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RESEARCH ARTICLE

Evaluation of the hepatoprotective activity of root extracts of *Millettia aboensis* on carbon tetrachloride [CCl₄] induced hepatotoxicity in rats

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Abstract

Purpose: The study was conducted to evaluate the hepatoprotective activity of the *Millettia aboensis* root extracts on carbon tetrachloride [CCl₄] induced hepatotoxicity in rats.

Method: Roots of *Millettia aboensis* were collected, shade dried pulverized and the different batches were 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 the CCl₄ (0.7 ml/kg) 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. Livers were weighed and subjected to histopathological studies. Similar studies were carried out with normal rats.

Result: Alcoholic and aqueous extracts at doses 431mg/kg body weight P.O and LIV-52^(R) (1 ml/kg P.O) produced reduction in the marker enzymes (AST, ALT, ALP and serum bilirubins).

Conclusion: Both alcoholic and aqueous extracts of *Millettia aboensis* has shown significant ($p < 0.05$) hepatoprotective activity in carbon tetrachloride induced hepatotoxicity and alcohol extract is found to be more effective than the aqueous extract.

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Introduction

Environmental pollutants affect our liver. It is therefore necessary we think what we can do to help the liver. The first thing we must do is to clean up our environment. In the meantime, the herbs and the natural medicinal plants are helpful. With the ever increasing cost of orthodox health care services, and with incidence of fake drugs and side effect of modern drug therapies, many patients seem to be more interested in the alternative herbal health care which they feel is safer, more accessible, more economical and which takes into consideration the people's socio-cultural values. *Millettia aboensis* (uturuekpa) are 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⁽¹⁾ and is mainly tropical and sub tropical family of tree, whose leaves are stipulate, nearly always alternate, and range from bipinnately or palmately compound to simple. In traditional setting, *Millettia aboensis* have many medicinal properties. The leaf is used by traditional herbalist for general healing including ulcer healing and laxatives while the root is used in treating gastro- intestinal disturbances and liver diseases. The phytochemical screening of both water and ethanolic extracts of *Millettia aboensis* roots show that the aqueous extract contained high amount of saponins, resins, proteins and amino acid and carbohydrate while ethanolic extract has high amount of flavonoids, alkaloids, steroids, and terpenoids⁽¹⁾.

The liver being the largest organ in the body plays a central role in transforming and clearing chemicals from the body. Therefore, it is susceptible to the toxicity from these agents. Certain medicinal agents when taken in overdoses and sometimes, even when introduced within therapeutic ranges may injure the liver. Chemical agents such as those used in laboratories and industries, natural chemicals (e.g. microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failure^[2]. The human body identifies almost all drugs as foreign substances (i.e. xenobiotics) and subjects them to various chemical processes (i.e. metabolism) to make them change biological activity. Although almost all tissue in the body have some ability to metabolize chemicals, smooth endoplasmic reticulum in liver is the principal metabolic clearing house" for both endogenous chemicals (e.g. cholesterol, steroid hormones, fatty acids, and proteins), and exogenous substance (e.g. drugs)^[3].

Carbon tetrachloride is the organic compound with the formula CCl₄. It was formerly widely used in fire extinguishers, as a precursor to refrigerants, and as very effective solvent and cleaner (cleaning agent), but unfortunately it may be quite toxic. Deaths have occurred due to accidental exposure, in the home and in the factory. It is a colourless liquid with a "sweet" smell that can be detected at low levels. Exposure to high concentrations of carbon tetrachloride (including vapor) can affect the central nervous system, degenerate the liver^[4] and kidneys^[5] and may result (after prolonged exposure) in coma and even death.^[6] Chronic exposure to carbon tetrachloride can cause liver^[7] and kidney damage and could result in cancer.^[7] The aim of this study is to evaluate the hepatoprotective activity of *Millettia aboensis* root extracts on tetrachloromethane 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.

Chemicals; The chemicals used in this study were of analytical grade products of BDH, England and Sigma Aldrich, Germany.

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 24 h maceration, then filtered and dried (yield 49.02% w/w).

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

Acute oral toxicity test: This was performed according to modified Dietrich Lorke method^[8].

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 \pm SEM and values of $P \leq 0.05$ were considered significant.

RESULTS

Acute oral toxicity study-

Acute oral toxicity was performed according to Modified Dietrich Lorke Method. Here an initial investigation involving 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.

Table 2: 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
Ethanollic extract	0/3	0/3	0/3

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 3**.

Table 3: 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 LD₅₀ was calculated as 2,154mg/kg, using the Dietrich lorke's method for calculation of LD₅₀.

Table 4: Administration of the drugs to the groups

GROUPS	TREATMENT
1	Treated with distilled water (1ml/kg per oral (PO)).
2	Treated with distilled water for nine days + CCl ₄ (0.7ml/kg IP) administered on the ninth day.
3	Treated with LIV 52 ^(R) 1ml/kg PO for nine days + CCl ₄ (0.7ml/kg IP) administered on the ninth day.
4	Treated with alcoholic extract of <i>Millettia aboensis</i> (215mg/kg PO) for nine days + CCl ₄ (0.7ml/kg IP) administered on the ninth day.
5	Treated with aqueous extract of <i>Millettia aboensis</i> (215mg/kg PO) for nine days + CCl ₄ (0.7ml/kg IP) administered on the ninth day.
6	Treated with alcoholic extract of <i>Millettia aboensis</i> (431mg/kg PO) for nine days + CCl ₄ (0.7ml/kg IP) administered on the ninth day.
7	Treated with aqueous extract of <i>Millettia aboensis</i> (431mg/kg PO) for nine days + CCl ₄ (0.7ml/kg IP) administered on the ninth day.

Food was withdrawn 12 h before carbon tetrachloride administration to enhance the acute liver damage in animals of groups 2,3, 4,5, 6, and 7 with CCl₄. The animals were sacrificed 24 h after the administration of CCl₄. Blood samples were collected and the serum assayed for marker enzymes such as aspartate aminotransferase (AST)^[9], alanine aminotransferase (ALT)^[9], alkaline phosphatase (ALP)^[10] and the liver immediately isolated and washed with normal saline, blotted with filter paper and weighed. The liver was then subjected to Histopathological examination

Carbon tetrachloride induced acute toxicity –

A significant difference in biochemical markers was observed between normal and CCl₄ 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)

Evaluation of hepatoprotective activity.

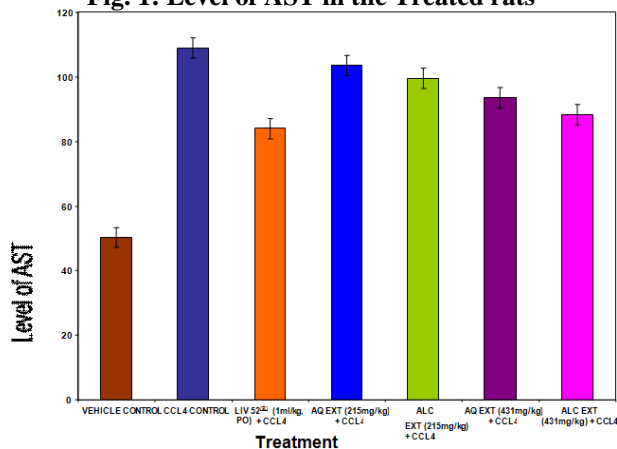
Sixty-five healthy, albino rats of either sex housed under standard conditions and fed with standard rodent diet with water were used and their livers were damaged using carbon tetrachloride. The CCl₄ was diluted with liquid paraffin (1:1) before administration. The rats were divided into 7 groups consisting of 5 rats per group. The animals were then subjected to either one of the following treatments for 9 days.

of *Millettia aboensis* showed protection against the hepatotoxins.

Liver enzyme analysis:

Fig 1 and 4 show the levels of AST, ALT, ALP, Total Billirubin and Conjugated bilirubin in CCl₄ induced hepatotoxicity.

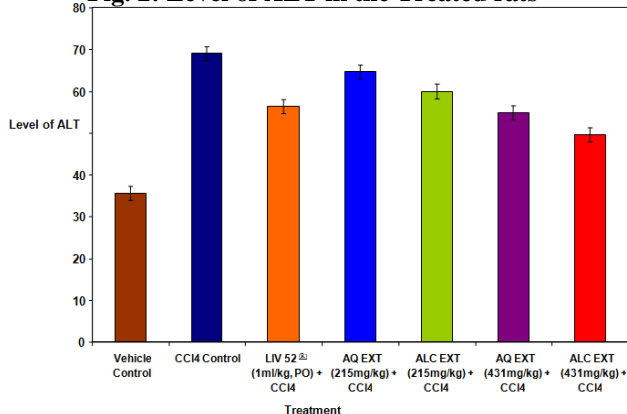
The effects of ethanolic and aqueous extracts of *Millettia aboensis* on CCl₄ induced hepatic damage in rats (mean ± SEM)

Fig. 1: Level of AST in the Treated rats

The liver is damaged by CCl_4 which induced oxidative stress. This is exhibited by the increase levels of the liver enzymes. The increased AST and other liver function enzymes are used for the indication of liver diseases. Rats that were given hepatoprotective drugs have decrease AST level when exposed to CCl_4 or intoxicant. The test drug mediated reduction in level of AST towards the normal values which is an indication of stabilization.

The effects of ethanolic and aqueous extracts of *Millettia aboensis* on the level of ALT (U/L) in CCl_4 treated rats

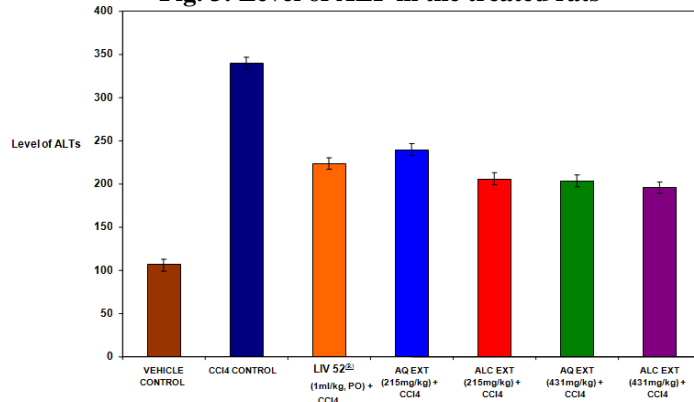
Fig. 2: Level of ALT in the Treated rats



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 CCl_4 alone. The observed effects were dose dependent and are significant at $p \leq 0.05$ with respect to negative controls (CCl_4). This shows that ethanolic and aqueous extracts of *Millettia aboensis* had hepatoprotective effect.

The effects of ethanolic and aqueous extracts of *Millettia aboensis* on the level of ALP (U/L) in CCl_4 treated rats

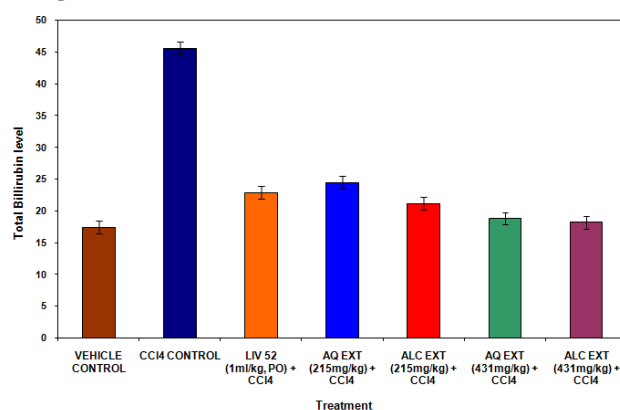
Fig. 3: Level of ALP in the treated rats



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 CCl_4 alone as shown in fig.7. The observed effects were dose dependent and are significant at $p \leq 0.05$ with respect to negative controls (CCl_4).

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

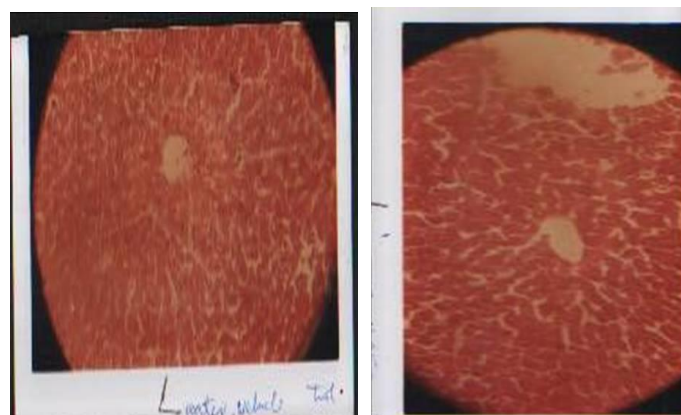
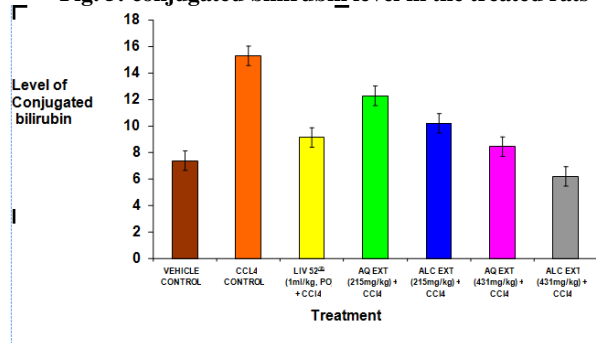
Fig 4: Total Billirubin Level in the treated rats



The results of the effect of ethanolic and aqueous extracts of *Millettia aboensis* root on the level of total bilirubin (mg/dl) in CCl_4 treated rats as shown in fig 8. 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 aqueous extract. However the total bilirubin in rats treated with CCl_4 alone was significantly higher in rats which receive extracts, Liv 52^(R) and vehicle treated rats.

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 CCl_4 in alcoholic and aqueous treated rats. CCl_4 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 bilirubin level in the treated rats

c)

d)

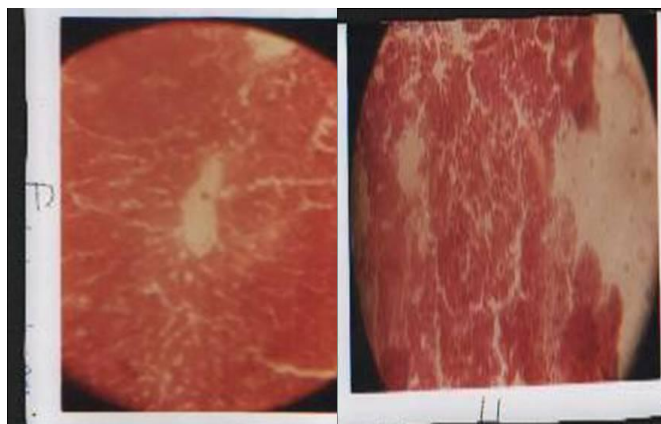
The extracts showed dose dependent activity. Alcoholic and aqueous extract at higher doses i.e. 431mg/kg body weight P.O and LIV-52^(R) (1 ml/kg P.O) produced a significant reduction ($p < 0.05$) in the marker enzymes (AST, ALT, ALP and serum bilirubin). Lower dose of both extracts 215mg/kg P.O also showed significant reduction in marker enzyme levels but the effect was less compared to the higher dose.

Histopathological examination

Administration of CCl₄ produced moderately significant increase in the liver weights. Higher doses of alcoholic and aqueous extract, LIV-52^(R), and lower dose of alcoholic extract produced significant decrease in the liver weights.



e)

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

a)

b)

The rats treated with vehicle control group-1 (Fig. 6a) show normal hepatic structure and visible portal tract. Histopathological examination of the liver tissues from CCl₄ intoxicated animals showed profound inflammation and congestion especially in the sinusoids. Hydropic degeneration and steatosis in the periportal region was also observed (Fig.6b). Pretreatment of animals with LIV-52^(R) (Fig.6c), 431mg/kg alcoholic extract of *Millettia aboensis* (Fig.6d) and 431mg/kg aqueous extract of *Millettia aboensis* (Fig.6e) showed reduction in inflammation of the skin and significantly prevented degeneration of hepatocytes .

DISCUSSION

Phytochemical screening of *Millettia aboensis* ethanolic extract has revealed the presence of high amount of flavonoids, alkaloids, steroids, and terpenoids¹. Reports have indicated that flavonoids contribute to the hepatoprotective effects of plant extracts. The increase in level of the liver biomarker

enzymes (AST, ALT, ALP, total bilirubin and conjugated bilirubin) observed in groups treated with CCl_4 indicate some extent of liver damage. From the result it is well established that CCl_4 induces hepatotoxicity by metabolic activation. Therefore it selectively causes toxicity in liver cells [5]. *Millettia aboensis* treated animals showed a protection against the injurious effects of CCl_4 free radicals. The tendency of these marker enzymes to return to near normal in group treated with higher dose of the extracts was a clear suggestion that the extracts have an anti hepatotoxic effects. The flavonoids and possibly, other phytoconstituents present in the plant studied could be responsible for the hepatoprotection shown, evident in the serum enzymes lowering. 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. The evaluation of the 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 5000mg/ kg.

CONCLUSIONS

The aqueous extract and alcoholic extract of *Millettia aboensis* roots have shown promising hepatoprotective activity. Both the extracts showed dose dependant activity. Higher dose of alcoholic extract (431 mg/kg) has shown the protective activity that is comparable to the reference drug LIV-52. Therefore, our results support the claims of the traditional herbalists that the *Millettia aboensis* root extracts have hepatoprotective effects.

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