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EVALUATION OF THE HEPATOPROTECTIVE ACTIVITY OF ROOT EXTRACTS OF *MILLETTIA ABOENSIS* ON PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Numerous plants are used widely by people on all continents as food and as source of drugs. The hepatoprotective activity of the Millettia aboensis root extracts on paracetamol induced hepatotoxicity in rats was evaluated. Root of Millettia aboensis was shade dried, pulverized and extracted with alcohol (90% v/v) and distilled water respectively. The extracts were concentrated and dried separately under vacuum .Hepatotoxicity was induced in albino rats by the administration of paracetamol and the hepatoprotective activity of the plant extracts was evaluated. Prior to the administration of the extracts, the liver biomarker enzymes were determined as the base line .LIV-52 ,a herbal marketed product used in treating liver ailments was taken as standard and other groups were treated with ethanolic and aqueous extracts(215mg/kg and 431mg/kg).After nine days ,the serum was analysed for the liver enzymes- serum aspartate aminotransferase (SGOT), alanine aminotransferase(SGPT), Alkaline phosphatase(ALP) and serum bilirubin .Liver tissues were weighed and subjected to histopathological studies. Similar studies were carried out with normal rats. Both alcoholic and aqueous extracts of Millttia aboensis has shown significant ($p<0.05$) hepatoprotective activity in paracetamol induced hepatotoxicity and alcohol extract is found to be more effective than the aqueous extract.

KEY WORDS

Millettia aboensis, Phytochemical constituents, Paracetamol, Hepatopotective activity, Hepatotoxicity, Rats.

INTRODUCTION

Millettia aboensis is mainly tropical and sub tropical family of tree. It is found commonly in low land rain forest, often on low lying marshy sites. Small trees of 30–40 feet high and up to 2 feet in girth but usually 12 m high with reddish-brown pubescence on the petioles, branches, inflorescence and fruits. This type of specie is found in Cameroon, Gabon and Nigeria (Nsukka, Udi, Enugu, Anambra etc.) In Ibo, it is called uturuekpa. Almost all the part of Millettia aboensis has medicinal properties. The leaf is used by traditional herbalist for general healing including ulcer healing and laxatives while the root is used in treating disturbances gastro intestinal and hepatic disease. Also the leaf, stem and roots mixed with other plant materials are used to cure veneral diseases such as syphilis and Chlamydia trachomatic infection. Liver is an important organ in the body system. It is responsible for many critical functions within the body. Some of these functions include .production of bile. Other functions are production of certain proteins for blood plasma, production of cholesterol and special proteins to help carry fats through the body, conversion of excess glucose into glycogen for storage, regulation of blood levels of amino acids, which form the building blocks of proteins, processing of hemoglobin for use of its iron contents,

conversion of poisonous ammonia to urea(Urea is one of the end products of protein metabolism that is excreted in the urine.), clearing the blood of drugs and other poisonous substances, regulating blood clotting, resisting infections by producing immune factors and removing bacteria from the blood stream, production of albumin and angiotensinogen and moreso, the liver stores a multitude of substances, including glucose (in the form of glycogen), vitamin A (1–2 years' supply), vitamin D (1–4 months' supply), vitamin B12 (1-3 years' supply), iron, and copper. Should the liver become diseased or injured, the loss of those functions can cause significant damage to the body.

Paracetamol or acetaminophen is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). It is commonly used for the relief of headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, paracetamol can also be used in the management of more severe pain such as post surgical pain and providing palliative care in advanced cancer patients.[1] While generally safe for use at recommended doses, acute overdoses of paracetamol can cause potentially fatal liver damage and the risk is heightened by alcohol consumption. Paracetamol toxicity is the foremost cause of acute liver failure in the Western world. Many individuals with paracetamol toxicity may have no symptoms at all in the first 24 hours following overdose. Others may initially have nonspecific complaints such as vague abdominal pain and nausea. With progressive disease, signs of liver failure may develop; these include low blood sugar, low blood pressure, easy bleeding, and hepatic encephalopathy. Some will spontaneously resolve, although untreated cases may result in death. Damage to the liver, or hepatotoxicity, results not from paracetamol itself, but from one of its metabolites, N-acetyl-p-benzoquinoneimine (NAPQI). NAPQI depletes the liver's natural antioxidant glutathione and directly damages cells in the liver, leading to liver failure. The toxic dose of paracetamol is highly variable. In adults, single doses above 10 grams or 200 mg/kg of bodyweight, whichever is lower, have a reasonable likelihood of causing toxicity.[2][3] Toxicity can also occur when multiple smaller doses within 24 hours exceed these levels.[3] Following a normal dose of 1 gram of paracetamol four times a day for two weeks, patients can expect an increase in alanine transaminase in their liver to about three times the normal value.[4] It is unlikely that this dose would lead to liver failure.[5] Studies have shown that significant hepatotoxicity is uncommon in patients who have taken greater than normal doses over 3 to 4 days.[9] In adults, a dose of 6 grams a day over the preceding 48 hours could potentially lead to toxicity,[6] while in children acute doses above 200 mg/kg could potentially cause toxicity.[10]. This study was conducted to evaluate the hepatoprotective activity of the root extracts of Millettia aboensis on the paracetamol induced hepatotoxicity in rats.

MATERIALS AND METHODS

PLANT MATERIAL

The roots of *Millettia aboensis* were harvested from Ehandiagu, Nsukka, Enugu State. The plant material was authenticated by Mr. A. Ozioko at Bio resource Development and Conservative Programme (BDCP) Nsukka, Enugu State.

Experimental Animals

The albino rats weighing 66-153g of either sex, obtained from the Faculty of Veterinary Medicine, UNN were used. The rats were allowed to acclimatize in the experimental animal house unit of the Department of Biochemistry, UNN, for 5 days, during which they

were fed with standard rodent diet. Water was given liberally

Chemicals;

The chemicals used in this study were of analytical grade products of BDH, England and Sigma Aldrich, Germany. They include ethanol, sulphuric acid, and sodium hydroxide. Solvents used are; for the extraction of the plant material were: ethanol and water, Formalin was for preservation of the dissected liver, Liv 52(R and, Paracetamol,

Extraction of plant materials:

The root of *Millettia aboensis* was shade - dried and pulverized to coarse powder using an electrically operated mill. It was extracted with 90 % ethanol in soxhlet extractor, concentrated under vacuum (yield 45.93 %). The aqueous extraction was done by placing 50 g of the powder in a 250 ml percolator which was initially plugged with a cotton wool at the base. A volume of 150 ml of distilled water was added and thoroughly mixed. The mixture was then allowed to macerate, filtered and dried (yield 49.02% w/w).

ACUTE TOXICITY DETERMINATION

Acute oral toxicity test:

This was performed according to modified Dietrich Lorke method[13]. Here an initial investigation involves administering (10, 100 and 1000 mg/kg) of the plant extract to three different groups of three mice each. After 24 h, the number of deaths was recorded, but there was no death. The result was compared to that in a reference table. Based on the result from the same table the doses to be chosen for a second acute oral toxicity test were extrapolated. In this second test three dose levels were used (1600, 2900 & 5000 mg/kg). Observations were made up to 14 days during which dead animals and toxic manifestations were noted. The LD_{50} was calculated as the geometric mean of the minimum dose that cause 0% death and the maximum dose that cause 100 % death. Aqueous and alcoholic extracts of *Millettia aboensis* produced death to the doses of 2900 mg/kg and 5000 mg/kg body weight. Hence, 1/5th and 1/10th of the lethal dose i.e. 215 mg/kg P.O and 431 mg/kg P.O of both the extracts were used for the next study.

Evaluation of hepatoprotective activity (Acute hepatitis model):

Sixty-five healthy, albino rats of either sex housed under standard conditions and fed with standard rodent diet with water were used and their livers were damaged using paracetamol. The paracetamol (350mg/kg) was administered. The rats were divided into 7 groups consisting of 5 rats per group. The animals were then subjected to either one of the following treatments for 9 days.

Table 1: Administration of the drug to the groups

GROUPS	TREATMENT
1	Treated with distilled water (1ml/kg per oral (PO)).
2	Treated with distilled water for nine days + Paracetamol (350 mg/kg IP) administered on the nineth day.
3	Treated with LIV 52(R) 1ml/kg PO for nine days + Paracetamol (350 mg/kg IP) administered on the nineth day.
4	Treated with alcoholic extract of *Millettia aboensis* (215mg/kg PO) for nine days + paracetamol (350mg/kg IP) administered on the nineth day.
5	Treated with aqueous extract of *Millettia aboensis* (215mg/kg PO) for nine days + paracetamol (350mg/kg IP) administered on the nineth day.
6	Treated with alcoholic extract of *Millettia aboensis* (431mg/kg PO) for nine days + paracetamol (350mg/kg IP) administered on the nineth day.
7	Treated with aqueous extract of *Millettia aboensis* (431mg/kg PO) for nine days + paracetamol (350mg/kg IP) administered on the nineth day.

Food was withdrawn 12 h before PCM administration to enhance the acute liver damage in animals of groups 2, 3, 4, 5, 6 and 7 for PCM. The animals were sacrificed 48 h after the administration of PCM. Blood samples were collected and the serum assayed for marker enzymes such as aspartate aminotransferase (AST)[14], alanine aminotransferasen (ALT)[14], alkaline phosphatase (ALP)[15] and the liver immediately isolated and washed with normal saline, blotted with filter paper and weighed. The liver was then subjected to Histopathological examination

Statistical analysis

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The values are expressed as means ± SEM and values of P≤ 0.05 were considered significant.

RESULTS AND DISCUSSION

Acute oral toxicity study-

The acute oral toxicity study was carried out and the results obtained are presented in **Table 3** and **4**. Acute oral toxicity was performed according to Modified Dietrich Lorke Method.

The actual acute toxicity test involved dose levels of 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg using 3 animals per dose-level. At the end of 14 days the surviving animals were all of 1600 mg/kg, two of 2900 mg/kg and one of 5000 mg/kg for both extracts as shown in **Table 4**.

Table 3: Initial acute oral toxicity test

Samples	Dose levels		
	10 mg/kg	100 mg/kg	1000 mg/kg
Aqueous extract	0/3	0/3	0/3
Ethanolic extract	0/3	0/3	0/3

Table 4: Main Acute Oral toxicity test

Dose level	1600 mg/kg	2900 mg/kg	5000 mg/kg
Surviving animal	3/3	2/3	1/3

Therefore, the LD_{50} was calculated as 2,154mg/kg, using the Dietrich lorke's method for calculation of LD_{50}. The evaluation of plant extracts orally administered in mice showed no toxic effect at doses less or equal to 5000mg/kg. This suggests that the crude extract is relatively safe for consumption and that LD_{50} is greater than 2000mg/kg.

Paracetamol (PCM) induced acute toxicity –
A significant difference in biochemical markers was observed between normal PCM treated groups. Comparative analysis of the effect of various extracts on ALT, AST and ALP levels revealed that alcoholic extract and aqueous extract (431 mg/kg body weight) of *Millettia aboensis* showed protection against the hepatoxin.

Figures 1-5 show the effect of Liv 52(R), ethanolic and aqueous extracts on serum AST, ALT, ALP, bilirubin levels in paracetamol induced hepatic damaged rats.

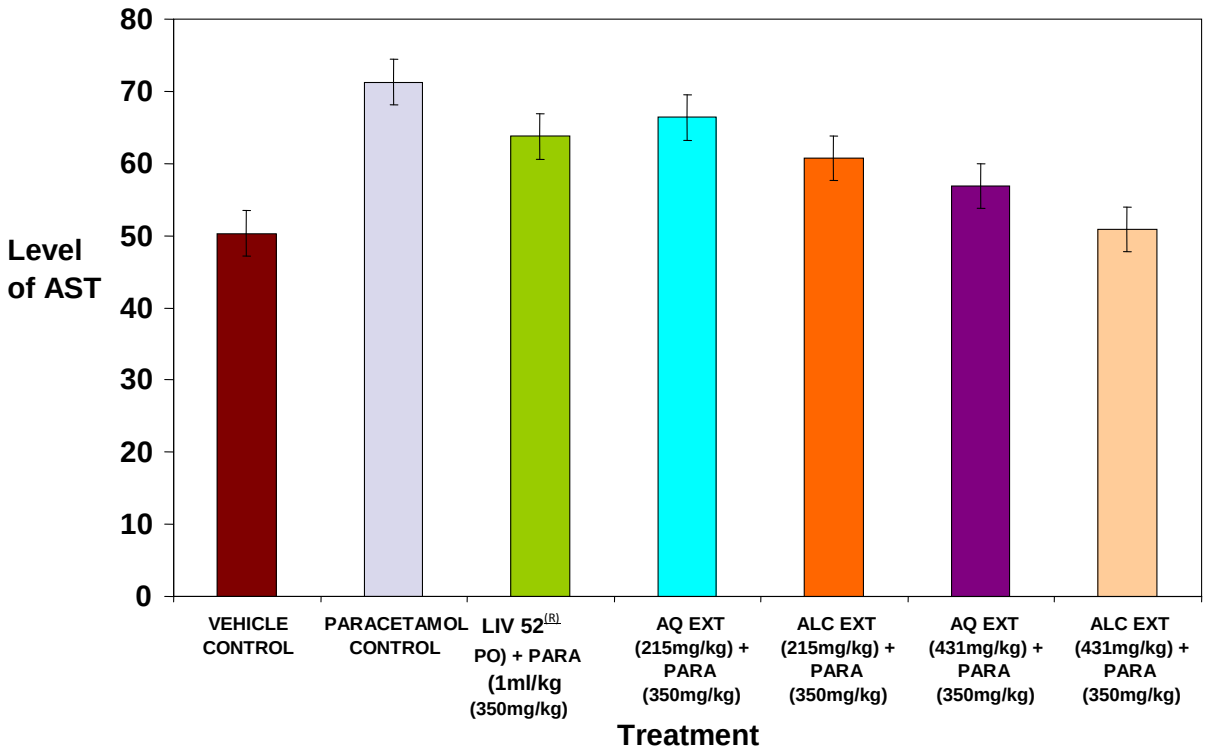

Fig. 1: AST` level in the treated rats

Fig 1: Effcets of ethanol and aqueous extracts of *Millettia aboensis* root on the level of AST (U/L) in paracetamol treated rats.

The effect of ethanolic and aqueous extracts of *Millettia aboensis* root on the level of AST (IU/L) in paracetamol treated rats is shown in **Fig.1**. The plot showed a dose dependent decrease in AST (IU/L) in

all treated rats. At all dose levels the alcoholic extract appears to have greater decrease in AST (IU/L) when compared to aqueous extract. However the AST (IU/L) in rats treated with Paraceamol alone was significantly higher than in rats which receive extracts, Liv 52(R) and vehicle treated rats

Effcets of ethanolic and aqueous extracts of *Millettia aboensis* root on the level of ALT (U/L) in paracetamol treated rats.

The effect of ethanolic and aqueous extracts of *Millettia aboensis* root on the level of ALT (IU/L) in paracetamol treated rats is shown in **Fig.2**. The plot shows a dose dependent decrease in ALT (IU/L) in all treated rats. At all dose levels the alcoholic extract appears to have greater decrease in ALT (IU/L) when compared to aqueous extract. However the ALT (IU/L) in rats treated with paracetamol alone was significantly higher than that in rats which receive the extracts, Liv 52(R) and vehicle treated rats.

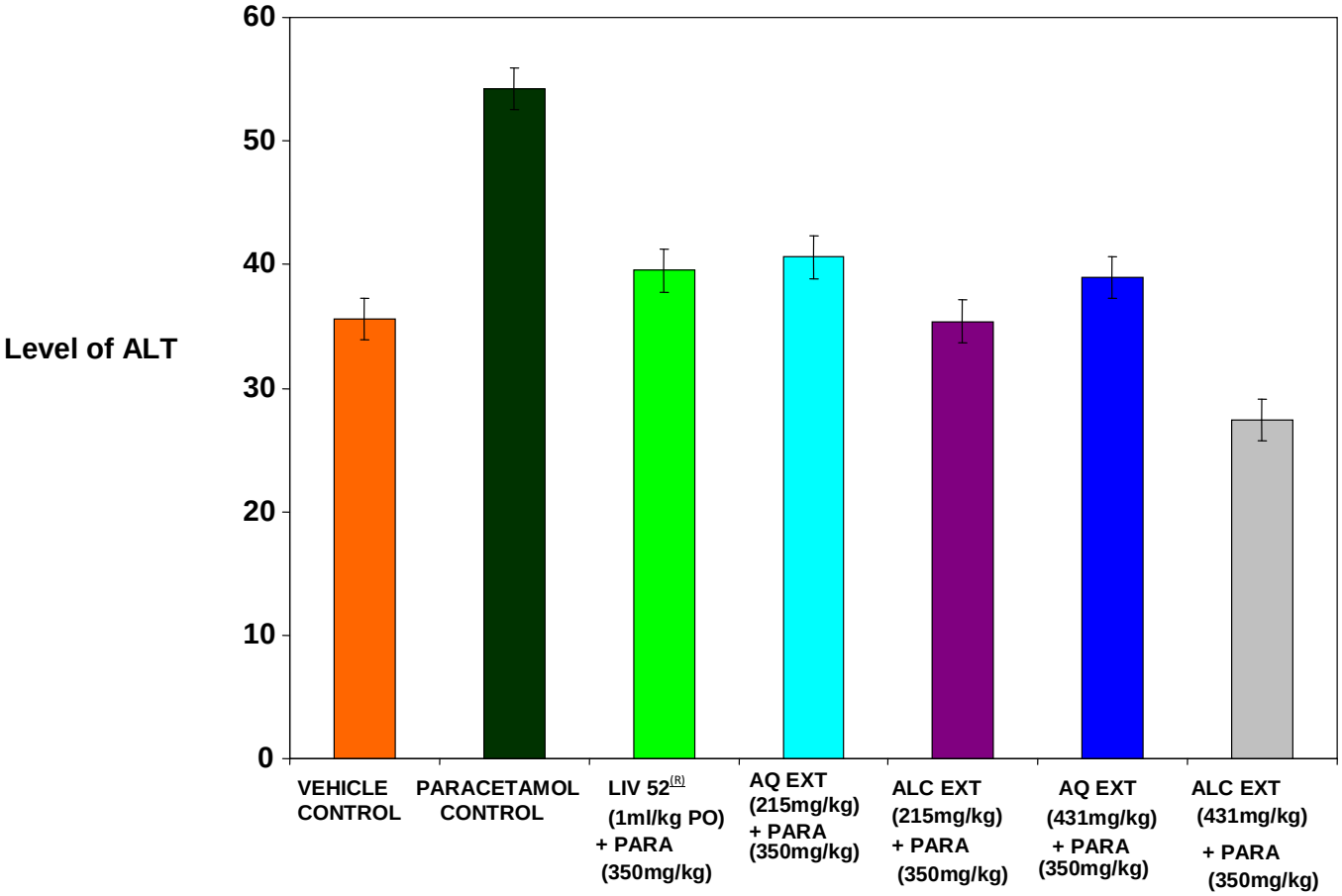

Fig 2: ALT Level in the treated rats

Fig.3: The effects of ethanolic and aqueous extracts of *Millettia aboensis* on the level of ALP (U/L) in paracetamol treated rats

Doses of 431 and 215 mg/kg ethanolic and aqueous extracts of *Millettia aboensis* root inhibited the increase of serum liver function biomarker when compared with those obtained from rats treated with paracetamol alone as shown in **Fig.3**. The observed effects were dose dependent and is significant at $p \leq 0.05$ with respect to negative controls (paracetamol).

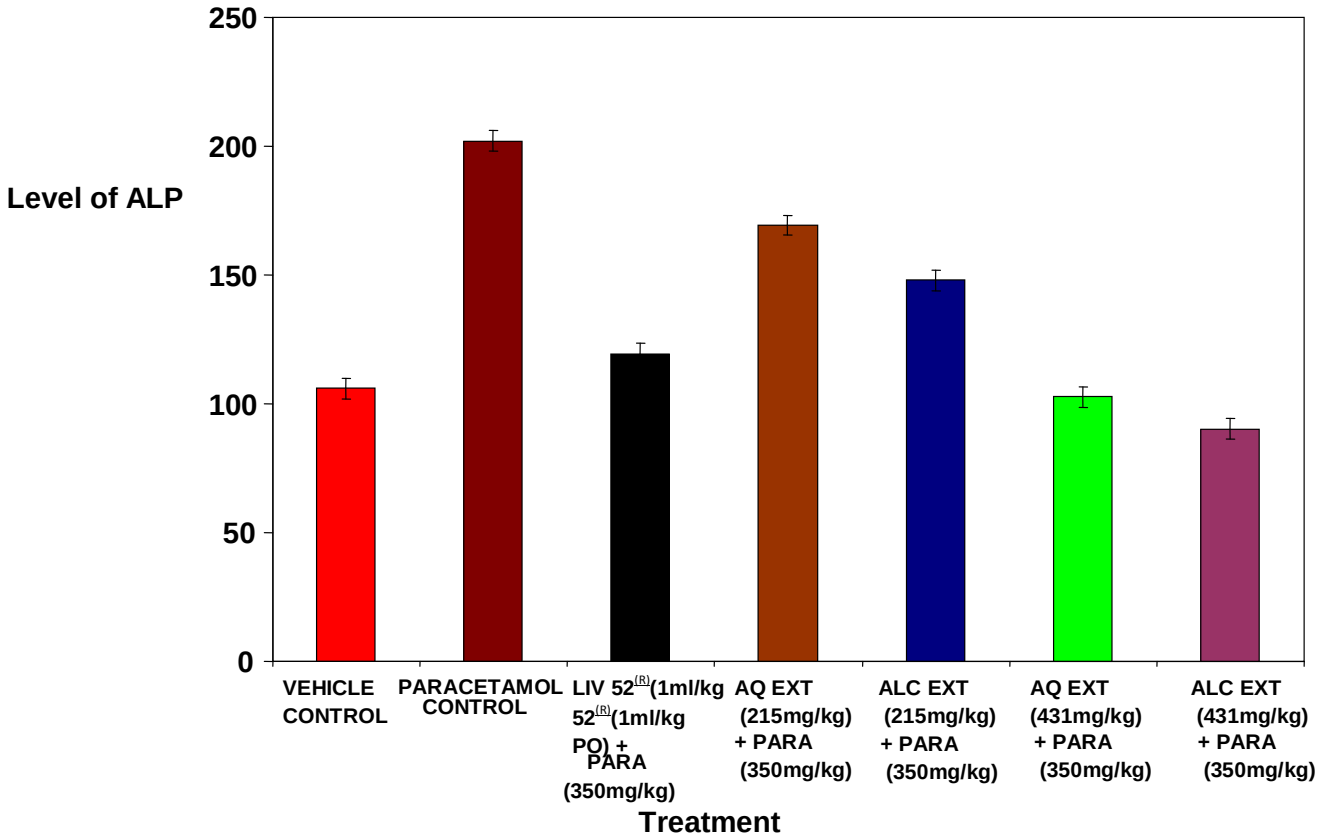

Fig. 3: ALP Level in the Treated rats

Fig. 4: The effects of ethanolic and aqueous extracts of *Millettia aboensis* on the level of total bilirubin (mg/dl) in treated rats

The results of the effect of alcoholic and aqueous extracts of *Millettia aboensis* root on the level of total bilirubin (mg/dl) in paracetamol treated rats are shown in **Fig.4**. The result showed a dose dependent decrease in total bilirubin in all treated rats. At all dose levels the alcoholic extract appears to have greater decrease in total bilirubin when compared to ethanolic extract. However the total bilirubin in rats treated with paracetamol alone was significantly higher than that in rats which received extracts, Liv 52(R) and vehicle alone.

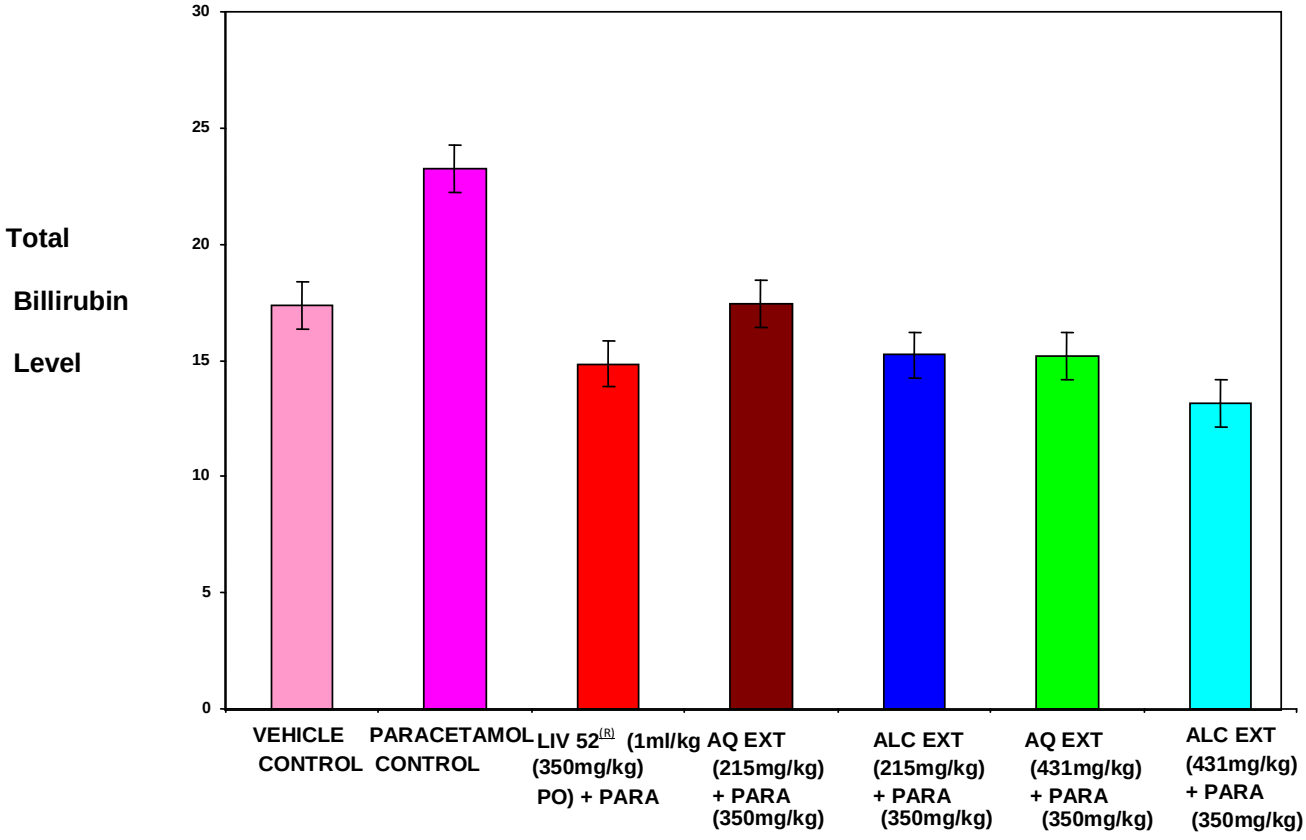

Fig. 4: Total Bilrubin Level in the treated rats

Fig.5: The effects of ethanolic and aqueous extracts of *Millettia aboensis* on the level of conjugated bilirubin (mg/dl) in treated rats

Hepatotoxicity was assessed by quantifying serum conjugated bilirubin in ethanolic and aqueous extracts treated rats after administration of paracetamol in alcoholic and aqueous treated rats. Paracetamol causes a severe liver toxicity which was higher than that treated with both extracts and vehicle alone. The level of conjugated bilirubin in alcoholic treated rats at 431 mg/kg was comparable with that of the control.

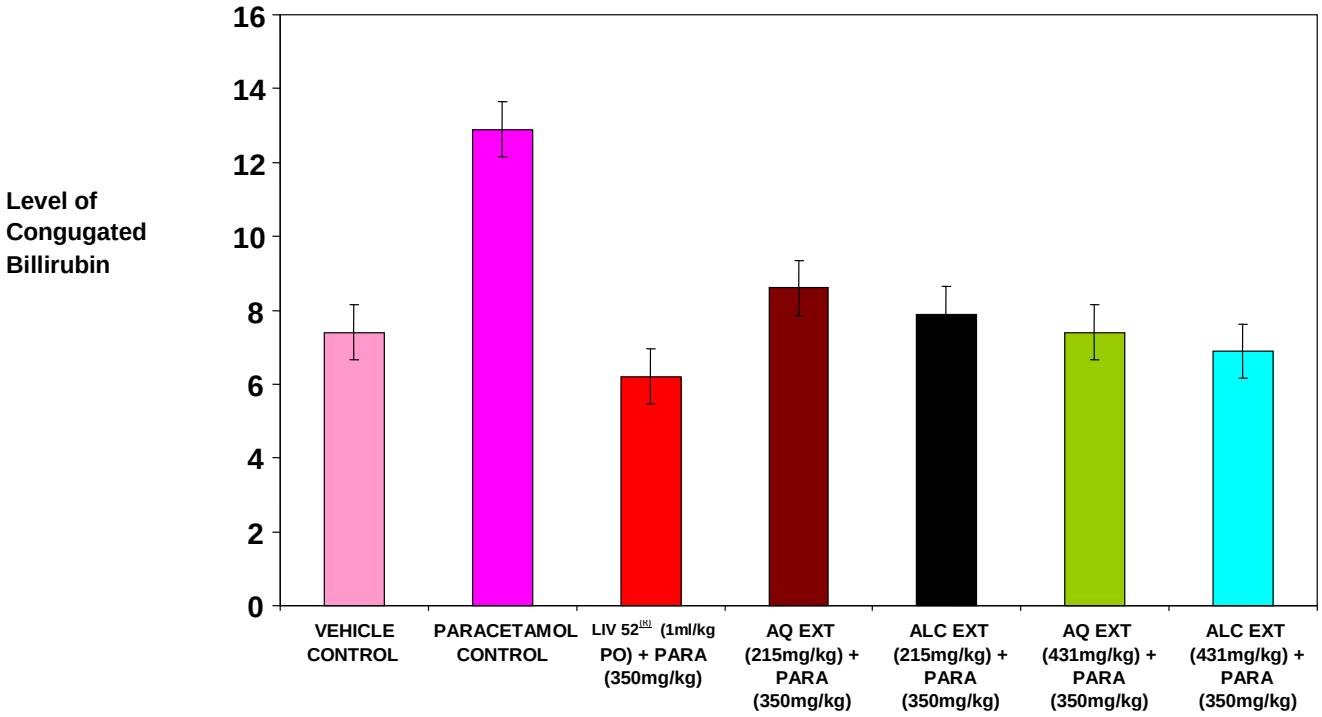

Fig. 5: Conjugated Billirubin Level in the treated rats

Pretreatment with ethanolic and aqueous extracts (215 mg/kg and 431 mg/kg) and Liv 52$^{(R)}$ significantly reduced the level of AST, ALT, ALP and serum billirubin after intoxication with paracetamol.

The increase in level of the biomarker enzymes (AST, ALT, ALP, total bilirubin and conjugated bilirubin) observed in groups treated with paracetamol indicate some extent of liver damage.

Millettia aboensis treated animals showed a protection against the injurious effects of paracetamol resulting in the hindrance of formation of hepatotoxic free radicals. The tendency of these marker enzymes to return to near normal in group treated with higher dose of the extracts treated animal was a clear suggestion that the extracts have an anti

hepatotoxic effects. The ethanolic and aqueous extracts of *Millettia aboensis* roots showed significant hepatoprotective activity at dose dependent level. The effect produced by ethanolic extract (431 mg/kg) of *Millettia aboensis,* was almost similar to that produced by Liv 52$^{(R)}$ (1ml/kg p.o.), a well known hepatoprotective agent. Damage induced in the liver is accompanied by the increase in the activity of some serum enzymes. Drugs having antioxidant activity are also effective in treating paracetamol induced hepatotoxicity by scavenging the free radicals.

HISTOPATHOLOGY

Histological studies of liver give a visual assessment of the hepatic structure. Comparing the normal liver with that with induced hepatotoxicity clearly showed degeneration of hepatocytes, necrotic areas and non visible portal tract resulting from the paracetamol .

Fig. 6 a-e; show the representative photomicrographs of liver section of rats

Histopathological examinations of the liver tissues showed severe congestion of blood vessels, mild hydropic degeneration, pyknosis of nucleus and occasional necrosis in PCM treated animals (**Fig 6 b**). Animals treated with ethanolic and aqueous extract of Millettia aboensis showed mild hydropic degeneration and there was no pyknosis or congestion (**Fig 6 d and e**). The paracetamol (PCM) induced a significant increase in liver weight, which was due to the blocking of secretion of hepatic triglycerides into the plasma. Ethanolic and aqueous extract of Millettiaaboensis prevented the increase in liver weight of rats pretreated with PCM.

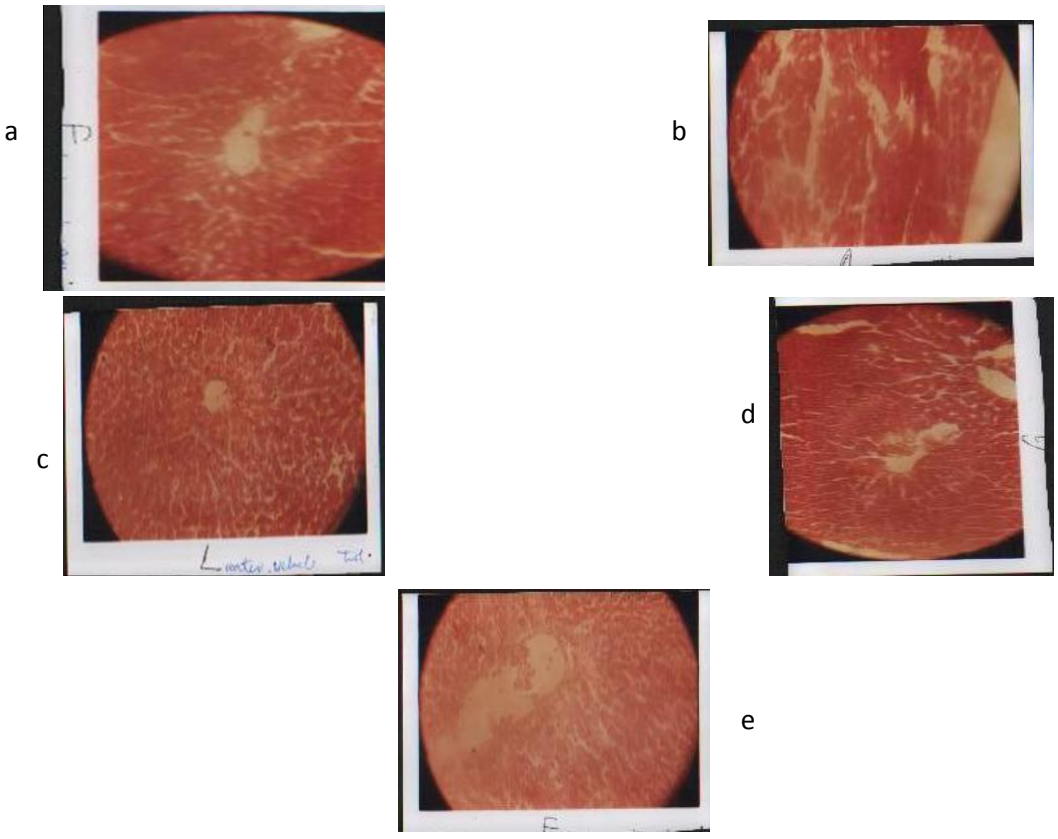

Fig. 6. Photographs of liver section taken from rats of group-1 (a), group-2 (b), group-3 (c), group-6 (d) and group-7 (e)

The mice treated with a vehicle control (group-1) (**Fig. 6a**) show normal hepatic structure and visible portal tract. Treatment of animals with paracetamol (350mg/kg) (group- 2) results acute hepatotoxicity as can be observed in fig. 6b where it shows as necrotic patches and degenerative hepatocytes with mild inflammation and unremarkable portal tract. Pre-treatment of animals with alcoholic extract of *Millettia aboensis* (431mg/kg, group 6) resulted in heptoprotection, as in fig.6d Similar results were observed with pre-treatment of animals with aqueous extract at 431mg/kg (group 6) (**Fig. 6 e**)

CONCLUSIONS

The aqueous extract and alcoholic extracts of *Millettia aboensis* root have shown promising hepatoprotective activity on PCM- hepatotoxicity in rats at the administered dose of 215 mg/kg and 431 mg/kg body weight, orally. Both the extracts showed dose dependant activity. The hepatoprotective activity exhibited by *Millettia aboensis* root extracts may be due to their antioxidant activity, since the extracts have shown the presence of flavonoids which is an antioxidant.

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