the ability to scavenge free hydrogen peroxide, superoxide anion and inhibit the formation of hydroxyl radical (Fashola *et al.*, 2011; Vyas *et al.*, 2010; Ogunlana and Ogunlana, 2008 and Chen *et al.*, 2010). *P. guajava* has been used for centuries in the folklore medicine without any adverse effects.

1.2. Paraquat

Paraquat (PQ) belongs to a group of redox cycling compounds capable of inducing mitochondrial damage, increasing reactive oxygen species (ROS) production and causes oxidative stress (Casteilo *et al.*, 2007). It is a toxic chemical widely used as a herbicide (plant killer) primarily for weed and grass control. Despite the fact that it has been banned in so many countries, some elements of it are still seen in and around us especially in the Agro chemical industries. Paraquat is highly toxic to animals by all routes of exposure such as dermal, oral and inhalation. Its mode of action in weeds is that it interferes with electron transfer, a process that is common in all life. It is an electron acceptor in redox and radical reactions. As an herbicide, paraquat acts by inhibiting photosynthesis. In light exposed plants, it accepts electrons from photosystem 1 and transfers them to molecular oxygen, and by so doing, destructive reactive oxygen species are produced. In forming these reactive oxygen species, the oxidized form of paraquat is regenerated, and is again available to shunt electrons from photosystem 1 to start the cycle again (Summers, 1980). Its mode of action in animals and humans is that it causes extensive damage to the mitochondria of cells through the production of free radicals and oxidative stress, resulting in the interruption of important biochemical processes, cell death, and multi organ failure (Suntress, 2002; and Cocheme and Murphy, 2009), and the organs mostly affected are the lungs, liver and kidney.

1.3. Oxidative stress

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. In humans, oxidative stress is thought to be involved in the development of degenerative diseases such as Asperger Syndrome (Singh *et al.*, 1995), cancer, Parkinson's disease, heart failure, chronic fatigue syndrome (Gwen *et al.*, 2005) and a host of other diseases. Oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses, such as glutathione (GSH) (Schafer *et al.*, 2001).

This study therefore is to assess the antioxidant properties of the ethanol extract of *Psidium guajava* on paraquat induced hepatotoxicity and oxidative stress in rats.

2. Materials and methods

2.1. Plant materials

Matured leaves of *Psidium guajava* freshly harvested were obtained from Umuagu, Umuguma in Owerri west Local Government Area of Imo State. The leaves were authenticated by Dr. Cyriacus Udah, former Director of Forestry, Imo State Ministry of Environment.

2.2. Safety study of *Psidium guajava* (PG) and dose administration

Safety study of PG and dose administration was according to Samistha and Swarnamoni (2010): Acute oral toxicity test for the ethanolic extract of leaves of PG was carried out as per (OECD guidelines 425). Two arbitrary doses of 250 mg/kg body weight and 500 mg/kg body weight were selected for the study, as the extract was found to be safe even at doses more than 5,000 mg/kg b.wt without any sign of toxicity or mortality.

2.3. Animals

Thirty healthy male albino rats weighing between 72-113 gm were obtained from the Department of Zoology and Environmental Biology, University of Nigeria Nsukka. The rats were acclimatized for two weeks before the commencement of the experiment. The rats were provided with their respective diets along with portable drinking water ad libitum throughout the experimental period. The rats were housed in cages under standard laboratory conditions. All experiments were carried out as approved by appropriate ethics committee (SOBS-BCH-EC) and all the animals received proper human care in accordance with the guidelines for ethical treatment of laboratory animals provided by the National Institute of Health, USA. The Animals were being weighed weekly, and their weights recorded.

2.4. Methods

2.4.1. Preparation of plant extract

The leaves of *P. guajava* were air dried at room temperature for one week to a constant weight. The dried leaves were ground to fine powder using a mill (BL-335Kenwood) and stored in air tight container. 400 g of the powdered leaves were soaked in 2.0 L, 70% ethanol. The whole set up was left to stand for four days with occasional agitation. They were filtered through a qualitative filter paper (number1: Whatman, England). The crude leaf solution was rotor evaporated at 49 °C (Buchi Rotavapour, Japan) and the extract was obtained. The extract was finally stored in the refrigerator throughout the experimental period and was prepared daily for administration.

2.5. Experimental design

The rats were divided into five groups of six animals each after two weeks of acclimatization. The plant extract was given at three different doses for 30 days.

Group 1: normal control group and received normal saline, food and water.

Group 2: intoxicated control and received only paraquat, food and water.

Group 3: 1st treated group and received paraquat with 200 mg/kg body weight of PG extract with food and water.

Group 4: 2nd treated group and received paraquat with 400 mg/kg body weight of PG extract with food and water.

Group 5: 3rd treated group and received paraquat with 800 mg/kg b.wt body weight of PG extract with food and water.

Toxicity was induced with 3.5 mg/kg b.wt of paraquat by Intraperitoneal injection on the 15th day of the experiment and antioxidant activity measured on the 30th day. Blood sample were collected by optical puncture using orbital technique for assay of biochemical parameters. Animals were anaesthetized and the liver was perfused and preserved with formal saline for histological studies.

2.6. Assay of biochemical parameters

Alanine amino transferase (ALT) activity was assayed by the method of Reitman and Frankel (1957), by monitoring the concentration of pyruvate hydrazine formed by the reaction of 2, 4-dinitrophenyl hydrazine and pyruvate. This reaction is measured at 546 nm. Aspartate amino transferase activity (AST) was assayed by the method of Reitman and Frankel (1957). It is measured by monitoring the concentration of oxaloacetate hydrazone formed by the reaction of 2, 4-dinitrophenyl hydrazine and oxaloacetate. This reaction is measured at 546 nm. The total protein concentration was determined by Biuret method as described by Gornall *et al.* (1949). Copper (II) ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a colored complex. The test was carried out using a protein test-kit (Randox, U. K.) that utilizes the Biuret method for protein determination. The albumin concentration was determined based on the method described by Doumas *et al.* (1971). The measurement of serum albumin was based on its quantitative binding to bromocresol green. The albumin-Bcg complex absorbed maximally at 578 nm and the absorbance was directly proportional to the concentration of the albumin in the sample. Globulin concentration was determined from the difference between serum total protein concentration and Serum albumin concentration. Superoxide dismutase assay was carried out according to the method described by Vijayalakshmi and Kumar (2013). The SOD was assessed by autoxidation of hydroxylamine at P^H 10.2, accompanied by reduction of nitro-blue tetrazoleum (NBT). Nitrite production in the presence of EDTA was detected colorimetrically. One enzymatic unit of SOD corresponds to the amount of proteins present in 100 μ l of serum required to inhibit the reduction of 24 mM NBT by 50%. Catalase activity was assayed by the method of Aebi (1974). Lipid peroxidation in the supernatant fractions was determined spectrophotometrically by assessing the concentration of thiobarbituric acid reactive substances (TBARS) as described by Liu *et al.* (1997). The results were expressed in malondialdehyde (MDA) formed relative to an extinction coefficient of 1.56×10^5 mol/cm. GSH (reduced) was measured according to the method of Ellman (1959) as described by Raja *et al.* (2006). Reduced GSH forms the bulk of non-protein sulfhydryl groups. The Red Blood Cell (RBC), (Hb) count was done with the sysmex K-21n automatic multi-parameter blood cell counter for *in vitro* diagnostic use in clinical laboratories. Counting of blood cells is based on the volumetric impedance method, directly measuring RBC, haemoglobin (HGB). Histological study was

carried out with liver samples from different groups. This was carried out to crosscheck the results that were obtained from the biochemical assays. The method described by Okoro (2002) was used with minor modifications.

2.7. Antimicrobial activity of *Psidium guajava*

Antimicrobial activity of leaf extract of *Psidium guajava* was determined by agar well diffusion method. Sterile nutrient agar plates were inoculated with 0.5 Mc farland standard of test organisms. The agar surface was left to dry for 5.0 mins. Holes of 5.00 mm were bored into the inoculated plates using sterile cork borer of 5.00 mm diameter. One gram (1 g) of the extract was dissolved in 1 ml of sterile water to give 1 g/ml concentration. Aliquot of 0.1 ml of the concentration gives 100 mg/ml. This was inoculated in the holes made on the agar media containing each isolate of test organisms. A control hole contain sterile distilled water. They were incubated at 37 °C for 18 h in the incubator. The ability of the various extracts to inhibit growth of the clinically significant bacteria was measured and recorded as diameter of inhibition in millimeter (mm).

2.8. Minimum inhibitory concentration of *Psidium guajava*

The Minimum Inhibitory Concentration (MIC) of the extract was determined by incorporating constant volume 0.1 ml of double fold dilutions as in zone of inhibition, (50 mg/ml, 25 mg/ml, 12.50 mg/ml, 6.25 mg/ml) into the already prepared agar wells. The MIC was determined by recording the least concentration of the extract (mg/ml) or the highest dilution that inhibited the growth of the organism.

2.9. Statistical analysis

Data were analyzed using appropriate software, Scientific Package for Social Sciences (SPSS). Results were presented as mean \pm standard deviation of six observations for biochemical parameters and statistically analyzed using one-way analysis of variance on statistical computer software program "Analyze it statistical software for Microsoft excel" (leeds U.K). The degree of statistical difference was accepted at $p < 0.05$.

3. Results

The results obtained are presented in Figures 1-13 respectively, it showed a significant increase of serum ALT and AST Activity, Alb/Glo ratio, Malondialdehyde concentration at ($p < 0.05$) and a significant decrease of Total protein concentration, Globulin concentration, Superoxide Dismutase and Catalase Activities, GSH concentration, HGB concentration, Packed Cell Volume (PCV), RBC concentration at ($p < 0.05$) and a non-significant variation of albumin concentration at ($p < 0.05$) after paraquat intoxication.

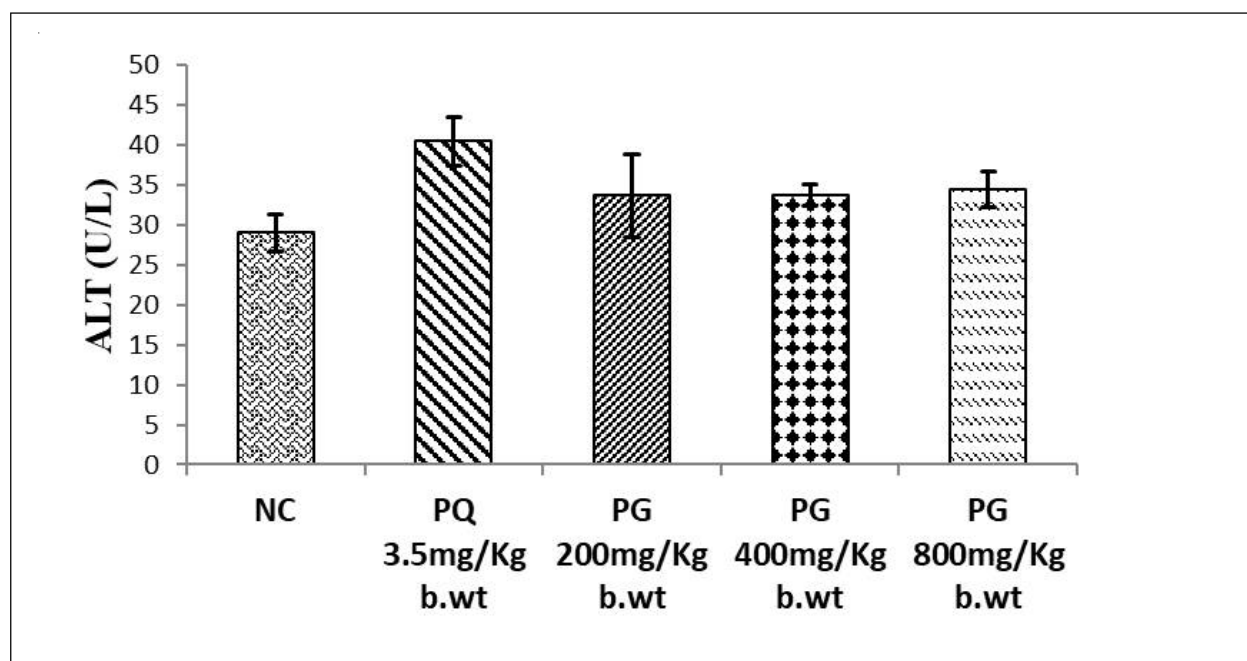


Figure 1: Effect of *Psidium guajava* leaf extract administration on serum alanine aminotransferase activities of male Wistar albino rats exposed to 3.5 mg/kg b.wt. Paraquat

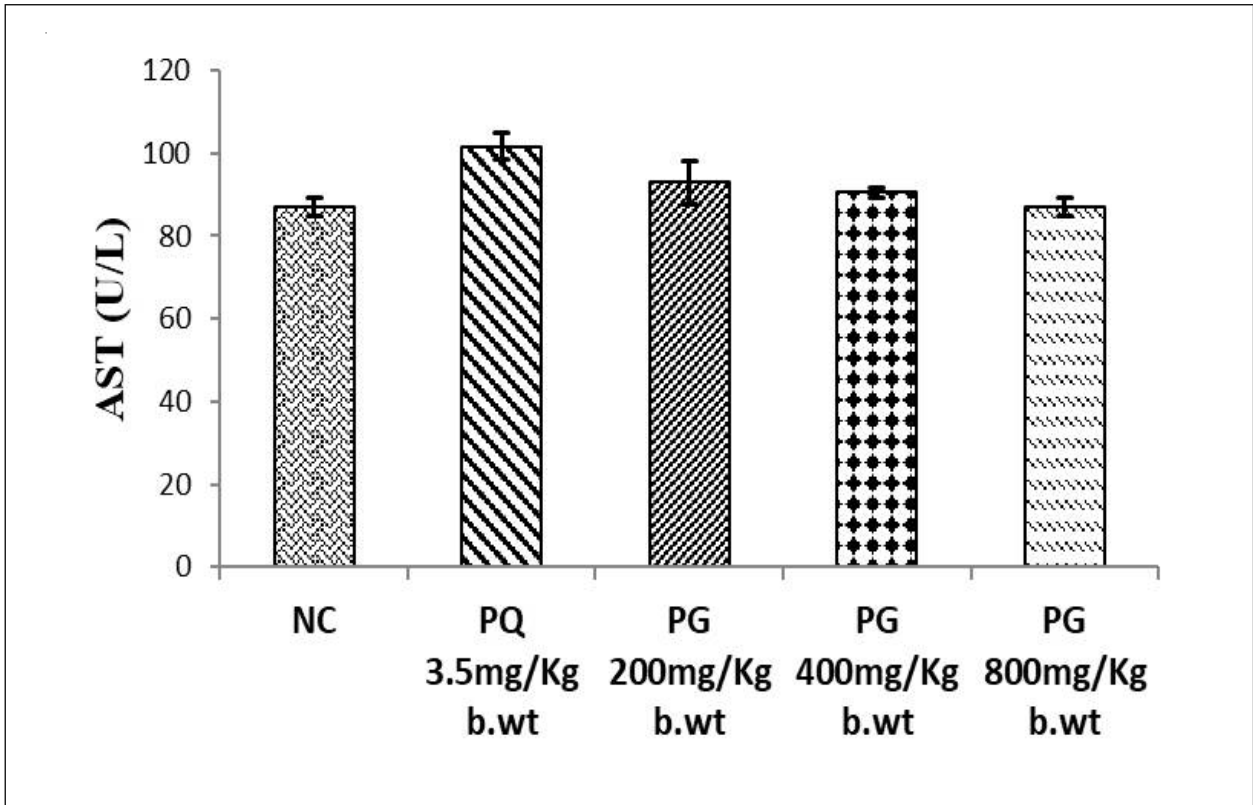


Figure 2: Effect of *Psidium guajava* leaf extract administration on serum aspartate aminotransferase activities of male Wistar albino rats exposed to 3.5 mg/kg b.wt Paraquat

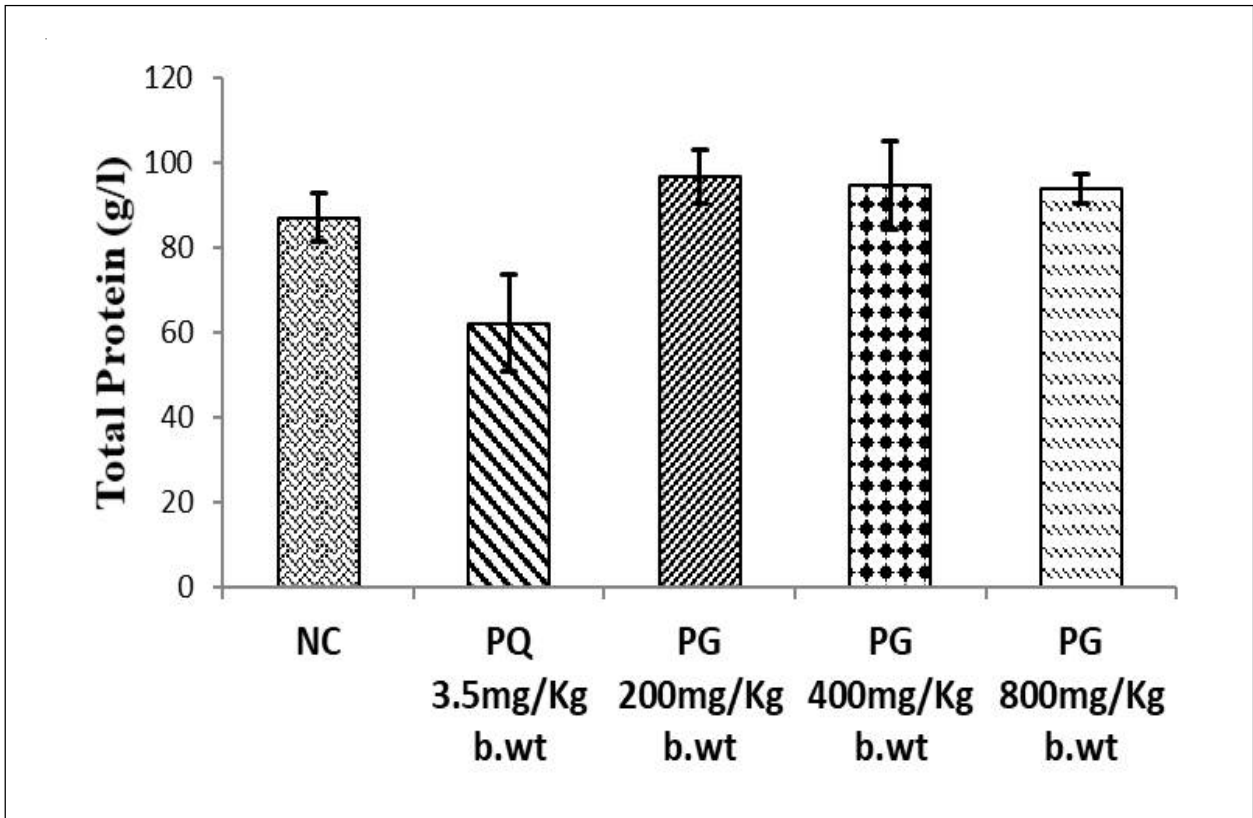


Figure 3: Effect of *Psidium guajava* leaf extract administration on serum total protein concentration of male Wistar albino rats administered 3.5 mg/kg b.wt. Paraquat

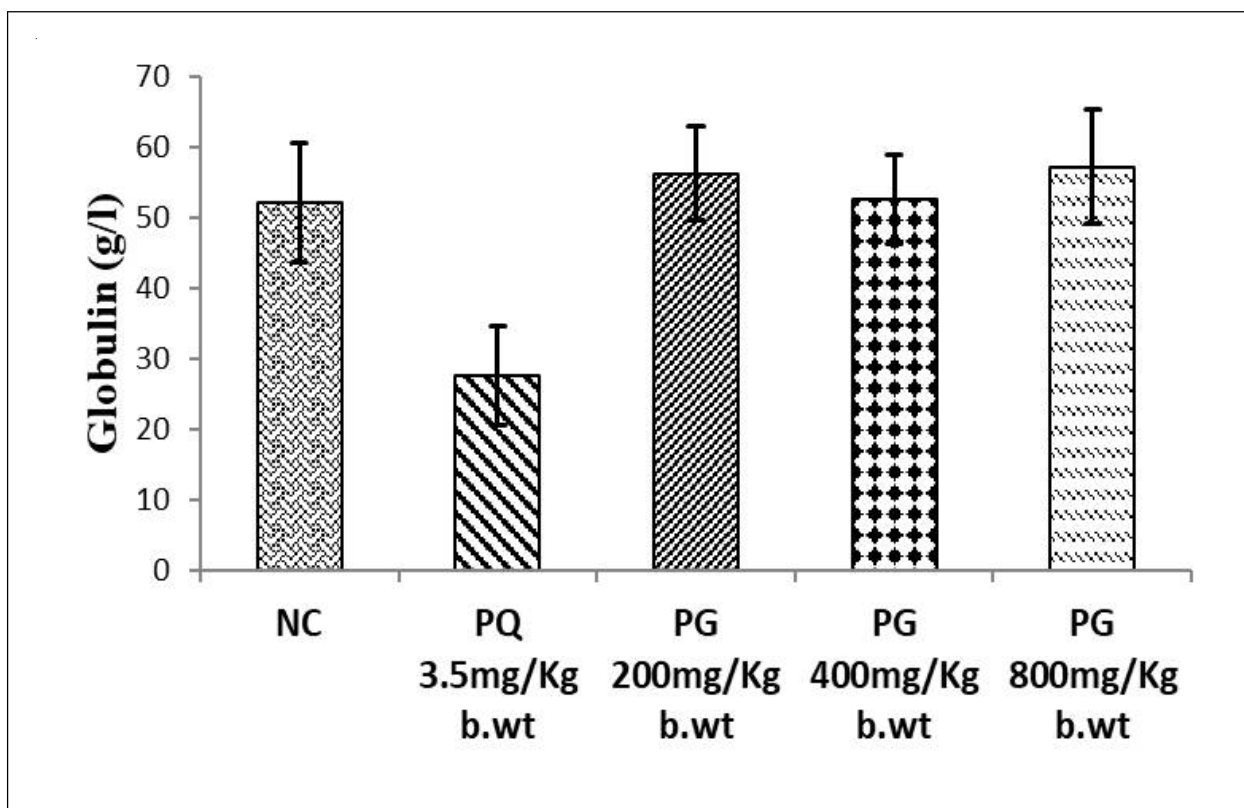


Figure 4: Effect of *Psidium guajava* leaf extract administration on serum globulin concentration of male Wistar albino rats administered 3.5 mg/kg b.wt. Paraquat

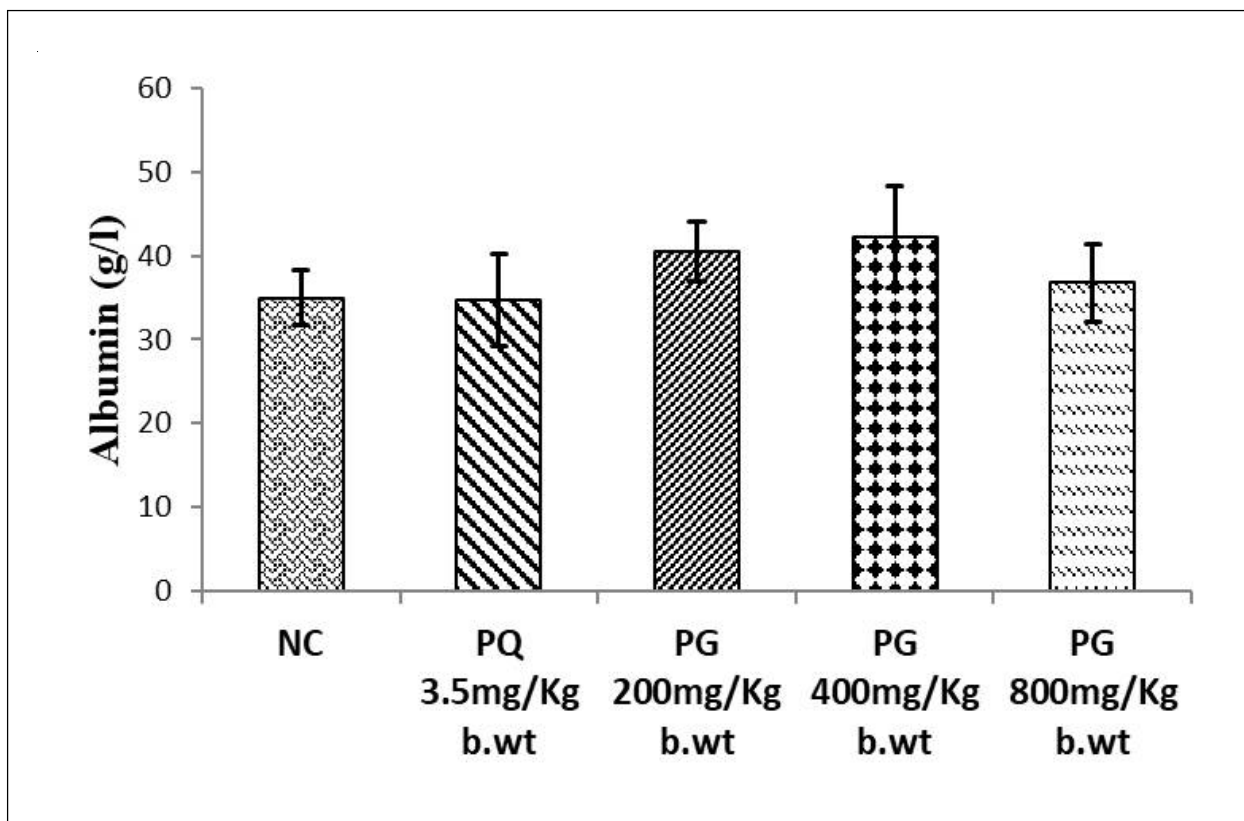


Figure 5: Effect of *Psidium guajava* leaf extract administration on serum albumin concentration of male Wistar albino rats administered 3.5 mg/kg b.wt. Paraquat

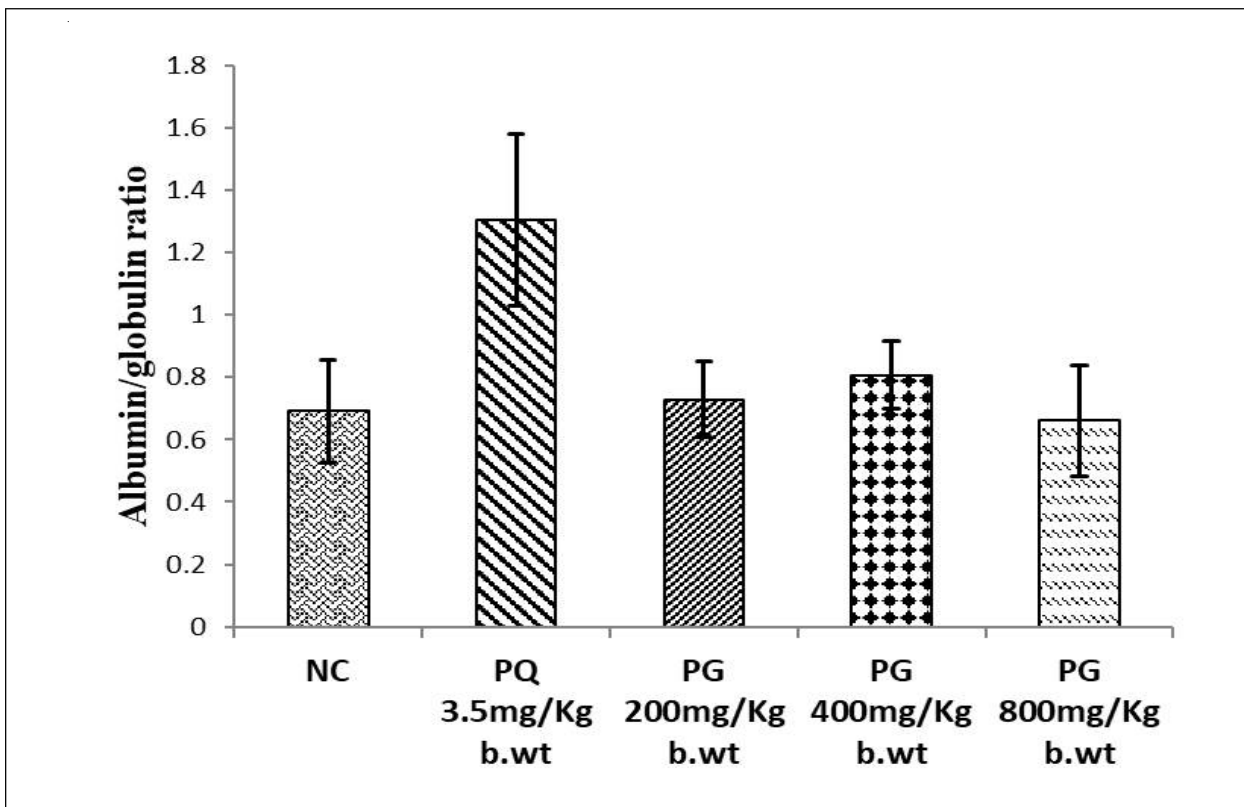


Figure 6: Effect of *Psidium guajava* leaf extract administration on serum albumin/globulin ratio of male Wistar albino rats administered 3.5 mg/kg b.wt. Paraquat

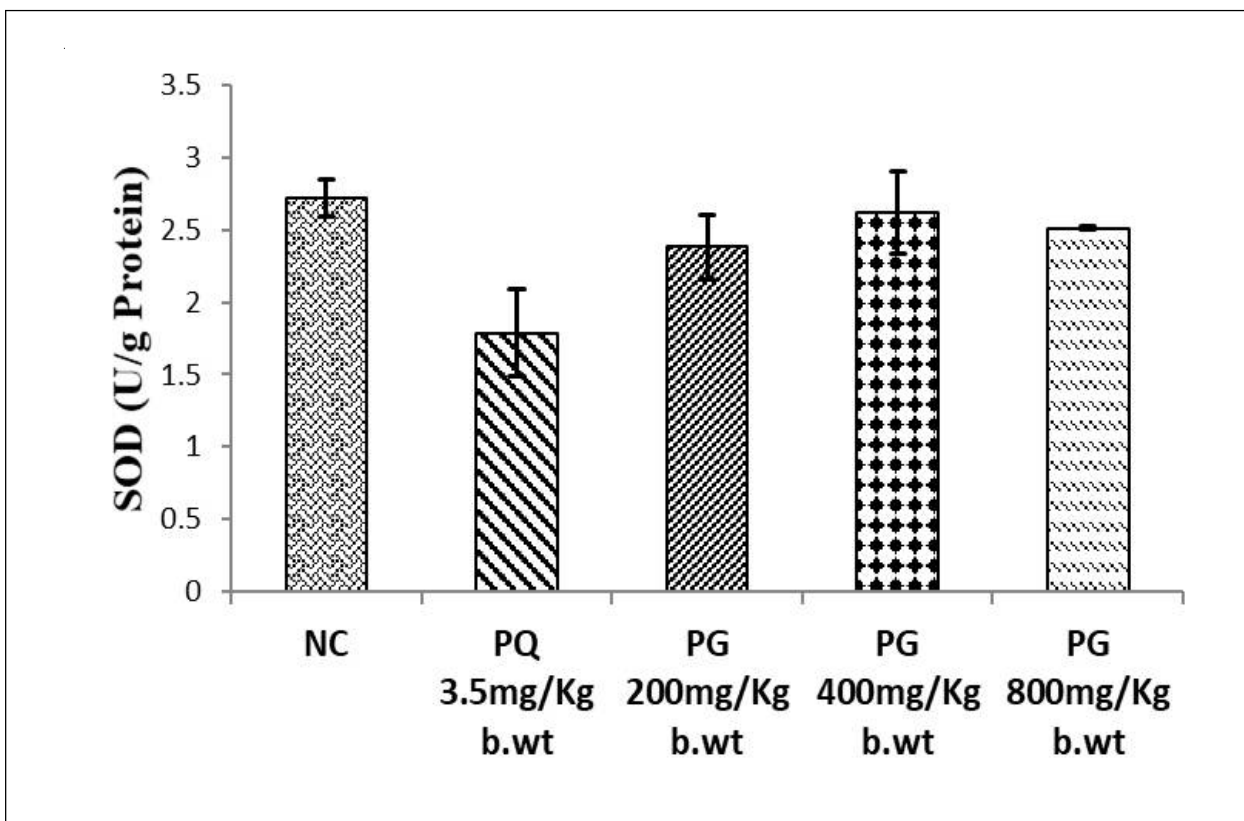


Figure 7: Effect of *Psidium guajava* leaf extract administration on serum superoxide dismutase activity of male Wistar albino rats administered 3.5 mg/kg b.wt. Paraquat

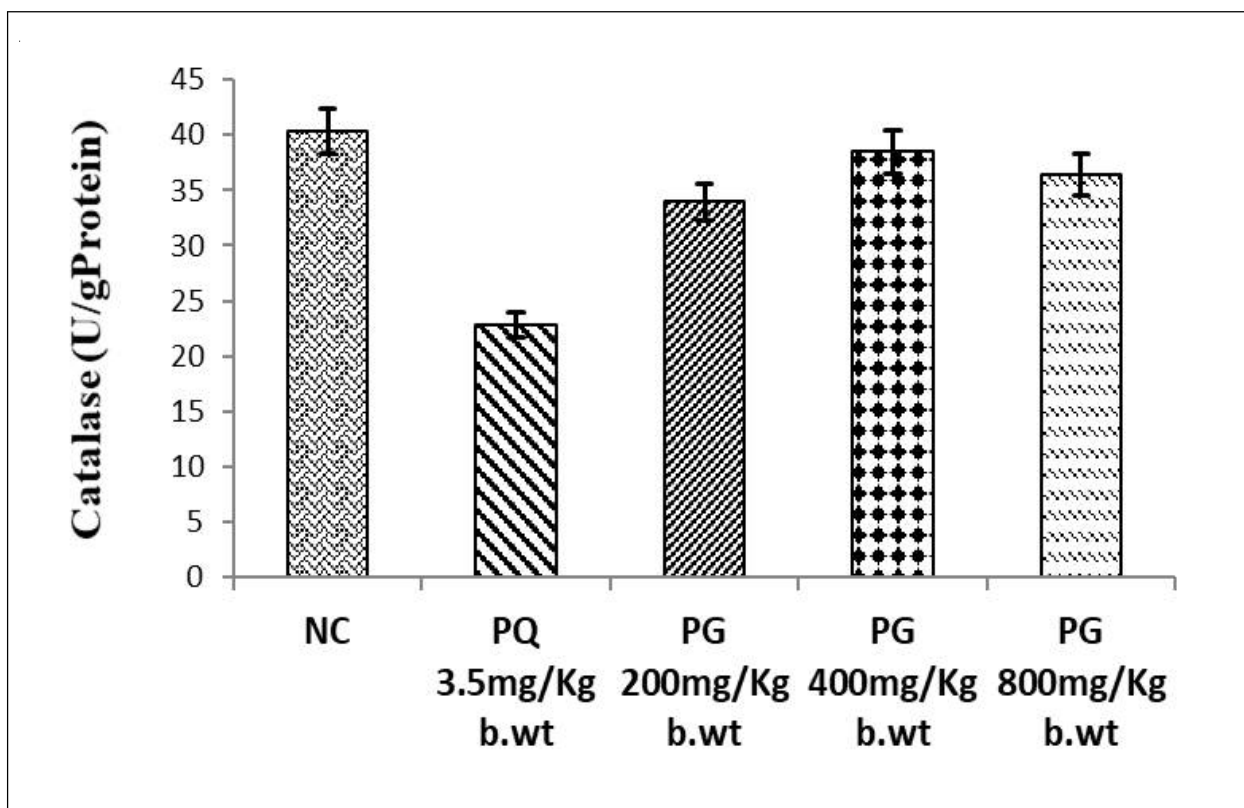


Figure 8: Effect of *Psidium guajava* leaf extract administration on serum catalase activity of male Wistar albino rats administered 3.5 mg/kg b.wt. Paraquat

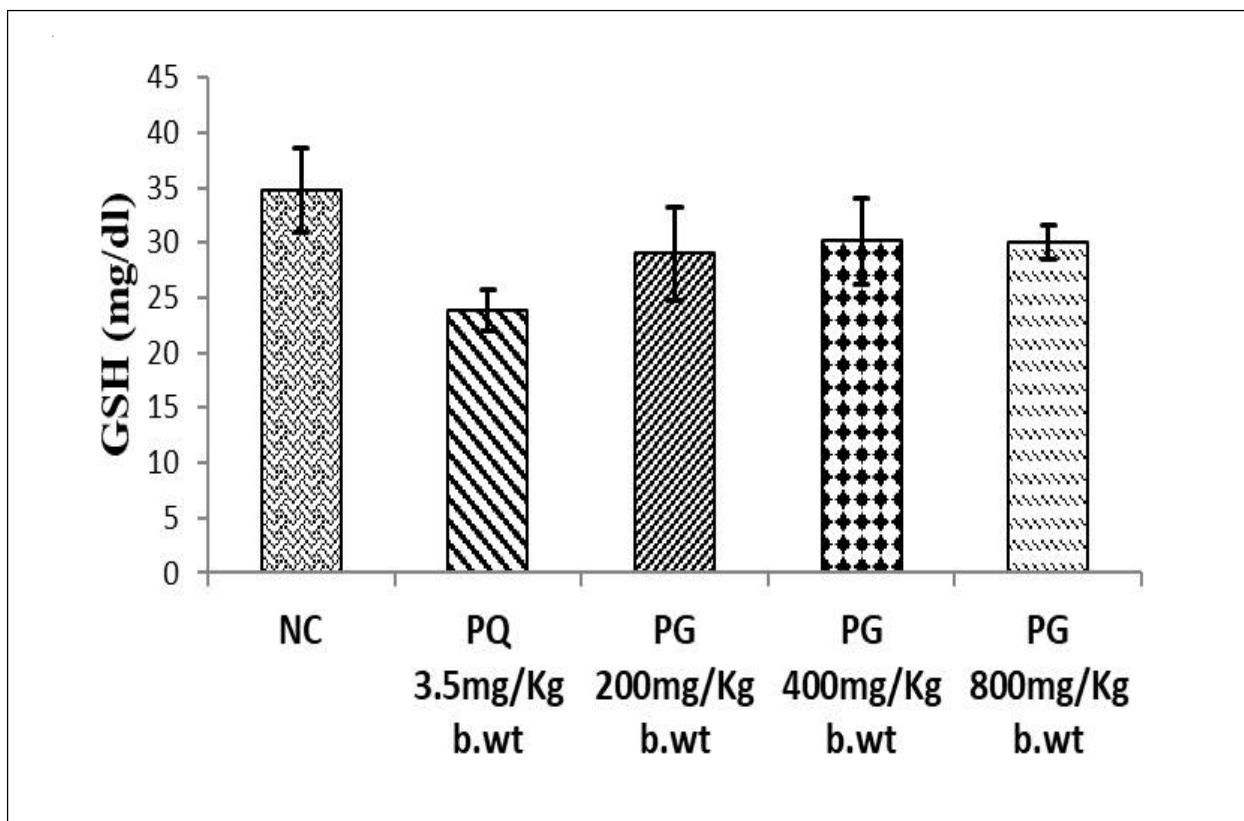


Figure 9: Effect of *Psidium guajava* leaf extract administration on serum glutathione concentration of male Wistar albino rats administered 3.5 mg/kg b.wt. Paraquat

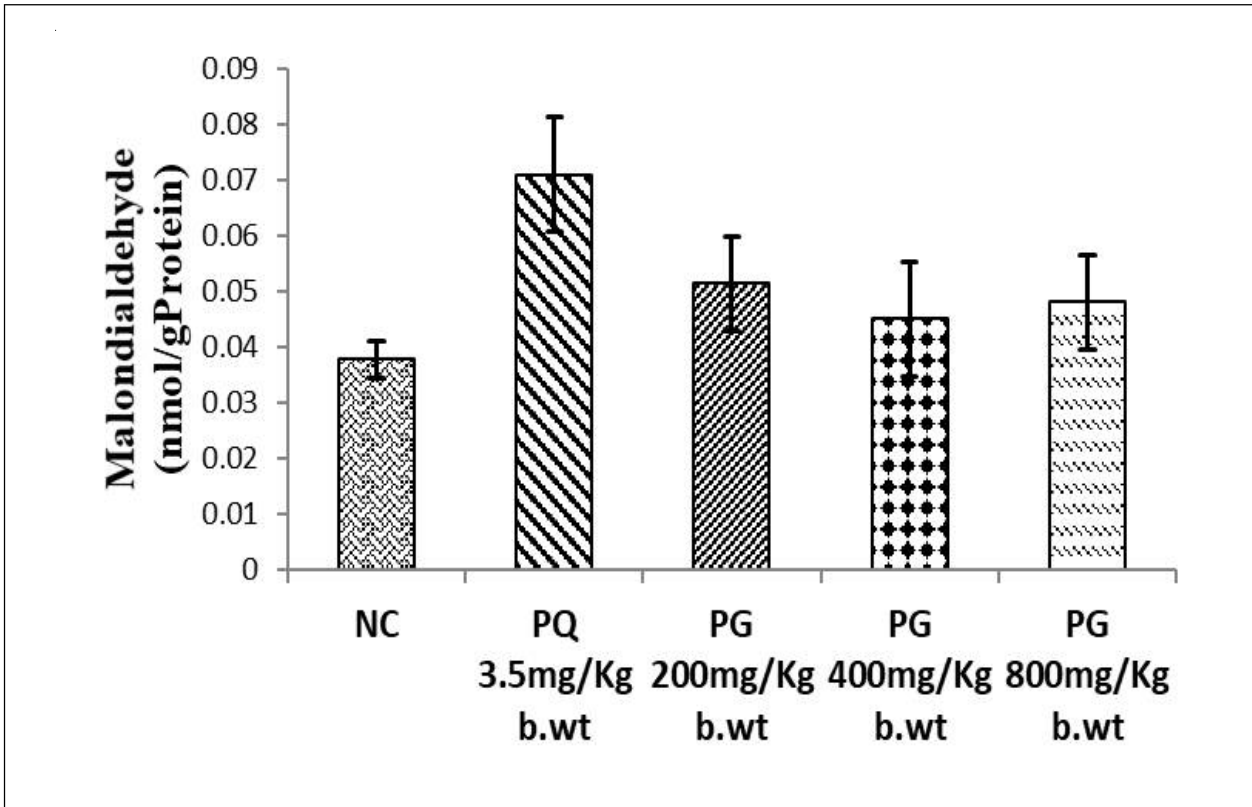


Figure 10: Effect of *Psidium guajava* leaf extract administration on serum malondialdehyde concentration of male Wistar albino rats administered 3.5 mg/kg b.wt. Paraquat

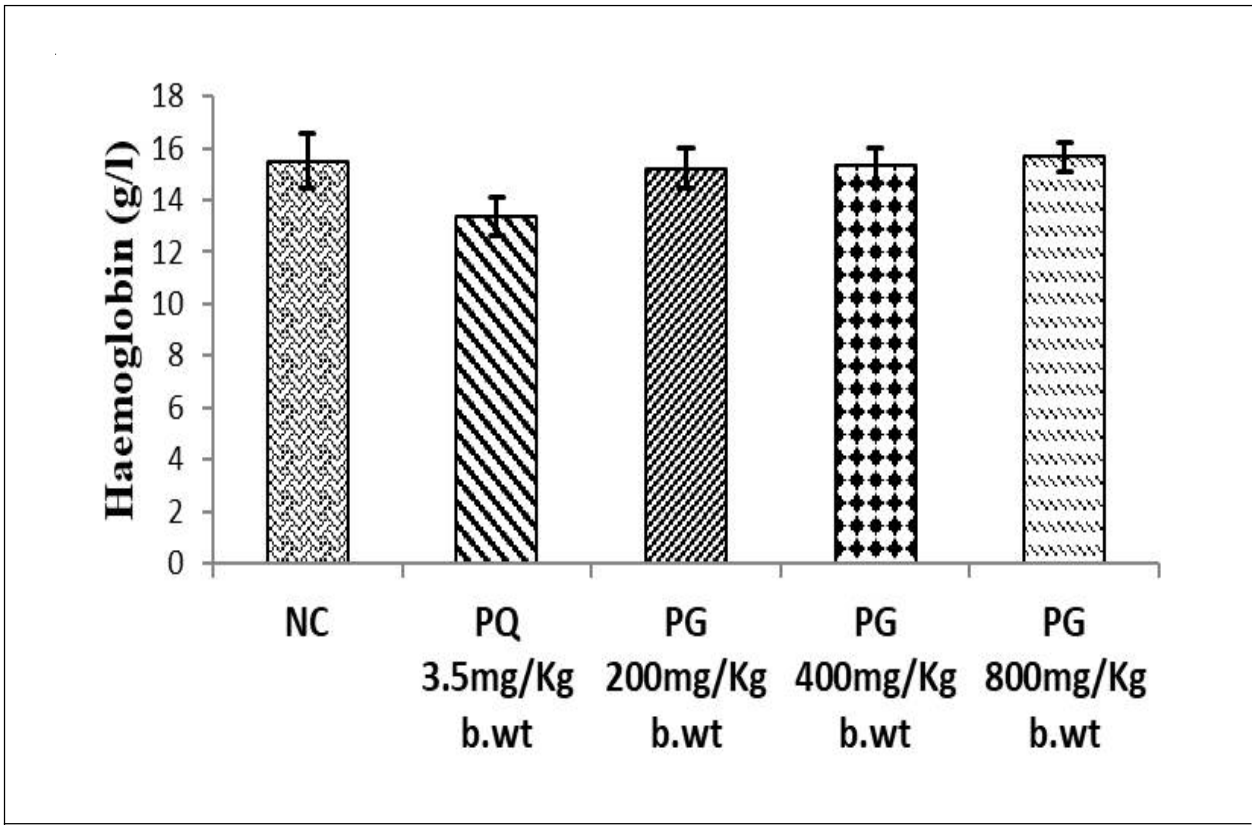


Figure 11: Effect of *Psidium guajava* leaf extract administration on hemoglobin concentration of male Wistar albino rats administered 3.5 mg/kg b.wt. Paraquat

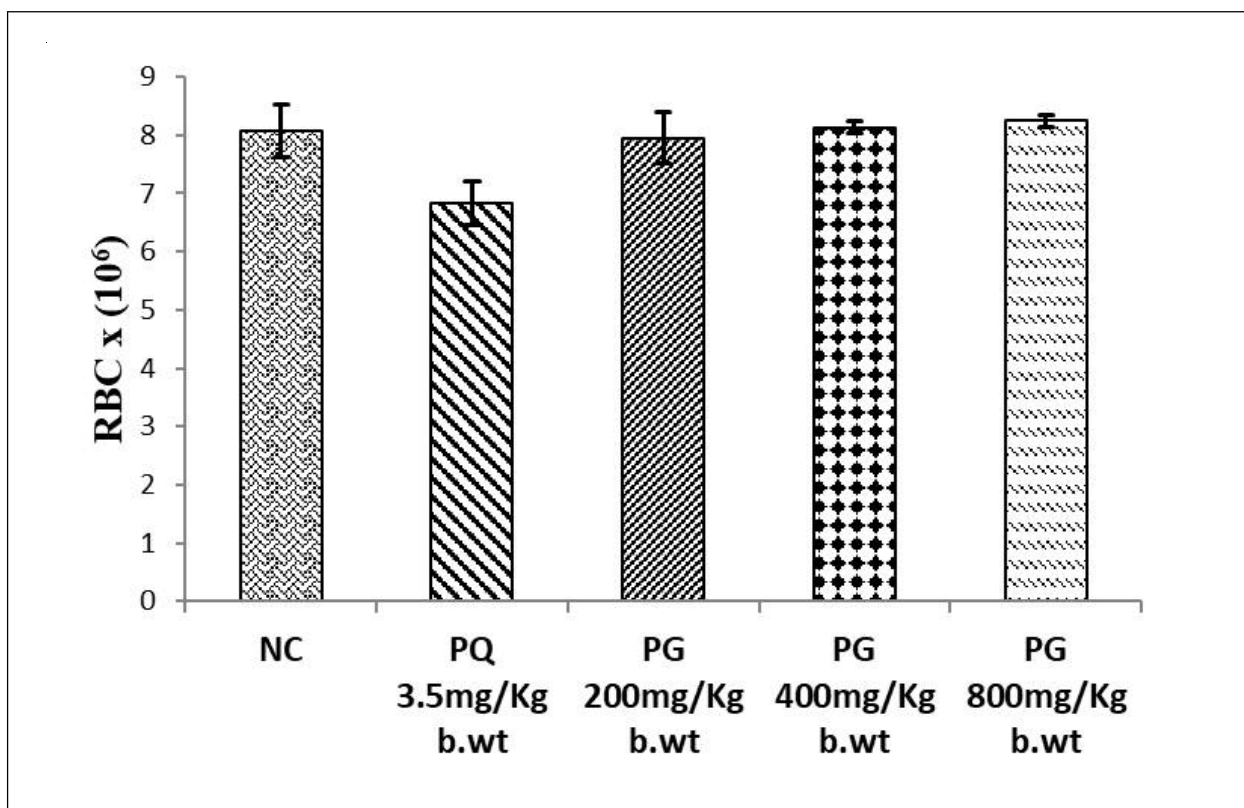


Figure 12: Effect of *Psidium guajava* leaf extract administration on red blood cells count of male Wistar albino rats administered 3.5 mg/kg b.wt. Paraquat

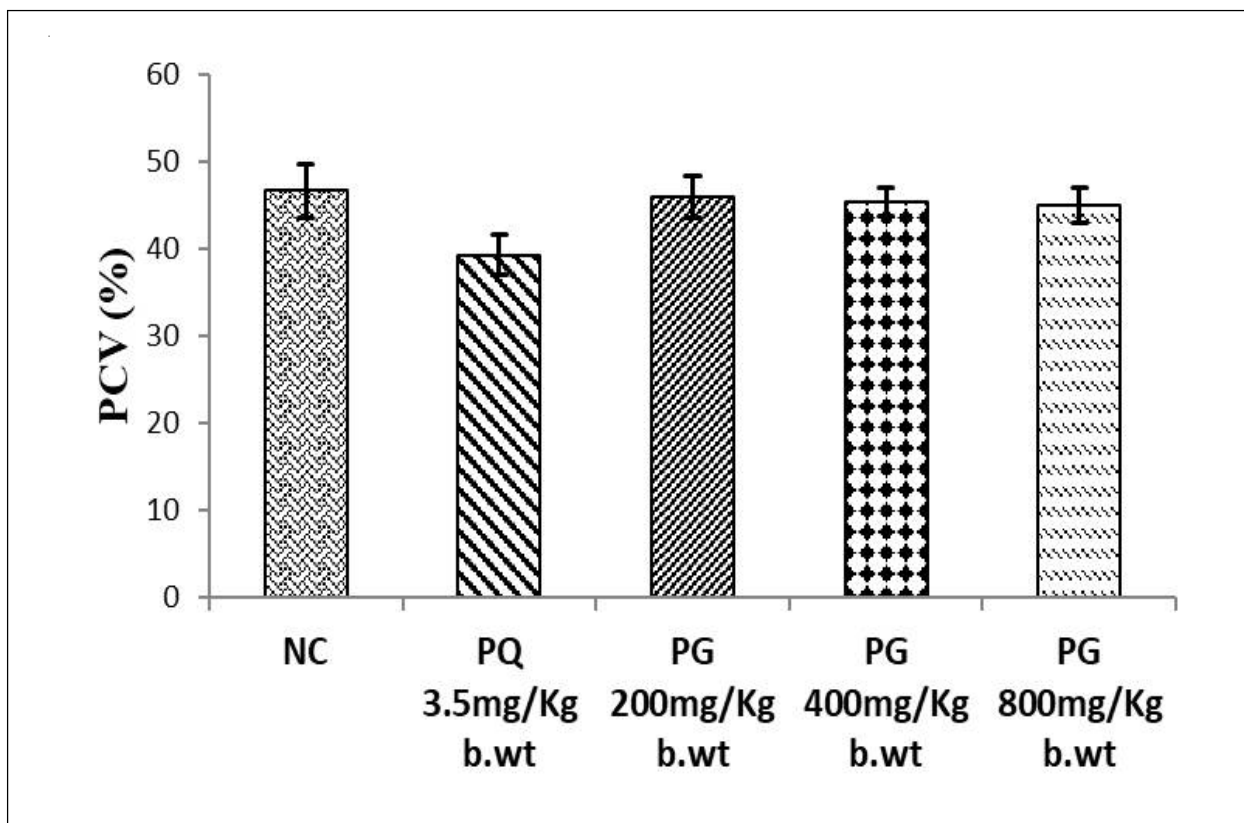
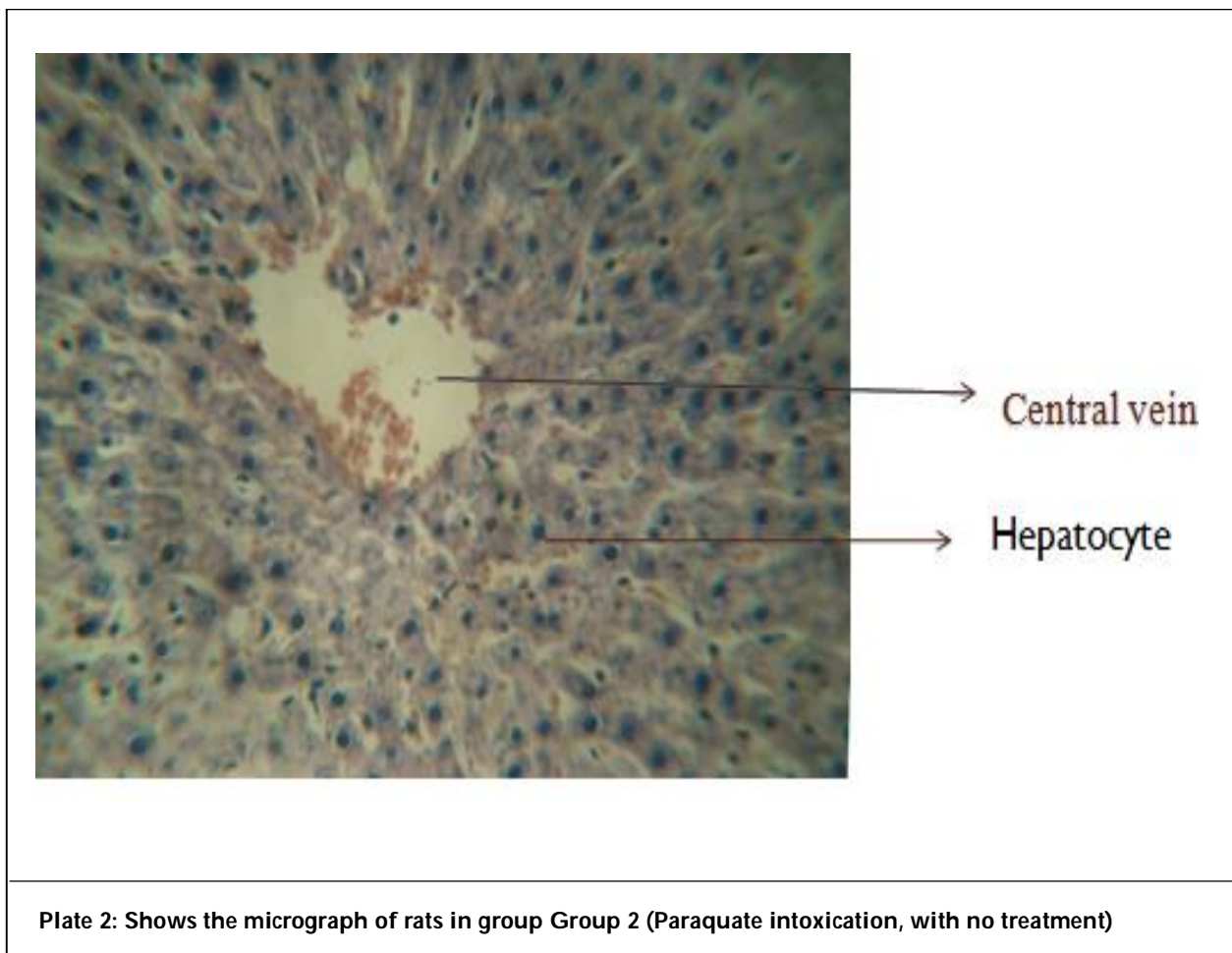
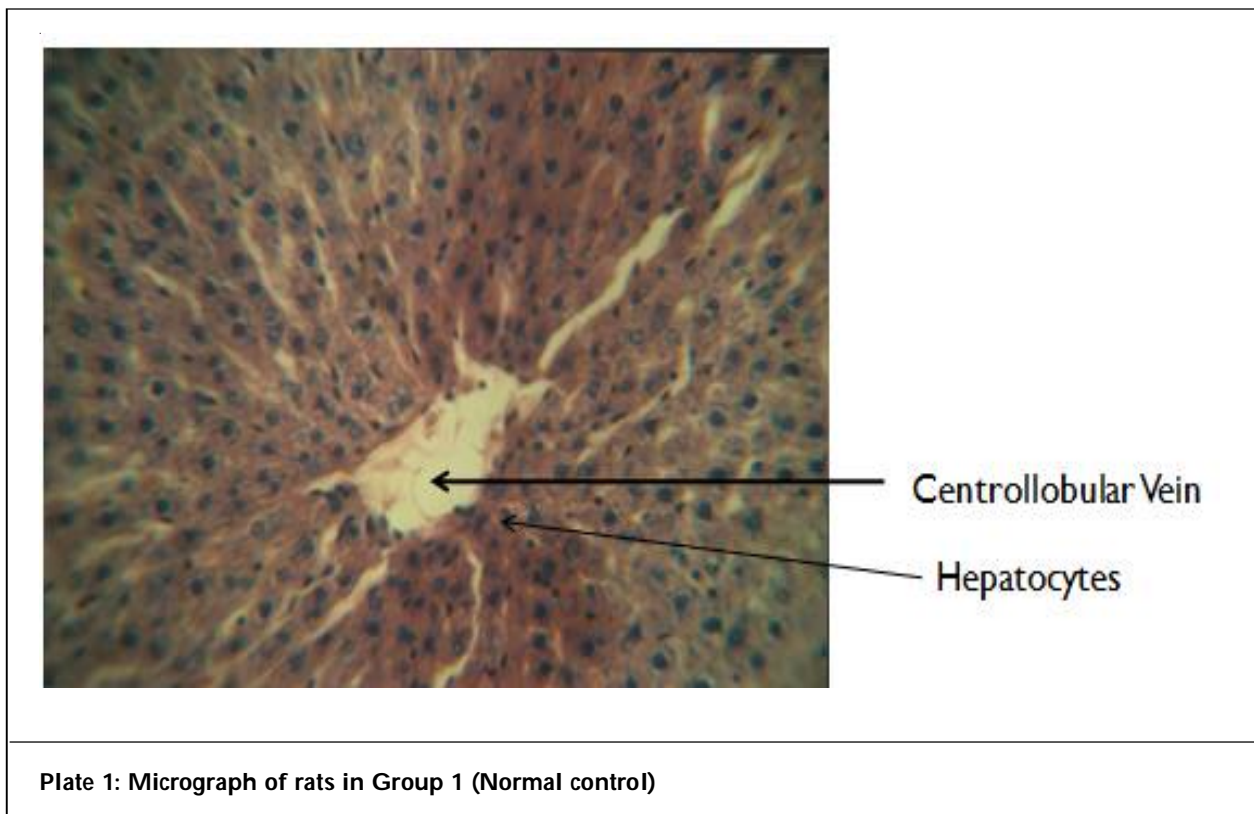


Figure 13: Effect of *Psidium guajava* leaf extract administration on packed cell volume of male wistar albino rats administered 3.5 mg/kg b.wt Paraquat

4. Histological results of the liver



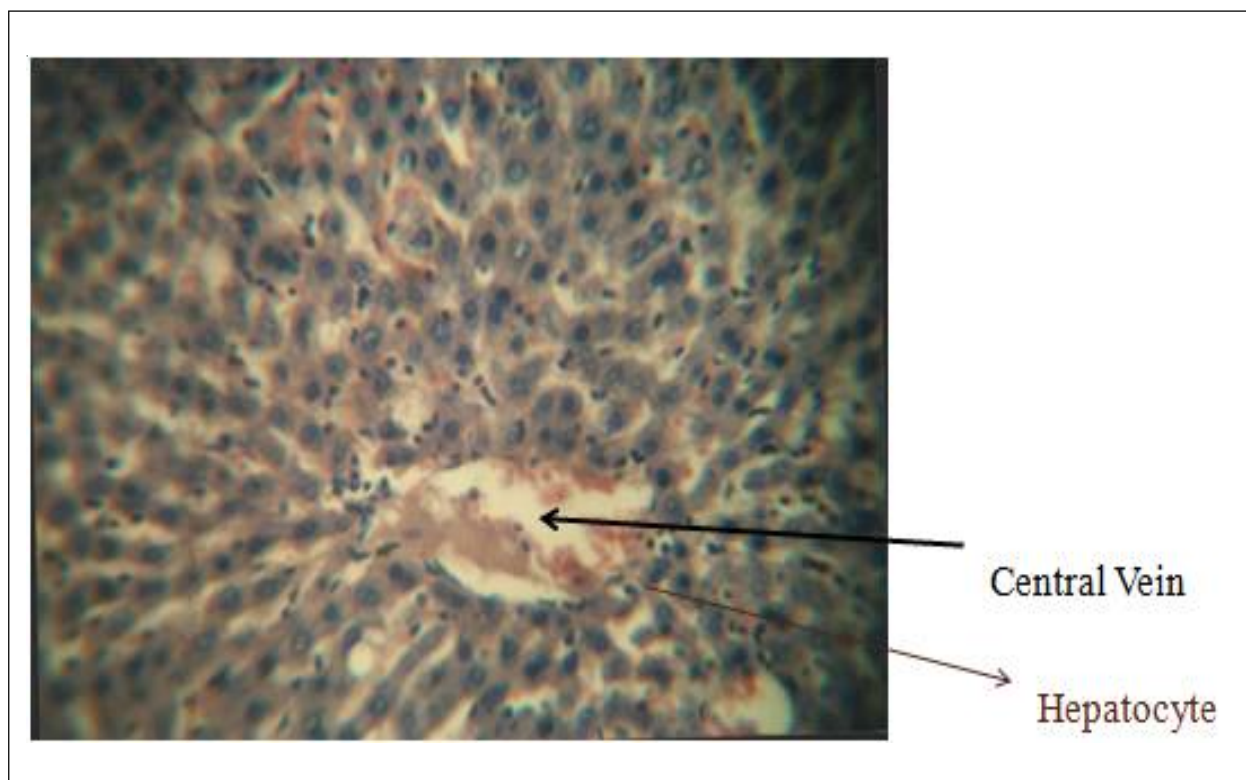


Plate 3: Shows the micrograph of rats in grp 3 (Paraquat intoxication, + 200 mg/ml guava extract treatment)

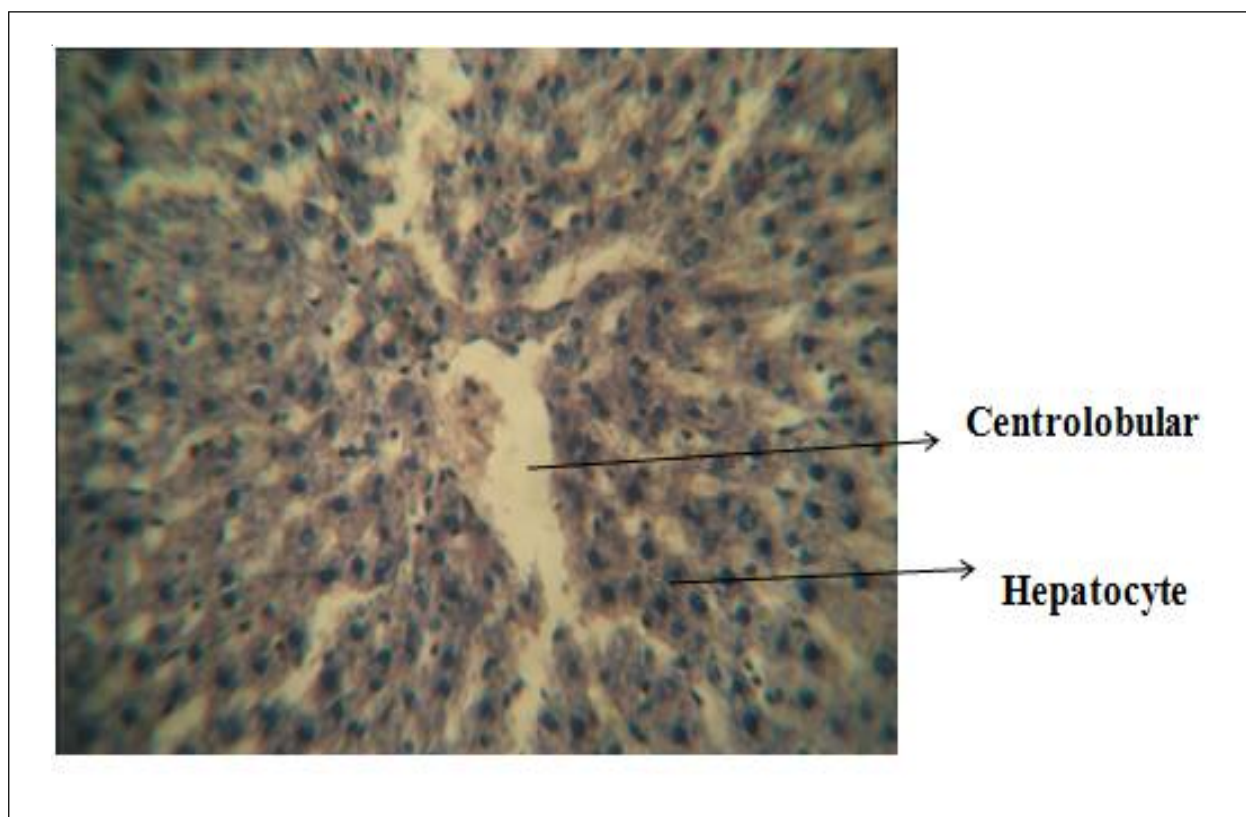
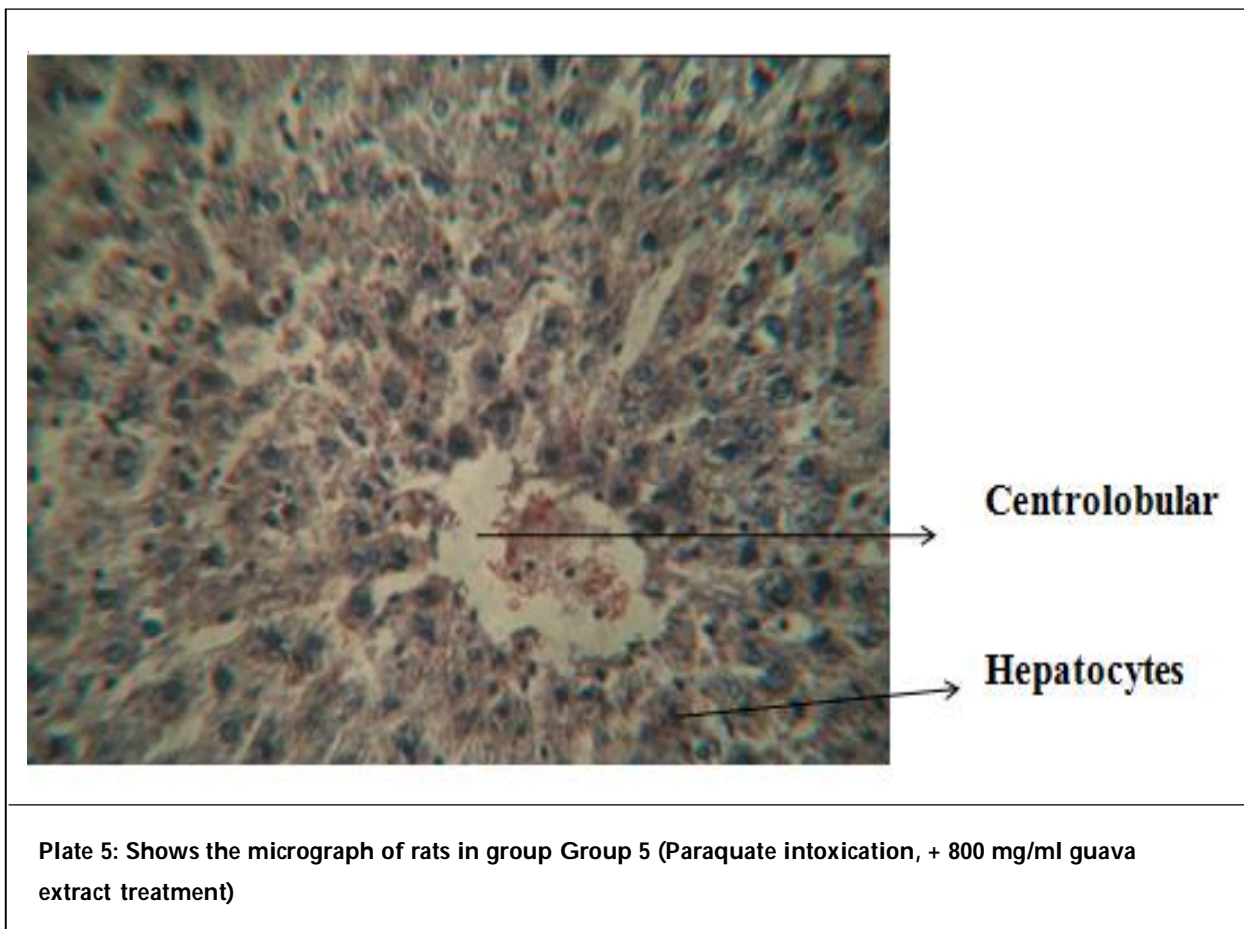


Plate 4: Shows the micrograph of rats in group Group 4 (Paraquate intoxication, + 400 mg/ml guava extract treatment)



The results of the antimicrobial activity of *Psidium guajava* leaf extract and the MIC of the leaf extract is represented in Tables 1 and 2.

Table 1: Antimicrobial activity of leaf extract of *Psidium guajava* on E.coli and S.aureus.

Concentration of crude extract (mg/ml)	Diameter zones of inhibition (mm) extract (mg/ml)	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
100 mg/ml	18.00 mm	24.00 mm
50 mg/ml	11.00 mm	16.00 mm
25 mg/ml	7.00 mm	10.00 mm
12.5 mg/ml	Nil	6.00 mm
6.25 mg/ml	Nil	Nil

Note: Nil = No Inhibition

Table 2: Minimum Inhibitory Concentration (MIC) of *Psidium guajava* leaf extract on test organisms

Test Organisms	MIC of <i>Psidium guajava</i> on test organisms
<i>Escherichia coli</i>	25.00 mg/ml
<i>Staphylococcus aureus</i>	12.50 mg/ml

4. Discussion

The dose of 3.5 mg/kg body weight (b.wt) of paraquat was chosen to induce toxicity and oxidative stress based on the dose optimization of paraquat according to Shanker *et al.* (2011). They recorded severe signs of toxicity, and 80% mortality when 10 mg/kg b.wt of PQ was used for intoxication and 60% and 30% mortality when 7.0 mg/kg b.wt and 3.5 mg/kg b.wt of PQ were used respectively, hence the need to choose the lesser toxic dose of 3.5 mg/kg b.wt. In this study, the enzyme, ALT and AST activities were found to increase in the paraquat group after paraquat intoxication, probably due to the toxicity and oxidative stress caused by paraquat in the liver which corresponded with the findings of Dere and Polart (2000), which says that paraquat alters the level and activity of various enzymes in liver and kidney. Treatment with *Psidium guajava* leaf ethanol extract was able to bring the activities of these enzymes, which are crucial in both amino acid degradation and biosynthesis back to normal as seen in the treated groups. This implies that the hepatic functions were significantly restored.

Decreased globulin concentrations are seen in hepatic dysfunction and renal disease, this study revealed that the total protein and globulin concentrations were significantly reduced at ($p < 0.05$) after paraquat intoxication, which signifies liver damage probably due to the effect of paraquat on liver and its parameters, according to Dere and Polart (2000). Treatment with *Psidium guajava* leaf ethanol extract significantly restored back the liver function of amino acid biosynthesis.

The result of some antioxidant and oxidative stress parameters showed that there was a significant reduction of SOD, Catalase activities and GSH concentration. There was also a significant increase of MDA concentration at ($p < 0.05$). GSH is the prominent endogenous antioxidant in mammalian cells and its GSH homeostasis relies on the activities of a number of antioxidant enzymes such as SOD, CAT and GR (Webb *et al.*, 2008). SOD and CAT are the most important enzymes involved in ameliorating the effects of oxygen metabolism. This study revealed, reduced concentration of GSH and reduced activities of SOD and CAT in the PQ intoxicated rats. PG treatment significantly improved the reduced GSH concentration and SOD and CAT activities at ($p < 0.05$) towards normalcy. This also corresponded with the findings of Anupama *et al.* (2011) that rats exposed to organophosphate pesticides resulted in decrease in the activities of antioxidant enzymes namely CAT, SOD and GPX.

MDA is a breakdown product that is usually quantified as a measure of lipid peroxidation. Its assay has been found to be one of the better predictor of oxidative damages and often show excellent correlation with other markers such as isoprostanes considered to be the most reliable markers of lipid peroxidation (Morrow, 2010). The present study revealed an elevated concentration of MDA in PQ intoxicated rats which indicate that PQ induced oxidative damage. Treatment with PG shows a remarkable reduction in the elevated MDA concentration which also indicates the ameliorative potentials of PG against PQ-induced stress which may probably be attributed to the antioxidant properties of PG.

Low Hb indicates the presence of anemia, low PCV also indicates presence of anemia which could be caused by any factor. In this study, treatment with PG significantly reversed the reduction in Hb Concentration, PCV and RBC at ($p < 0.05$).

The histological result of the liver revealed that the photomicrograph of rats in group1 (NC) shows a central vein in the center with arrays of bi-nucleated hepatocytes which are uniformly distributed throughout the cytoplasmic matrix. 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 the paraquat group showed a dilated central vein, necrotic or constricted hepatocytes adjacent the central vein. There was distortion of the sinusoid and enlargement of distant hepatocytes which indicates that paraquat altered the morphological features and caused necrosis of the liver cells.

The photomicrograph of rats in group 3 (paraquat intoxication +200 mg/kg body weight PG extract) showed reduction in the dilation of the central vein, regeneration of necrotic or constricted hepatocytes adjacent the central vein and elsewhere which indicates that treatment with 200 mg/kg body weight of PG leaf ethanol extract was able to reduce the dilation of the central vein, regenerate most of the constricted hepatocytes and elsewhere.

The photomicrograph of rats in group 4 (paraquat intoxication+400 mg/kg body weight) showed a central vein without enlargement and few population of necrotic hepatocytes which indicates that treatment with 400 mg/kg b.wt of PG leaf ethanol extract was able to remove entirely the dilated central vein and more cells were regenerated.

The photomicrograph of rats in group 5 (paraquat + 800 mg/kg b.wt PG extract) showed an enlarged central vein, necrosis of the hepatocytes and distortion of the sinusoid probably due to liver intoxication. The histological result which is represented in plate 1-5 correlated with the result of the biochemical parameters.

The result of the antimicrobial properties shows that *Psidium guajava* extract concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.50 mg/ml, inhibited *Escherichia coli* by diameter zones of inhibition of 18.00 mm, 11.00 mm, and 7.00 mm respectively while it inhibited *Staphylococcus aureus* by diameter zones of inhibition of 24.00 mm, 16.00 mm, 10.00 mm and 6.00 mm respectively. Also the MIC of *Psidium guajava* on *E. coli* was 25.00 mg/ml while that of *S. aureus* was 12.50 mg/ml. This shows that the leaf extract has antimicrobial properties against gram positive and gram negative organisms such as *E. coli* and *S. aureus* which corresponds with the findings of Manosroi *et al.* (2006) that certain extracts from leaves and bark of *Psidium guajava* can prevent bacterial growth.

5. Conclusion

From the result of the biochemical parameters and some of the antioxidant parameters, it could be deduced that paraquat caused toxicity of the liver and the antioxidant enzymes were drastically affected which serves as an enlightenment to our Agro-based industries and/or subsistence farmers who are into constant use of herbicides such as paraquat, while the *Psidium guajava* leaf extract was able to restore back close to normalcy the hepatic functions and also improved the activities of the antioxidant enzymes. *Psidium guajava* leaf extract could be recommended in hepatotoxicity or any other form of toxicity since it is an excellent antioxidant.

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