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Research Article

Evaluation of the Hepatoprotective Effect of Aqueous and Ethanolic Root Extracts of *Millettia Aboensis* on Acetaminophen-Induced Hepatotoxicity in Experimental Rats

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ABSTRACT

The objective of the research was to study the hepatoprotective activity of ethanol and aqueous root extracts (EREMA and AREMA) of *Milletia aboensis* Hook F. (Fabaceae) against paracetamol-induced liver damage in rats. Preliminary phytochemical screening of the extracts (AREMA and EREMA) was carried out using standard procedures while oral acute toxicity was evaluated using a modified Lorke's method. Hepatotoxicity was induced by administration of paracetamol (350 mg/kg) and the biochemical parameters such as serum glutamate oxalate transaminase (SGOT/AST), serum glutamate pyruvate transaminase (SGPT/ALT), alkaline phosphatase (ALP), total and conjugated bilirubin and histopathological changes in liver were studied along with Liv-52®, a standard hepatoprotective agent. Alkaloids, glycosides, resins, steroids, flavonoids, terpenoids and saponins were detected in the phytochemical screening. LD_{50} of the extracts was determined to be 2,154 mg/kg. The extracts at a dose level of 215 mg/kg and 431 mg/kg produced significant ($P<0.05$) hepatoprotection by dose-dependently decreasing the levels of serum enzymes (for EREMA), total and conjugated bilirubin (for AREMA). Overall, while the hepatoprotective effect of AREMA was less than that of EREMA, at all dose levels, hepatoprotective effect of the latter (431 mg/kg) was comparable to the standard reference Liv-52® (1 mL/kg). The hepatoprotective effect was confirmed by histopathological examination of the liver tissue of control and treated animals. *Millettia aboensis* root conferred hepatoprotection against paracetamol-induced liver damage in rats and this activity was better in ethanol than aqueous extract.

INTRODUCTION

The physiological functions of the liver, including metabolism, detoxification and excretion of various endogenous and exogenous substances such as xenobiotics, result in the generation of highly reactive free radicals that induce oxidative tissue damage either by inducing lipid peroxidation or causing inflammation [1-3]. However, in-built protective mechanisms (i.e. in-built antioxidant systems) such as superoxide dismutase (SOD), tissue glutathione (GSH) etc or exogenous administration of antioxidant(s) may be useful in protecting the organs against the deleterious effects of the reactive oxygen species, which are important pathological mediators in clinical disorders such as heart disease, diabetes, gout and cancer [4-7].

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Liver diseases remain one of the serious health problems the world over. About 20, 000 deaths are found every year due to liver disorder [8]. Hepatic disease may be caused by drugs, persistent viral infections, metabolic diseases, autoimmune hepatitis, or due to unknown factors [9]. Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione depletion [10]. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses [11]. Despite overwhelming advances made in modern medicine, there is lack of satisfactory hepatoprotective drugs in orthodox medical practice for serious liver disorders [12]. Presently, only a few allopathic hepatoprotective drugs with significant action like silymarin and those from natural sources are available for the treatment of liver disorders [13, 14]. Scientific research in herbal medicine serves as an

alternative therapy in liver diseases [15]. Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver [16]. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in Nigeria [12]. Many plants have been evaluated for their hepatoprotective action in the light of modern medicine [16].

Millettia aboensis (Family Fabaceae), commonly called 'uturuekpa'in South Eastern Nigeria, is a small tree of about 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 [17]. The plant which is found commonly in low land rain forest, has conspicuously rusty-hairy leaves and beautiful purple flowers in erect terminal racemes at branches [18]. Almost all parts of the plant possess medicinal properties. The leaf is used by traditional herbalist for general healing including ulcer healing and as laxatives while the root is used in treating gastrointestinal disturbances and liver diseases. Also the leaf, stem and roots mixed with other plant materials (herbs) is used in Nigerian folkloric medicine to cure veneral diseases such as gonorrhoea, syphilis etc [17-20].

Liv 52®, a herbal medicine commonly used to treat liver ailments, was developed in mid 1950s by the Himalaya Herbal Healthcare group of India and it contains the following: Capers (*Capparis spinosa*, 65 mg), a hepatic stimulant and protector which improves the functional efficiency of the liver; Wild Chicory (*Cichorium intybus*, 65 mg), a powerful hepatic stimulant which increases bile secretion and acts on liver glycogen to promote digestion; Black Nightshade (*Solanum nigrum*, 32mg), which promotes liver and kidney health and has shown hepatoprotective activity in cases of toxicity induced by drugs and chemicals; Arjuna (*Terminalia arjua*, 32 mg), a tonic for heart and liver and regulates hepatic cholesterol biosynthesis; Negro Coffee (*Cassia occidentalis*, 16 mg), a digestive and hepatic tonic; Yarrow (*Achillea millefolium*, 16 mg), a stimulant tonic for liver; Tamarisk (*Tamarix gallica*, 16 mg), a hepatic stimulant which also provides digestive support [21]. It possesses both antioxidant and enzyme-regulating properties and also works as a cholesterol regulator. Liv-52® maintains the functional effectiveness of the liver by protecting the hepatic parenchyma. In many parts of the world, this wonder drug is used as a preventive measure for risks of contracting life-threatening liver diseases. Alcoholics take it as a vitamin to maintain a healthy liver amid load of toxins they take each day. Patients suffering from liver disorders also find Liv 52® useful in improving their health condition [21].

However, no work has been reported on the hepatoprotective properties of *Millettia aboensis*. With this in view, the present study was undertaken to investigate the hepatoprotective activity of the ethanolic and aqueous root extracts of *Millettia aboensis* (EREMA and AREMA) on paracetamol-induced liver damage in rats.

MATERIALS AND METHODS

Plant materials and preaparation of extracts

The roots of *Millettia aboensis* were harvested from Ehandiagu in June, 2010, in Nsukka Local Governement Area of Enugu State, Nigeria. Their botanical identities were determined and authenticated by Mr. A. Ozioko, a taxonomist with the International Centre for Ethnomedicine and Drug Development (INTERCEDD), Nsukka. The voucher specimen was deposited at the centre for future references. The root of *Millettia aboensis* was shade-dried and pulverized to coarse powder using an electrically operated mill. A 0.5 kg of shade-dried pulverized roots was extracted with 2 L of ethanol (90 %v/v) using a soxhlet apparatus and concentrated under vacuum. 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 concentrated under vacuum.

Determination of yield of the extracts

The percentage yield of the extracts (AREMA and EREMA) was determined by weighing the coarse plant part before extraction and after concentration and calculated using the formula below.

$$Yield\ (\%) = \frac{W_E}{W_P} \times 100 \quad \ldots\ldots\ldots\ldots (1)$$

Where W_E = Weight of extract (g),
W_P = Weight of drug powder (g).

Preliminary phytochemical analysis

Standard procedures [19, 22] were followed in the tests for the phytochemical constituents of AREMA and EREMA. The following constituents

were tested for: alkaloids, resins, reducing sugars, acidic compounds, fats and oil, steroids, terpenoids, glycosides, flavonoids, tannins, carbohydrates, saponins, proteins and amino acids.

Experimental Animals

Wistar albino rats of either sex weighing between 66 g and 153 g and mice weighing between 25 g and 30 g procured from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used. They were housed in well ventilated stainless-steel cages at room temperature (25 ^{0}C) in hygienic conditions under natural light and dark schedule and were fed on standard laboratory diet. Food and water were given ad libitium. Permission for the use of animal and animal protocol was obtained from the Ethics Committee of the University of Nigeria, Nsukka. The animal studies were carried out in accordance with Ethical Guidelines of Animal Care and Use Committee (Research Ethics Committee) of University of Nigeria, Nsukka, following the 18th WMA General Assembly Helsinki, June 1964 and updated by the 59th WMA General Assembly, Seoul, October 2008.

Drugs and Chemicals

Paracetamol was obtained as gift sample from JUHEL Pharmaceutical Company Limited, Awka, Anambra State, Nigeria. Liv-52® was procured from the Himalaya Herbal Healthcare group of India. SGOT, SGPT, ALP, Total Bilirubin and Conjugated Bilirubin kits were procured from Span Diagnostics, Surat, India. 5, 5'- dithiobis-2-nitrobenzoic acid (DTNB) was obtained from Sisco research laboratories, Mumbai, India. All other reagents used for the experiments were of high analytical grade.

Acute Toxicity Studies

The safety of the extracts (AREMA and EREMA) to the mice was evaluated by determining the oral acute toxicity (LD_{50}) using a modified Lorke's method [23]. The treatment and control animals were weighed and marked appropriately. The acute toxicity test of the plant extracts was tested on mice using six doses (10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight) administered orally for each plant extract. The study was carried out in two phases. In the phase one of the study, nine mice were randomized into three groups of three mice each and were given 10, 100 and 1000 mg/kg b. wt. of each of the extract orally. The mice were observed for paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in the first 4 h and subsequently daily for 7 days. In the second phase of the study, another fresh set of nine mice were randomized into three groups of three mice each and were given 1600, 2900 and 5000 mg/kg b. wt. of each of the extract orally based on the result of the first phase. These were observed for signs of toxicity and mortality for the first critical 4 h and thereafter daily for 7 days. The LD_{50} was then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose, i.e. the geometric mean of the consecutive doses for which 0 and 100 % survival rates were recorded in the second phase. The oral median lethal dose was calculated using the formula:

LD_{50} = √Minimum toxic dose x Maximum tolerated dose …….. (2)

Experimental Design for Hepatoprotective Activity (Acute Model)

The hepatoprotective activity of the extracts of the plant (AREMA and EREMA) was tested using paracetamol-induced hepatotoxicity model. Liv-52® (1.0 mL/kg) was used as a reference standard drug in this study [11]. Liv-52®, a herbal medicine commonly used to treat liver ailments, was developed in the mid 1950s by the Himalaya Herbal Healthcare group of India, and has been introduced fairly recently as a hepatoprotective agent [21]. The rats were divided randomly into seven groups of six animals per group according to the following protocol.

Treatment Protocol

GROUP I: Normal control (n=6, the animals were given 1 mL/kg distilled water only).

GROUP II: Hepatotoxic control (n=6, the animals were given 350 mg/kg paracetamol).

GROUP III: Treatment control (n=6, the animals were given 350 mg/kg paracetamol and 1 mL/kg Liv-52®)

GROUP IV: Treatment group (n=6, the animals were given 350 mg/kg paracetamol and 215 mg/kg of aqueous root extract of *Millettia aboensis*, AREMA).

GROUP V: Treatment group (n=6, the animals were given 350 mg/kg paracetamol and 215 mg/kg of ethanol root extract of *Millettia aboensis*, EREMA).

GROUP VI: Treatment group (n=6, the animals were given 350 mg/kg paracetamol and 431 mg/kg of aqueous root extract of *Millettia aboensis*, AREMA).

GROUP VII: Treatment group (n=6, the animals were given 350 mg/kg paracetamol and 431 mg/kg of ethanol root extract of *Millettia aboensis*, EREMA).

Rats were treated as per the treatment protocol for a period of nine days. The drugs were given orally. Food was withdrawn 12 h before parcetamol administration to enhance the acute liver damage in the animals, which were sacrificed 48 h after the administration of paracetamol.

Assessment of Hepatoprotective Activity

The hepatoprotective activity of the extracts (AREMA and EREMA) was determined biochemically by using parecetamol-induced hepatotoxic rat model [16]. After nine days of drug treatment, the animals were sacrificed under ether anesthesia and dissected. Blood from each rat was withdrawn from carotid artery at the neck and collected in previously labeled falcon tubes and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2,500 rpm at 30 ^{o}C for 15 min and utilized for the estimation of some biochemical parameters namely alanine aminotransferase (ALT/ SGPT), aspartate aminotransferase (AST/SGOT), alkaline phosphatase (ALP), total bilirubin and conjugated bilirubin using standard kits [16, 24-27].

Serum Hepatospecific Markers

Activities of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by the method of Reitman and Frankel [24]. Briefly, 0.05 ml of serum with 0.25 ml of substrate (aspartate and α-ketoglutarate for SGOT; alanine and α - keto glutarate for SGPT, in phosphate buffer, pH 7.4) was incubated for 1 h in case of SGOT and 30 min for SGPT. A 0.25 ml of DNPH solution was added to quench or stop the reaction and kept for 20 min in room temperature. After incubation, 1 ml of 0.4 N NaOH was added and the absorbance was read at 505 nm in a UV-Vis spectrophotometer (UNICO 2102 PC UV-Vis Spectrophotometer, USA). Activities were expressed as IU/L. Based on the method of King and Armstrong [25] alkaline phosphatase activity was assayed using disodium phenyl phosphate as substrate. The colour developed was read at 510 nm in a UV-Vis spectrophotometer (UNICO 2102 PC UV-Vis Spectrophotometer, USA) after 10 min. Activities of ALP was expressed as IU/L. Serum total bilirubin level was estimated based on the method of Malloy and Evelyn [26]. Diazotised sulphonilic acid (0.25 ml) reacts with bilirubin in diluted serum (0.1 ml serum + 0.9 ml distilled water) and forms purple coloured azobilirubin, which was measured at 540 nm in a UV-Vis spectrophotometer (UNICO 2102 PC UV-Vis Spectrophotometer, USA). Activities of total bilirubin were expressed as mg/dL. The level of conjugated bilirubin was estimated based on the method of Gornall *et al* [27]. Biuret reagent (1.0 ml) reacts with serum (10 µL) and the colour developed was read at 578 nm in UV-vis spectrophotometer. Activities of conjugated bilirubin were expressed as mg/dL.

Histopathological Studies

One animal from each group was utilized for this purpose. Small pieces of liver tissues in each group were collected in 10 % neutral buffered formalin for proper fixation for 24 h. These tissues were processed and embedded in paraffin wax. Sections of 5- 6 µm in thickness were cut and stained with hematoxylin and eosin (H&E). These sections were examined photomicroscopically for necrosis, steatosis and fatty changes of hepatic cells [16, 11].

Statistical Analysis

The experimental results were expressed as the Mean ± SEM for six animals in each group. Differences between means were determined statistically using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test (DMCT). P value of < 0.05 was considered as statistically significant**.**

RESULTS AND DISCUSSION

Extraction yields (w/w) were 49.0 % and 45.93 % for AREMA and EREMA respectively in terms of dry weight. Thus AREMA had higher yield than EREMA. Preliminary phytochemical tests of the extracts of *Millettia aboensis* revealed the presence of the phytoconsituents presented in Table 1. It could be seen from Table 1 that AREMA contained high amount of saponins, resins, proteins, amino acids and carbohydrate while EREMA had high amount of flavonoids, alkaloids, steroids, and terpenoids. The extracts had equal amount of glycosides and reducing sugar, but tannins, acidic compounds and fats and oil were absent in the extracts.

The LD_{50} of AREMA and EREMA was calculated as 2,154 mg/kg. The extracts were found to be safe in the dose used and there was no mortality up to a dose of 2,154 mg/kg, b.w. for both extracts. Hence, 1/5th and 1/10th of the lethal dose i.e. 215 and 431 mg/kg b.w. p.o. of both extracts were selected for further evaluation. Behavioural signs of toxicity observed in mice given 5000 mg/kg and above include drowsiness, weakness, reduced activity in treated mice and paw licking. The necropsy and autopsy were performed and signs of toxicity included diarrhea and congested lungs with focal areas of necrosis.

Table 1: Phytochemical constituents of EREMA and AREMA.

Constituent	Observation	
	EREMA	***AREMA***
Alkaloids	***+++***	+
Resins	-	+++
Reducing sugars	+	*+*
Acidic compounds	**-**	-
Fats and Oil	-	-
Steroids	***++***	*+*
Terpenoids	***++***	*+*
Glycosides	***+++***	*+++*
Flavonoids	***+++***	*+*
Tannins	**-**	-
Carbohydrates	*++*	*+++*
Saponins	+	*++++*
Proteins and amino acids	+	+++

KEYS:+++ = relatively high abundance of compound,++ = moderate abundance of compound,+ = relative low presence of compound, ND = not detected, EREMA = ethanolic root extract of Millettia aboensis, AREMA = alcoholic root extract of Millettia aboensis

The effects of the various extracts of *Millettia aboensis* on the levels of hepato-specific serum markers in paracetamol-induced liver damage in rats are summarized in Table 2. Administration of paracetamol (350 mg/kg, p.o.) induced a marked increase in the serum hepatic enzyme levels, ALT, AST, ALP, total bilirubin and conjugated bilirubin as compared to normal control indicating liver damage (centrilobular necrosis). Pretreatment with EREMA and AREMA (215 mg/kg and 431 mg/kg) and Liv 52® significantly ($P<0.05$)) reduced the level of AST, ALT, ALP and serum total and conjugated bilirubin after intoxication with paracetamol (Table 2). AREMA and EREMA exhibited dose-dependent liver protective activity. Comparative analysis of the effect of various extracts on ALT, AST and ALP levels revealed that AREMA and EREMA at dose level 431 mg/kg showed protection against the hepatoxins. At all dose levels EREMA caused greater decrease in AST, ALT and ALP when compared to AREMA. Conversely, at all dose levels, AREMA had greater decrease in total bilirubin and conjugated bilirubin when compared to EREMA. The level of conjugated bilirubin in EREMA treated rats at a dose level of 431 mg/kg was comparable with that of the control (Liv-52®).

The hepatoprotective effect of AREMA and EREMA was confirmed by histopathological examination of the liver tissue of control and treated animals. Histopathological results indicate that histological architecture of paracetamol-treated liver sections showed severe congestion of blood vessels, mild hydropic degeneration, pyknosis of nucleus and occasional necrosis. Comparing the normal liver with that exposed to paracetamol-induced hepatotoxicity clearly showed degeneration of hepatocytes, necrotic areas and non visible portal tract resulting from the hepatokin in the latter (Group II). Animals treated with AREMA and EREMA showed mild hydropic degeneration but absence of pyknosis or congestion. Animals treated with a vehicle control (Group I) showed normal hepatic structure and visible portal tract. Treatment of animals with paracetamol (350 mg/kg) resulted in acute hepatotoxicity as detected by necrotic patches and degenerative hepatocytes with mild inflammation and unremarkable portal tract. Pre-treatment of animals with EREMA at dose level 431 mg/kg (Group VII) resulted in hepatoprotection as was pre-treatment of animals with AREMA at dose level 431 mg/kg (Group VI).

In living systems, liver is considered to be highly sensitive to toxic agents, including paracetamol (acetominophen), a commonly and widely used analgesic and antipyretic agent. A part of paracetamol is metabolized by hepatic cytochrome P_{450} co-enzyme system to a minor yet significant alkylating metabolite known as N-acetyl-p-benzo quinoneimine (NAPQI), which covalently binds irreversibly to cysteine groups on proteins to form 3-(cystein-S-yl) acetaminophen adducts [28]. At normal levels of hepatic glutathione, glutathione protects hepatocytes by combining with the reactive metabolite of paracetamol thus preventing their covalent binding to liver proteins [29].

Table 2: Effect of EREMA, AREMA and Liv-52® on serum enzymes, total bilirubin and conjugated bilirubin on paracetamol induced liver damage in rats.

Groups and treatment	Dose	SGOT (IU/L)	SGTP (IU/L)	SALP (IU/L)	Total Bilirubin (mg/dL)	Conjugated Bilirubin (mg/dL)
Group I-Normal (Distilled water)	1 mL/kg	50.00 ± 2.03	25.17 ± 0.99	110.78 ± 4.65	17.92 ± 0.87	7.24 ± 0.31
Group II-paracetamol control	350 mg/kg	70.24 ± 1.92	55.71 ± 1.39	200.48 ± 4.75	24.85 ± 1.26	13.51 ± 0.73
Group III-LIV-52® + paracetamol	1 mL/kg + 350 mg/kg	65.48 ± 2.76	40.15 ± 1.58	120.92 ± 3.57	15.43 ± 1.24	6.22 ± 0.91
Group IV-AREMA + paracetamol	215 mg/kg + 350 mg/kg	68.05 ± 2.83	42.16 ±1.05	175.73± 4.81	18.25 ± 1.46	9.73 ± 0.17
Group V-EREMA + paracetamol	215 mg/kg + 350 mg/kg	60.48 ±1.67	35.95 ±1.81	160.19 ± 3.56	16.41 ± 1.97	8.87 ± 0.93
Group VI-AREMA + paracetamol	431 mg/kg + 350 mg/kg	57.69 ± 2.61	40.92 ±1.55	100.75 ±3.53	16.93 ±1.62	7.13 ± 0.74
Group VII-EREMA + paracetamol	431 mg/kg + 350 mg/kg	51.34 ±2.56	28.10 ±1.62	100.97 ± 2.86	14.75 ± 1.17	6.59 ±0.92

Values are Mean ± SEM; n = 6 animals in each group; EREMA = ethanolic extract of Millettia aboensis, AREMA = alcoholic extract of Millettia aboensis.

Hepatotoxic doses of acetominophen deplete glutathione [10]. Thus, paracetamol hepatotoxicity is caused by NAPQI, which causes oxidative stress and glutathione depletion. Depletion of glutathione initiates covalent binding to cellular proteins leading to the disruption of calcium homeostasis, mitochondrial dysfunction, and oxidative stress and may eventually culminate in cellular damage and death [11]. In this study, paracetamol was employed as an experimental hepatotoxic agent and the potential hepatoprotective activity of *Millettia aboensis* ethanolic and aqueous root extracts against hepatic injury produced by paracetamol in rats, was evaluated. The extent of toxicity was estimated by histopathological studies and biochemical enzyme markers like SGOT, SGPT, SALP, total bilirubin and conjugated bilirubin levels. The study of different enzyme activities such as SGOT, SGPT, SALP, total bilirubin and conjugated bilirubin have been found to be of great value in the assessment of clinical and experimental liver damage [30]. In the present investigation, it is obvious that the animals treated with acetaminophen (Group II) showed significant liver damage as shown by the increased levels of serum biomarker enzymes. These changes in the marker levels will reflect in hepatic structural integrity. The rise in the SGOT is usually accompanied by an elevation in the levels of SGPT, which plays a vital role in the conversion of amino acids to keto acids [16].

Millettia aboensis treated animals (Groups IV to VII) showed a protection against the injurious effects of paracetamol resulting in the hindrance of formation of hepatotoxic free radicals. In other words, damage induced in the liver by paracetamol, a hepatotoxin, was accompanied by increase in the activity of the serum biomarker enzymes. Pretreatment with AREMA and EREMA especially with 431mg/kg and 215mg/kg, significantly attenuated the elevated levels of the serum markers. The normalization of serum markers by these extracts of *Millettia aboensis* suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against acetaminophen-induced leakage of marker enzymes into the circulation. AREMA and EREMA exhibited dose-dependent hepatoprotective activity as was evident from the high tendency with which the serum biomarker enzymes returned to near normal in the group treated with higher dose of the extracts (Groups VI and VII). The effect produced by EREMA (421 mg/kg) was almost similar to that produced by Liv-52® (1ml/kg), a standard herbal hepatoprotective agent [11, 21]. Comparatively, EREMA caused greater decrease in biomarker enzymes than the AREMA, which caused greater decrease in total bilirubin and conjugated bilirubin than the former. The level of conjugated bilirubin in EREMA treated rats at 431 mg/kg was comparable to that of the control (Liv-52®). The above changes can be considered

as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells. Serum ALP and bilirubin levels, on the other hand, are related to hepatic cell damage. Increase in serum level of ALP is due to increased synthesis in presence of increasing billiary pressure [31]. Effective control of bilirubin level and alkaline phosphatase activity points towards an early improvement in the secretory mechanism of the hepatic cell [16]. Drugs having antioxidant activity are effective in treating paracetamol induced hepatotoxicity by scavenging the free radicals produced by paracetamol metabolism, thereby preventing liver damage induced by both paracetamol metabolite and that due to depletion of glutathione. Previous reports indicate that *Millettia aboensis* root could be used in Nigerian folkloric medicine as an antioxidant and a hepatoprotective [17-20] and this activity may be responsible for its effect in paracetamol-induced hepatotoxic model. Paracetamol induced a significant increase in liver weight, which was due to the blocking of secretion of hepatic triglycerides into the plasma. EREMA and AREMA prevented the increase in liver weight of rats pretreated with paracetamol.

The histopathological observations of paracetamol-treated rats showed severe congestion of blood vessels, mild hydropic degeneration, pyknosis of nucleus and occasional necrosis. This may be due to the formation of highly reactive radicals because of oxidative threat caused by paracetamol [16]. All these changes were very much reduced histopathologically in rats treated with AREMA and EREMA. Based on the above results, it could be concluded that AREMA and EREMA exerts significant hepatoprotection against paracetamol-induced toxicity. Moreover, Liv-52® maintained the normal architecture with minimal injuries and similar protection to higher dose of EREMA alone.

Phytochemistry of the extracts showed that AREMA contains high amounts of saponins, resins, proteins, amino acid and carbohydrates while EREMA had high amounts of flavonoids, alkaloids, steroids and terpenoids. Both extracts had equal amounts of glycosides and reducing sugars. Reports have shown that flavonoids, alkaloids and steroids might be responsible for hepatoprotective effect of the plant extracts [32-34]. Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage [35, 36]. Perhaps the hepatoprotective effect of the extracts could be attributed to high amounts of these phytoconstituents present in the extracts.

The results of acute toxicity test indicated that the LD_{50} of the extracts was 2,154 mg/kg. This implies that doses below this could be safe while doses above it could be detrimental to the human system [23]. Hence, 1/5th and 1/10th of the lethal dose i.e. 215 and 431 mg/kg b.w. p.o. of both extracts were selected for the hepatotoxicity test (acute model). The acute toxicity results indicated that the extracts were practically non-toxic acutely [23] and thus provided a guide in the choice of doses for further *in vivo* studies. This high safety profile might have contributed to the widespread use of *Millettia aboensis* in different ethno-therapeutic interventions, particularly as a hepatoprotective.

CONCLUSIONS

Results of this study have shown that *Millettia aboensis* is a potential hepatoprotective against paracetamol-induced hepatic damage in rats. AREMA and EREMA at the administered dose of 215 mg/kg and 431 mg/kg body weight could be useful in the attenuation of paracetamol-induced lipid peroxidation. Although the hepatoprotection offered by the extracts were concentration-dependent, EREMA produced greater effect than AREMA. Higher dose of EREMA (431 mg/kg) exhibited hepatoprotective effect which was comparable to the reference standard drug, Liv-52®. Liver protective activity of EREMA may be due to the presence of high amount of flavonoids, an antioxidant, in the extract. The present study thus justifies the traditional use of *Millettia aboensis* root in the treatment of liver disorders in Nigerian folkloric medicine. Further studies are underway to isolate phytoconstituent(s) in the extracts responsible for the observed hepatoprotective effect and deterimine the mechanism(s) of action.

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