

Evaluation of the hepatoprotective effect of the methanol extract of the root of *Uvaria afzelii* (Annonaceae)

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ABSTRACT

The claimed hepatoprotective effect of the root of *Uvaria afzelii* Sc. Elliot (Annonaceae) was investigated using the Carbon tetrachloride induced hepatotoxicity in albino rats as a template. The methanolic extract of *U. afzelii* co-comittantly administered with carbon tetrachloride at doses of 125, 250 and 500mg/kg was found to significantly ($p < 0.05$) reduce the levels of alanine aminotransferase (AST), aspartate transaminase (AST), alkaline phosphatase (ALP), total and un-conjugated bilirubin, while levels of total protein and albumin were significantly increased in a non dose-dependent pattern, compared to animals treated with carbon tetrachloride alone, where the activity of ALT, AST, ALT, total and un-conjugated bilirubin increased and that of total protein and serum albumin decreased. Histopathological examinations supported the biochemical results. This study conclusively provides scientific validation for the use of extract of the root of *U. afzelii* in ethnomedicine to manage jaundice and liver injury.

INTRODUCTION

Uvaria afzelii Sc Elliot (Annonaceae) is widely distributed and grown in the south and eastern part of Nigeria, where it is known by various local names such as “gbogbonishe” (Yoruba), “Umimi ofia” (Igbo) and “Osu-umimi” (Ukwani) (Odugbemi, 2008). Locally it is used in the treatment of cough, vaginal tumour, breast aches, swollen hands and feets, diabetes as well as leucorrhoea and gonorrhoea (Verger, 1995; Kayode *et al.*, 2009).

Other ethnomedicinal uses of the plant include its benefit as a remedy for jaundice, infections of the liver, kidney and bladder (Kerharo and Bouquet, 1950; Bouquet and Debray, 1974; Gills, 1992). A number of investigations carried out to ascertain the claimed uses of the plant include its reported bacteriocidal activity against gram positive and acid fast bacteria (Okoli, 2004; Lawal *et al.*, 2011), anti-helminthic and anti-parasitic activities (Okpekon, 2004).

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A number of compounds have been isolated from the plant which include; Syncarpic acid, Dimethoxymatteucinol, Emorydone, 2-hydroxydemethoxy matteucinol, Uvafzelic acid, syncarpurea and Afzeliindanone (Hufford *et al.*, 1981; Hufford *et al.*, 1984; Okpekon *et al.*, 2009). Some of these compounds have been credited with specific biological activities e.g. Afzeliindanone and Emorydone are reported to possess potent activity against *trypanosomiasis brucei*. A steady increase in cases of liver diseases has been reported (Mushawar, 2004).

This has been attributed to a number of reasons, some of which include wide spread distribution of the hepatitis B and C viral infections, increased alcohol consumption as a result of rising affluence, primary hepatocellular carcinomas, amongst others (Williams, 2006). Medicinal plant and their derivatives continue to be a source of natural remedy for the treatment of liver disease and other infections. This study was aimed at evaluating the claimed liver protective effect of the root of *U.afzelii* against acute liver injury induced by carbon tetrachloride in rats as there is no report to support its use in the management of jaundice and liver injury in ethnomedicine.

MATERIALS AND METHODS

Plant Materials

The root of *U. afzelii* was bought from a local herb seller in Lagos Street in Benin City in July 2012 and it was identified by Mr. Sunny Nweke of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, authentication was carried out at the Forestry Research Institute of Nigeria (FRIN) Ibadan by Dr Olufemi Shasanya, where a voucher specimen was deposited and the number FHI-170839 was issued.

Preparation of plant and extraction

The plant material was dried in the oven (40°C) and milled with the aid of an attrition mill to obtain the powder. 500g of the powdered plant material was macerated with 2L of methanol for 72hrs, filtered, and the filtrate concentrated in vacuo (40°C) to obtain a dark brown mass. The yield was 56.30g (11.26%) and this was stored in the refrigerator (4°C) till needed for use. The methanolic extract was subjected to test for toxicity, photochemical evaluation and hepatoprotective activity.

Phytochemical evaluation

Qualitative screening of the extract was carried using methods of Harborne (1984) and Trease and Evans (2002) for alkaloids, flavonoids, saponins, tannins, polyphenols, reducing compounds, anthracenes, glycosides, terpenes and sterols.

Animals

Albino rats (*Rattus norvegicus*) of both sexes (180 – 220g) were obtained from the Pharmacology Department of the Faculty of Pharmacy University of Benin and maintained in the animal house of same department under standard housing conditions. They were exposed to normal cycles of night/day and fed with pellitized chow (Life flour mills, Ibadan), and had free access to water ad libitum. Ethical approval was sought and obtained from the ethical review committee on the use of laboratory animals of the Faculty of Pharmacy, University of Benin.

Acute Toxicity Test

Acute toxicity of the extract was carried out as per the organization for Economic Cooperation and Development (OECD) guideline 423 (OECD guideline, 2000). A total of five animals were fasted overnight and each received a single dose of 2000mg/kg of the methanolic extract of *U. afzelii* in 5% tween 80 orally. The rats were observed individually 30minutes, 1hrs, 2hrs, 4hrs and 24hrs after the administration of the extract and daily thereafter for 14days. Any changes with regards to respiratory, autonomic, circulatory and central nervous effects were noted.

Hepatoprotective Effect

The method of Lin and Lin (1993) with a slight modification was adopted for this study. Rats were fasted over night and divided into five groups of five animals each. Group I

served as the control and received 5% Tween 80 (10ml/kg/day) only. Group II, III and IV animals received 125, 250 and 500 mg/kg/day respectively of the extract orally plus 1.5 ml/kg b.w. of CCl₄ (in 50% olive oil) intraperitoneally daily. Group V animals received 1.5ml/kg body weight of CCl₄ (in 50% olive oil). Administration of control, extract and CCl₄ was for 7days.

After the experimental period of 7days, the animals were sacrificed on the 8th day under light ether anaesthesia and blood was obtained by cardiac puncture from each animal. Blood was allowed to coagulate for 1hr at room temperature and thereafter centrifuged for 5minutes at 3000rpm (Ogbu and Okechukwu, 2001).

The serum was removed by pipetting it off and it was used to assay for the levels of aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) as well as serum albumin, total protein, total bilirubin and un-conjugated bilirubin.

Biochemical Evaluation

The determinations of AST, ALT AND ALP were carried out according to the method described by Reitman and Frankel (1957), while total protein was estimated by the method of Plummer (1978). The serum albumin was determined according to the method of Gowenlock *et al.*, (1988), the total bilirubin and unconjugated bilirubin by the method of Malloy and Evelyn (1937).

Histopathological Studies

The method according to Baker *et al.*, (1998) was adopted for this evaluation. The liver was exercised from each animal in the group and washed with ice cold normal saline. Thereafter, it was preserved in 10% buffered formalin solution for 48hrs and the procedure for embedding was carried out manually. 5µm microtome section of the liver were stained with heamatoxylin and eosin and subjected to histopathological examination.

Statistical Analysis

Values are presented as means ± standard error of mean (S.E.M) and analyzed statistically by the two way (paired) student t-test and the Duncan multiple range test. P value < 0.05 was considered significant.

RESULT

The results of acute toxicity test showed no mortality or physical changes after 14days. There were also no changes in respiratory rate, circulatory signs, autonomic effects and central nervous system. Administration of CCl₄ to animals in the negative control group produced significant increases in the levels of AST, ALT, ALP, total and un-conjugated bilirubin and, a significant decrease in total protein and albumin as show in table 1 and 2. Concomitant administration of 125, 250 and 500mg/kg of the methanol extract of *U.afzelii* respectively produced significant decreases in the activities of the serum hepatic enzymes, total and

un-conjugated bilirubin, while an increase was observed in the total protein and albumin levels compared to the control. Of note was the observation that these changes in the enzymatic activities were not dose dependent. Histopathological examinations (Fig 1a-e), of liver of control animals showed the normal architecture with partial triad (A) and hepatocytes (B) separated by sinusoids with partial triad (A) and hepatocytes (B) separated by sinusoids

(C). The liver of animals intoxicated with CCl_4 showed the presence of fat globules of mixed variety indicating injury and inflammation. Animals treated with 125, 250 and 500mg/kg of the extract presented with mild, marked and moderate reduction in liver injury respectively, as evidenced by the presence of micro and macro fat vesicles.

Table. 1: Effects of methanolic extract of root of *U.afzelii* on serum total bilirubin, un-conjugated bilirubin, albumin and total protein on experimental animals.

Experimental group	Total bilirubin (mg/dl)	Unconjugated bilirubin (mg/dl)	Albumin (mg/dl)	Total protein (mg/dl)
Control (5% tween 80)	1.53±0.14	1.04±0.1	5.33±0.18	6.43±0.55
125mg/kg + CCl_4	1.81±0.19 ^a	1.35±0.16 ^a	3.53±0.8 ^a	5.03±0.21
250mg/kg + CCl_4	1.73±0.12 ^a	1.35±0.21 ^a	4.85±0.14 ^a	5.68±0.51 ^{bcd}
500mg/ + CCl_4	1.78±0.21 ^a	2.33±0.13 ^a	4.38±0.31	5.38±0.21 ^{ac}
CCl_4 alone	2.95±0.16	3.10±0.11 ^a	2.70±0.11 ^a	4.21±0.09 ^{ad}

Values are expressed as means ± standard error of means (n=5). Values having different superscripts are significantly different (p < 0.05).

Table. 2: Effect of methanolic extract of root of *U.afzelii* on serum ALT, AST and ALP in experimental animals.

Experimental group	ALT (U/L)	ALP (U/L)	ALP (U/L)
Control (5% tween 80)	42.51±4.33	103.52±3.031	133.75±11.43
125mg/kg + CCl_4	131.68±1.22 ^a	138.00±4.45	183.50±14.84 ^d
250mg/kg + CCl_4	91.00±3.54 ^b	112.75±5.60 ^a	153.00±22.39 ^a
500mg/ + CCl_4	116.17±4.02 ^a	142.00±4.69 ^a	155.15±17.95 ^a
CCl_4 alone	173. ±37±2.00 ^a	185.50±7.26 ^d	209.11±18.51 ^a

Values are expressed as means ± standard error of means (n = 5). Values having different superscripts are significantly different (P < 0.05).

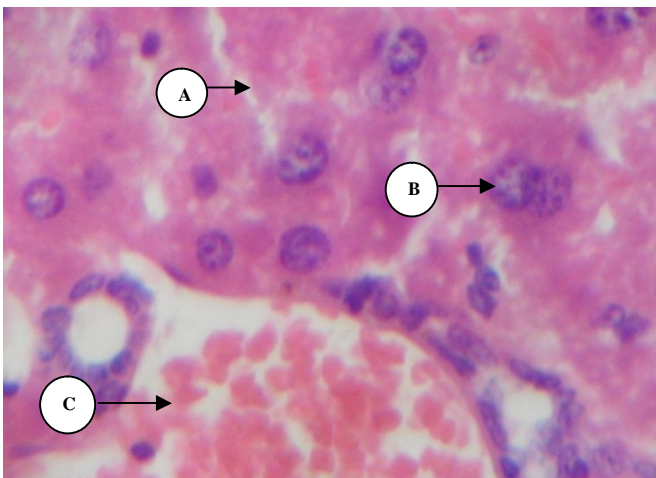


Fig. 1a : Control- Normal Rat liver showing portal triad (A), hepatocytes (B), separated by sinusoids (C) (H&EX40).

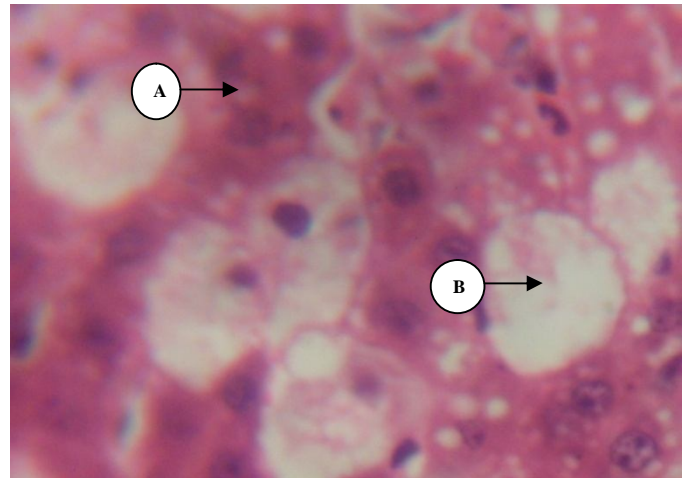


Fig. 1b: Negative control- Liver of rat intoxicated with CCl_4 showing fat globules of mixed variety, macro (A) and micro (B) vesicles (H&E X40).

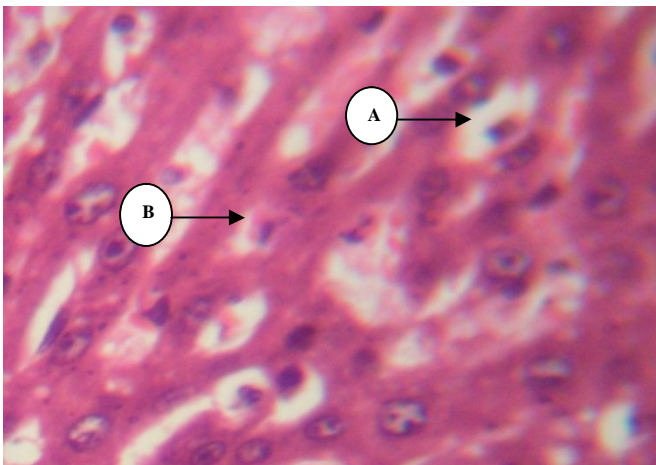


Fig. 1c: Liver of rat treated with 125mg/kg *U. afzelii* root extract for 1week showing moderate reduction in both macro(A) and micro(B) fat vesicles (H&EX40).

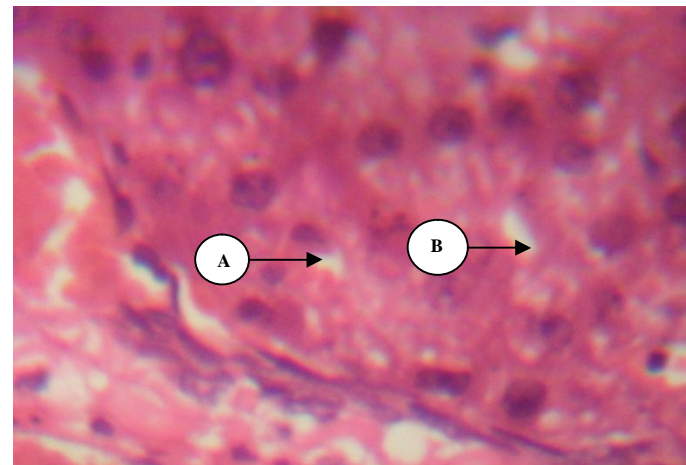


Fig. 1d: Liver of rat treated with 250mg/kg of *U. afzelii* root extract for 1 week showing marked reduction in macro(A) and micro (B) fat vesicles (H&E X40).

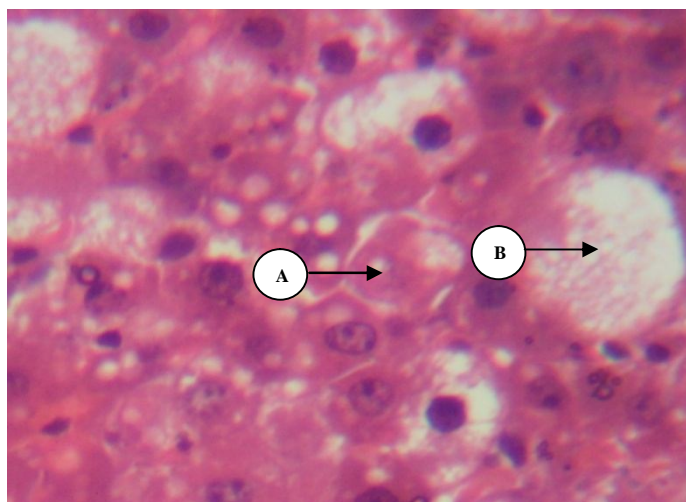


Fig. 1e: Liver of rat treated with 500mg/kg of *U. afzelii* root extract for 1 week showing mild reduction in both macro (A) and micro (B) vesicles (H&E X40).

DISCUSSION

The result of the acute toxicity test suggests that the crude methanolic extract of the plant under consideration is non-toxic to rats. Bilirubin is the main bile pigment that is formed from the breakdown of heme in the red blood cells. It is transported to the liver where it is extracted into the bile, however, conjugation of bilirubin is a pre-condition for its excretion (Nelson and Cox, 2000). Liver injury was evidenced by significant increase in the level of un-conjugated bilirubin in animals intoxicated with CCl_4 compared to control animals. Increase in the level of un-conjugated bilirubin in the blood is believed to be due to a defect in the ability of the liver to conjugate bilirubin (Marks and Dennis, 1996). The significant reduction in the serum levels of un-conjugated bilirubin when the methanolic extract of root of *U. afzelii* was concomitantly administered with CCl_4 at 125, 250 500mg/kg doses respectively compared to levels of un-conjugated bilirubin when CCl_4 alone, was administered, suggest that there was a tendency towards normalization of the liver function to conjugate bilirubin.

The ability of concomitant administration of CCl_4 and methanolic extract of root of *U. afzelii* at 125, 250 and 500mg/kg to reduce serum levels of bilirubin significantly compared with those of animals treated with CCl_4 alone may also suggest the potential effectiveness of the extract in lowering elevated bilirubin level by clearing it from the serum. The result obtained for the serum albumin and total protein followed the same pattern (Table I) suggesting that the extract may be exerting its effect by the same mechanism. The administration of CCl_4 may probably be interfering with the synthesis of protein such as albumin in the liver, hence the reduced levels of albumin and total protein. This trend is reversed to near normal with the concomitant administration of CCl_4 with various doses of the root extract of *U. afzelii* suggesting hepatoprotective activity by the extract against CCl_4 induced hepatotoxicity.

Significant increase in the level of serum AST, ALP and ALT in the group treated with CCl_4 alone compared to control

group is an evidence of hepatotoxicity. The increased levels of AST, ALP and ALT in serum are indicative of cellular liver leakage and loss of functional integrity in the cell membrane of the liver (Drotmann and Lowhorn, 1978). Concomitant administration of the extract at various dose levels mediated a reduction in the levels of these enzymes towards the normal value indicating a stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4 . This effect is in agreement with the common view that serum levels of transaminase return to normal following healing of liver parenchyma and regeneration of hepatocytes (Thabrew *et al.*, 1987).

Histopathological examination of the liver of animals treated with CCl_4 alone reveals massive fatty changes with loss of cellular boundary indicating hepatotoxicity, whereas liver of animal treated with 125, 250 and 500 mg/kg of the extract showed moderate, marked and mild reduction in the observed changes in the CCl_4 treated group. This observation is in tandem with that observed with biochemical results, as the root extract of *U. afzelii* is seen to exhibit hepatoprotective activities in CCl_4 induced liver damage, but in a non-dose dependent manner, as the 250mg/kg dose is observed to be more effective in protecting the liver compared to the 500mg/kg dose.

CCl_4 is known to produce experimental liver damage that histologically resembles viral hepatitis (James, *et al.*, 1996), with the mechanism of action thought to be due to the enzymatic activation of CCl_4 by cytochrome P_{450} to produce trichloromethyl radicals (CCl_3) (Reinke *et al.*, 1980). This lipid oxidation causes the disruption of the lipid bi-layer and cellular integrity accompanied by leakages of serum marker enzymes into the blood. This mechanism suggests an underlying process of oxidation which *U. afzelii* root extract may counter. Phytochemical evaluation of the root of *U. afzelii* indicated the presence of glycoside, flavonoid, terpenoids, phenols and sterols. Phytoconstituents such as flavonoids, triterpenoids and phenols have been reported to exhibit hepatoprotective activity (Pietha, 2000; Jovanovic *et al.*, 1994; Mehta *et al.*, 1999), it is therefore not out of place to assume that the hepatoprotective effect observed with the methanolic extract of *U. afzelii* may be due to any of these constituents or a combination of them.

CONCLUSION

The methanol extract of *U. afzelii* possess significant protection against carbon tetrachloride induced liver injury in a non-dose dependent manner. However more studies will be needed to isolate active component(s) responsible for this hepatoprotective activity. This study provides scientific validation for the use of the plant in ethno medicine to remedy jaundice and liver injury.

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