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Effect of ethanol extract of *Pyrenacantha staudtii* leaves on carbontetrachloride induced hepatotoxicity in rats

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Abstract

The effect of ethanol extract of *Pyrenacantha staudtii* leaves on carbontetrachloride (CCl_4) induced hepatotoxicity in rats was studied. Sixteen male wistar rats of 100-170g body weight divided into four groups of four rats each, designated – group I, II, III and IV were used. Groups II, III and IV were injected intraperitoneally with 5ml/kg body weight of CCl_4; control was injected with 5ml/kg body weight corn oil. After 48hrs, groups III and IV were administered with 750mg/kg and 1500mg/kg body weight of the extract respectively and the rats sacrificed after 5 days. Phytochemical analysis of the extract revealed the presence of alkaloids, glycosides, saponnins, carbohydrates, tannins, flavonoids and resin. Result from the study showed that both concentrations of the extract (750mg/kg and 1500mg/kg body weight) significantly reduced ($P < 0.05$) CCl_4 induced elevations in the liver enzymes- alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in a dose dependent manner. CCl_4 induced increases in total and conjugated bilirubin were also significantly lowered by the extract. These results show that the extract of *Pyrenacantha staudtii* leaves has protective effect against CCl_4 induced liver toxicity and damage.

Keywords: *Pyrenacantha staudtii*, ethanol extract, rats, liver toxicity

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INTRODUCTION

The use of plants in Nigerian traditional medicine as either extracts or infusion is a common practice in the treatment and cure of various types of infections and diseases; respiratory, urinary, skin, gastrointestinal, liver disease, among others[1,2]. *Pyrenacantha staudtii* plant has been claimed by many traditional medicine practitioners to be effective in the treatment and management of many ailments, such as malaria, ulcer, gastrointestinal tract infections and threatened abortion. This plant which grows by climbing up trees in the tropical rainforest it inhabits, is a shrub characterized by the warty appearance of the inner wall of the endocarp of its fruits[3,4].

Studies on the pharmacological properties of some of the active components of the plant show that the methanol extract of the leaves has antimalarial properties[5], exerted high smooth muscle relaxant activity on isolated rat uterus and therefore can be used as a remedy in threatened abortion and dysmenorrhoea[6]. The ethanol extract of the plant was reported to protect rats from developing gastric ulcers induced by various experimental models[7]. From our literature search, no report has been given on the effect of the plant on liver disorders. This study was therefore aimed at evaluating the effect of *Pyrenacantha staudtii* leaf extract on carbontetrachloride (CCl_4) induced hepatotoxicity in rats.

MATERIALS AND METHODS

Animals

Male Wistar rats of weight 100-170g were used for the experiment. They were obtained from the animal house of the Faculty of Biological sciences, University of Nigeria, Nsukka. The rats were maintained *ad libitum* on water and growers mash bought from Nsukka market.

Plant material

Pyrenacantha staudtii leaves were collected from the environs of Nsukka, Nigeria and identified by Mr. Alfred Ozioko of the department of Botany, University of Nigeria, Nsukka. Voucher specimens were deposited in the herbarium unit of the department.

Chemicals and Reagents

Carbontetrachloride (CCl_4) was purchased from Aldrich Chemical Co. All other chemicals used in this study were of analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstadt, Germany. Reagents used for all the assays were commercial kits and products of Randox, USA; QCA, Spain; Teco (TC), USA; Biosystem Reagents and Instruments, Spain.

Extraction

The leaves of *Pyrenacantha staudtii* were dried under room temperature ($25^oC \pm 15^oC$) for two weeks, pulverized into coarse form with a milling machine and macerated in absolute ethanol for 24hrs before filtering with a white filter cloth. The resulting ethanol extract was evaporated to dryness using a rotary evaporator at an optimum temperature of $40^oC \pm 5^oC$. The weight of the dry extract was determined (35.05g). This was made into 1 litre aqueous solution and stored in the fridge until used.

Phytochemical analysis

Preliminary phytochemical tests were carried out on the ethanol extract of the leaves using standard methods[8,9].

Acute toxicity tests

The acute toxicity tests were carried out by the method of Lorke[10]. The extract was found to be relatively safe. Doses of 750mg/kg and 1500mg/kg were then chosen as concentrations of the extract to be administered to the rats.

Experimental Design

Sixteen adult male wistar rats were equilibrated for seven days, randomly divided into four groups of four rats each and housed in separate cages. They were fasted for 12hrs prior to the experiment. CCl_4 was dissolved in corn oil in the ratio of 3:1 (v/v) and administered to the rats. Rats in group I (control) were intraperitoneally injected (i.p.) with 5ml/kg corn oil. Rats in groups II, III and IV were injected (i.p.) with 5ml/kg CCl_4 (single dose). After 48hrs, 750mg/kg and 1500mg/kg body weight of *Pyrenacantha staudtii* leaf extract was administered to groups III and IV respectively. The rats were sacrificed after 5 days.

Preparation of serum

Blood was obtained from the rats by heart puncture technique into centrifuge tubes. Serum was prepared by centrifugation for 10mins at 3000rev/hr in a bench centrifuge. The clear supernatant was used for the biochemical tests carried out.

Biochemical analysis

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum alkaline phosphatase were estimated colorimetrically using Randox reagent enzyme kits based on the methods of Reitman and Frankel[11], and King and Kind[12] respectively. Total protein based on Lowry[13] and Bilirubin based on Jendrassic reaction[14] were also determined using reagent kits.

Statistical analysis

Data were analysed as mean ± SD. Difference between means was assessed by a two-tailed Student's T- test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical analysis of the ethanol extract of *Pyrenacantha staudtii* leaves (Table 1) show the presence of alkaloids, glycosides, saponnins, carbohydrate, tannins, flavonoids and resins.

Table 1: Result of phytochemical tests of the ethanol extract of *Pyrenacantha staudti* leaves.

Constituent	Relative abundance
Alkaloids	++
Glycosides	+++
Saponnins	+
Acid compounds	+
Carbohydrates	++++
Tannins	+
Reducing sugar	_
Protein	_
Flavonoids	+++
Resin	+++
Fats and oil	_
Steroids	_
Terpenoids	_

++++ - *Abundantly present*
+++ - *Present in high concentration*
++ - *Present in moderate concentration*
\+ - *Present in small concentration*
\- - *Absent*

Phytochemicals such as protein, fats and oil, steroids and terpenoids were however not detected. The extract was also found to be slightly acidic. Earlier studies on the plant[6,7] also reported the presence of alkaloids, tannins and triterpenoid saponnins from the methanol and ethanol leaf extracts. These secondary metabolites are known to exhibit diverse biochemical and pharmacological effects in animals. Alkaloids present in plants are known to have numerous beneficial pharmacological effects[8]. Some bioflavonoids have been reported to possess antioxidant properties which help to combat free radical induced oxidative stress[15]. Results from the acute toxicity test of the ethanol extract of the plant (Table 2) show that the plant has no toxic effect on the animal (LD_{50} > 5g/kg body weight).

Table 2: Result of acute toxicity test for *Pyrenacantha staudti* leaf extract.

Phase one

Groups	Number of mice	Dose mg/kg	Dead (%)
1	3	10	0
2	3	100	0
3	3	1000	0

Phase two

Groups	Number of mice	Dose mg/kg	Dead (%)
4	3	1600	0
5	3	2900	0
6	3	5000	0

An earlier report[5] also observed no significant toxic effect for the dry aqueous extract of the plant. Results from Table 3, show increases in the liver enzymes – aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) at the administration of CCl_4. Treatment of the animals with different concentrations of the plant extracts (750mg/kg and 1500mg/kg body weight) significantly reduced ($P < 0.05$) the CCl_4 induced elevations in AST and ALT. The extract showed no significant effect on alkaline phosphatase. CCl_4 is a well known hepatocarcinogenic agent in laboratory animals[16]. Exposure to CCl_4 has been reported to induce free radical generation in tissues such as liver, heart, lung, testis, brain and blood[17].

Table 3: Effect of the ethanol extract of *Pyrenacantha staudti* leaves on some biochemical parameters of rats after CCl_4 administration.

PARAMETERS	GROUPS			
	Control	CCl_4 5ml/kg body weight	CCl_4 + 200mg/kg *Pyrenacantha staudti* extract	CCl_4+ 400mg/kg *Pyrenacantha staudti* extract
Aspartate aminotransferase (U/L)	104.75±20.30[a]	127.25 ± 14.84 [a]	88.50 ± 8.54 [b]	86.25 ± 13.32 [b]
Alanine aminotransferase (U/L)	30.00 ± 5.40[a]	42.50 ± 9.71 [a]	26.00 ± 5.66 [b]	21.00 ± 3.16 [b]
Alkaline phosphatase (U/L)	76.50 ± 6.35[a]	89.75 ± 5.19 [b]	85.75 ± 2.21 [a,b]	89.75 ± 9.36 [b]
Total protein (g/dl)	80.00 ± 4.08[a]	67.75 ± 4.50 [b]	60.75 ± 7.00 [b,c]	55.25 ± 7.41 [c]
Total bilirubin (mg/dl)	0.23 ± 0.50 [a]	0.45 ± 0.19 [b]	0.22 ± 0.10 [a]	0.27 ± 0.19 [a]
Conjugated bilirubin (mg/dl)	0.55 ± 0.25 [a]	1.15 ± 0.26 [b]	0.19 ± 0.07 [a]	0.19 ± 0.06 [a]

Values are mean ± SD; Results with different superscript ([a, b, c]) on the same row are statistically significant (P < 0.05)

The first metabolite of CCl_4; trichloromethyl free radical, is believed to initiate the biochemical processes leading to oxidative stress, which is the direct cause of many pathological conditions such as diabetes mellitus, cancer, hypertension, kidney damage, liver damage and death[17-19]. Liver damage caused by acute exposure to CCl_4 shows clinical symptoms such as jaundice, swollen and tender liver and elevated levels of liver enzymes in the blood[20,21]. The liver enzymes found within organs and tissues are released into the bloodstream following cellular necrosis and cell membrane permeability and are used as diagnostic measure of liver damage[22]. Result from this study showing a reduction in CCl_4 induced elevations of the liver enzymes, at the administration of *Pyrenacantha staudtii* extract, suggests a protective effect of the extract against CCl_4 induced toxicity and therefore amelioration of the liver damage. Total protein concentration of the CCl_4 treated rats (Table 3) was significantly reduced ($P < 0.05$) as compared to control. This suggests a reduction in the protein synthetic function of the liver, which could be as a result of possible damage to the hepatocytes induced by CCl_4. Most protein found in the plasma are synthesized by the hepatocytes and secreted into circulation. Reduction in total protein level at the administration of CCl_4 to male Fisher rats was also reported[20]. Administration of *Pyrenacantha staudti* extract lead to an increased reduction in the total protein level in a dose dependent manner as compared to control. This result shows that the extract enhanced, rather than modulate, the reduced protein synthetic function of the liver. We could not explain this trend in the result. Total and conjugated bilirubin levels (Table 3) were significantly increased in the CCl_4 treated rats as compared to control. Administration of the extracts (750mg/kg and 1500mg/kg body weight) lead to a significant reduction ($P < 0.05$) in their levels. Report from Tirkey *et al*[21] also showed a marked rise in bilirubin levels after CCl_4 administration. Bilirubin, a major breakdown product of haemoglobin rises when there is liver injury or damage; leading to the discolouration of the skin known as jaundice[22].

Elevation of total bilirubin which results from decreased uptake and conjugation of bilirubin by the liver is caused by liver cell dysfunction, while increased levels of direct or conjugated bilirubin is due to decreased secretion from the liver or obstruction of the bile ducts[22]. Reduction of CCl_4 induced increases in total and conjugated bilirubin by *Pyrenacantha staudti* extract further show its protective effect against CCl_4 induced liver toxicity. The extract perhaps protects the liver cell from damage, thereby enhancing bilirubin uptake and conjugation by the liver and subsequent secretion into the bile ducts. These reports from our study show that the ethanol extract of *Pyrenacantha staudti* possess antihepatotoxic activity as demonstrated

by its reduction of CCl_4 induced elevations in the levels of ALT, AST, total and conjugated bilirubin. This hepatoprotective effect suggests that the plant may also possess antioxidant properties that helped to combat the CCl_4-induced oxidative stress in the liver. The ability of natural compounds to attenuate carcinogen – induced hepatotoxicity is believed to be related to their intrinsic antioxidant properties[23]. Phytochemical result from this study revealed the presence of flavonoids, which has been reported to protect against toxicity induced by environmental toxicants[23] such as CCl_4. The chemoprotective activities of flavonoids are related to their ability to inhibit peroxidative damage caused by environmental toxicants. In conclusion, the flavonoids present in *Pyrenacantha staudti* plant may have, perhaps, played a major role in the hepatoprotective action of the plant.

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