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Phytochemical analysis, hepatoprotective and antioxidant activity of *Alchornea cordifolia* methanol leaf extract on carbon tetrachloride-induced hepatic damage in rats

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ABSTRACT

Objective: To investigate the hepatoprotective and antioxidant activities of *Alchornea cordifolia* (*A. cordifolia*) leaf extract. **Methods:** Various solvent fractions of the methanol extract of the leaf of the plant *A. cordifolia* Mull. Arg (Fam: Euphorbiaceae) were evaluated for hepatoprotective activity by carbon tetrachloride-induced liver damage in rats. The degree of protection was measured by using biochemical parameters such as serum glutamate oxalate transaminase (SGOT/AST), serum glutamate pyruvate transaminase (SGPT/ALT), alkaline phosphatase (ALP) and total bilirubin. The *in vitro* antioxidant activity of the extract was also evaluated by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. The extract was subjected to preliminary phytochemical screening. **Results:** The ethyl acetate and chloroform fractions, at a dose of 300 mg/kg, produced significant ($P<0.05$) hepatoprotection by decreasing the activities of the serum enzymes and bilirubin while there were marked scavenging of the DPPH free radicals by the fractions. The effects were comparable to those of the standard drugs used for the respective experiments, silymarin and ascorbic acid. Alkaloids, flavonoids, saponins and tannins were detected in the phytochemical screening. **Conclusion:** From this study, it was concluded that the plant of *A. cordifolia* possesses hepatoprotective as well as antioxidant activities and these activities reside mainly in the ethyl acetate and acetone fractions of methanol leaf extract.

1. Introduction

The liver is the major organ involved in the metabolism, detoxification and excretion of various endogenous and exogenous substances such as xenobiotics. Such physiological activity of the liver results in the generation of highly reactive free radicals which covalently bonds with membrane lipids causing lipid peroxidation. Lipid peroxidation alters the membrane permeability and causes tissue damage. Since the liver is involved in various biochemical reactions, the liver cells are prone to attack and necrosis by the free radicals[1]. In addition, these radicals have been implicated as important pathological mediators in many clinical disorders such as heart disease, diabetes, gout and cancer[2]. However, inbuilt antioxidant systems such as superoxide dismutase (SOD), tissue glutathione (GSH) *etc*. protect the tissues from free radical attack. Excessive release of reactive oxygen species overcome this system resulting in organ damage. Strengthening of the in-built protective mechanisms or exogenous administration of antioxidant may be useful in protecting the organs[1]. However, there is lack of satisfactory liver protective drugs in orthodox medical practice for serious liver disorders. Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver. Furthermore, there is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body and to prevent the deterioration of fats and other constituents of food stuffs. In both cases, there is a

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preference for antioxidant from natural rather than synthetic source because of the safety of the natural drugs. Many plants have been evaluated for their hepatoprotective and antioxidant action in the light of modern medicine[3-4].

Alchornea cordifolia (A. cordifolia) Mull. Arg (Fam: Euphorbiaceae) is an erect, sometimes scrambling, bushy, perennial shrub or small tree, up to 4 m high reproducing from seeds. The plant is reportedly used in African traditional medicine as topical anti-inflammatory, antibacterial and antifungal agent[5-6].

In order to investigate the efficiency of hepatoprotective and antioxidant substances, we use various methods of free radical scavenging such as reduced glutathione, glutathione peroxidase and S-transferase, lipid peroxidation, catalase peroxidation, superoxide dismutase or in vitro techniques such as the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging models. Hepatic damage is usually done using such drugs as acetaminophen, isoniazid and rifampicin or chemicals such as carbon tetrachloride. The present study was undertaken to assess the hepatoprotective and antioxidant activities of *A. cordifolia* leaf extracts in rats against CCl_4 as a hepatoxin to prove its claims in folklore practice against liver disorders. The preliminary phytochemical analysis of the extract was also carried out.

2. Materials and methods

2.1. Plant materials and preparation of extracts and fractions

Fresh leaves of the plant *A. cordifolia* were collected from Oba in Nsukka Local government Area of Enugu State, Nigeria. Their botanical identities were determined and authenticated by Mr. A. Ozioko, a taxonomist with the Bio-resource Development and Conservative Centre, Nsukka. The voucher specimen was deposited at the centre for future references. The leaves were washed with water, cut into pieces and air-dried at room temperature. The dried leaves were then pulverized into coarse powder in a grinding machine. 1 kg of air-dried pulverized leaves was extracted with 2 liters of methanol using soxhlet apparatus. Solvent from the sample was filtered and evaporated off under reduced pressure in a rotary evaporator to obtain crude methanol extract (ME). 150 g of dried ME was triturated with 400 g of silica gel to increase the surface area. This was transferred into an air-tight bottle and partitioned successively with solvents according to increasing order of polarity starting with *n*-hexane, chloroform, ethyl acetate, acetone to methanol. Their corresponding soluble fractions were labeled A-E.

2.2. Animals

24 Wister albino rats of either sex weighing between 110 and 165 g were used in this study. These rats were procured from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were housed in well ventilated stainless-steel cages at room temperature (24 ± 2) ℃ in hygienic condition 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.

2.3. Experimental design for hepatoprotective activity

The hepatoprotective activity of the leaf extract of the plant was tested using carbon tetrachloride (CCl_4) model. Silymarin (2.5 mg/kg bw) was used as a standard drug in this study[7]. Silymarin is a flavonolignan that has been introduced fairly recently as a hepatoprotective agent. It is extracted from the seeds and fruits of *Silybum marianum*. The rats were divided randomly into eight groups according to the following protocol.

2.4. Treatment protocol

GROUP I: Normal control (n=3, the animals were given normal saline only)

GROUP II: Hepatotoxic control (n=3, the animals were given 1.25 mL/kg CCl_4).

GROUPS III: Treatment control (n=3, the animals were given 1.25 mL/kg CCl_4 and silymarin)

GROUP IV-VIII: Treatment groups (n=3 in each case, the animals were given 1.25 mL/kg CCl_4 and 300 mg/kg of the fractions A-E of the plant extract respectively).

Rats were treated as per the treatment protocol for a period of 7 d. While CCl_4 was administered intraperitoneally, the drugs were given orally.

2.5. Assessment of hepatoprotective activity

In the present study, the hepatoprotective activity was evaluated biochemically. After 7 d of drug treatment, the animals were dissected under ether anesthesia. Blood from each rat was withdrawn from carotid artery at the neck and collected in previously labeled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 2 500 rpm for 10 min. The separated serum was used for the estimation of some biochemical parameters like Alanine aminotransferase (ALT/SGPT), Aspartate aminotransferase (AST/SGOT), Alkaline phosphate (ALP) and Total bilirubin using standard kits[8].

2.6. Antioxidant activity

The antioxidant activity of leaf of *A. cordifolia* was determined using the 1, 1-diphenyl- 2-picrylhydrazyl (DPPH) free radical scavenging assay by the method of Blois[9]. Ethyl acetate and acetone fractions of the leaf extract of the plant were used in this investigation. DPPH offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants[9]. A stock solution (50 mg/mL) of ethyl acetate and acetone

fractions each was prepared in methanol and test samples were prepared from stock solution by dilution with methanol to obtain different concentrations ranging from 1.56 mg/mL to 25 mg/mL. DPPH solution was prepared in methanol. 2 mL each of a given concentration of each fraction, including the stock solution, was put into two test tubes, one containing 1 mL of freshly prepared DPPH solution while the other contained 1 mL of methanol. After 30 min the absorbance was taken at 520 nm using a spectrophotometer (6505, Jenway, England). Ascorbic acid was used as a positive control. Concentrations of ascorbic acid used ranged from 0.50 mg/mL to 3.0 mg/mL. The absorbance readings were recorded as "with DPPH" and "without DPPH" (control) respectively. Percent scavenging of the DPPH free radical was measured using the following equation[9]:

%DPPH radical scavenging (or % DPPH inhibition = [1/(As/Ac)]×100)

Here Ac = absorbance of control, As = absorbance of sample solution.

2.7. Preliminary phytochemical analysis

Standard procedures[10] were followed in the tests for the phytochemical constituents of the plant. The following constituents were tested for-saponins, alkaloids, carbohydrates, reducing sugar, tannins and flavonoids.

2.8. Statistics

The results were expressed as Mean ± SEM. Statistical analysis was carried out by Student's *t* test and SPSS (Version 14) statistical program. The obtained results were considered significant at $P<0.05$.

3. Results

3.1. Extraction yield of plant material

Extraction yields (w/w) were 4.33% for *n*-hexane fraction (A), 0.81% for chloroform fraction (B), 17.41% for ethyl acetate fraction (C), 22.15% for acetone fraction (D) and 3.59% for methanol fraction (E) in terms of dry weight. Thus acetone fraction had the highest yield.

3.2. Hepatoprotective effect of the plant

The effects of the various fractions of *A. cordifolia* on serum transaminase, alkaline phosphatase, and bilirubin levels in CCl_4-induced liver damage in rats are summarized in Table 1. Administration of CCl_4 resulted to a significant ($P<0.05$) elevation of hepatospecific serum markers ALT, AST, ALP and bilirubin in CCl_4-treated group (Group II), in comparison with the normal control group (Group I). On administration of the test fractions (Group IV to VIII) and silymarin (Group III) the level of some of these enzymes were found to decrease, especially in ethyl acetate (Group VI) and chloroform fractions (Group V) and the sylimarin-treated group (Group III).

3.3. Antioxidant effect of the plant

The result of the *in vitro* antioxidant test is presented in Table 2. There was a marked reduction of free radical activity by ethyl acetate and acetone fractions by the various concentrations in a non-concentration dependent manner. The results are comparable with those obtained from the standard antioxidant, ascorbic acid.

3.4. Phytochemical constituents of the plant

Preliminary phytochemical tests revealed the presence of alkaloids, saponins, tannins and flavonoids while carbohydrates and reducing sugars were absent.

4. Discussion

Carbon tetrachloride-induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plants and drugs[11]. Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP and bilirubin which are enzymes originally present in high concentration in cytoplasm[11]. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage[12]. When rats were treated with carbon tetrachloride, it induced hepatotoxicity by metabolic activation, therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Carbon tetrachloride is metabolically activated by the cytochrome

Table 1
Effect of various fractions of *A. cordifolia* leaf extract on different biochemical parameters in CCl_4- induced hepatotoxic rats.

Group	SGOT/AST (IU/L)	SGPT/ALT (IU/L)	ALP (IU/L)	Total bilirubin (mg/L)
I (Normal control)	0.15±0.01	0.21±0.01	63.03±0.58	1.33±0.45
II (CCl_4-induced hepatotoxic group)	115.40±1.47	75.07±0.58	96.07±1.10	1.36±0.32
III (Sylimarin treated control)	41.00±1.15	27.00±1.15	141.33±0.88	1.26±0.43
IV (*n*-hexane fraction, A)	120.00±1.15	87.43±0.86	133.40±0.87	1.67±0.37
V (Chloroform fraction, B)	98.83±0.93	85.03±1.13	123.10±1.75	0.90±0.05
VI (Ethyl acetate fraction, C)	120.40±0.88	20.00±0.58	115.00±1.15	1.04±0.02
VII (Acetone fraction, D)	146.90±0.88	82.03±4.06	91.53±0.84	2.45±0.38
VIII (Methanol fraction, E)	125.90±0.88	74.53±1.02	114.00±0.58	1.58±0.34

Table 2

Effect of ethylacetate and acetone fractions of *A. cordifolia* leaf extract on the inhibition of DPPH in in vitro DPPH antioxidant model.

Group	Concentration (mg/mL)	% Inhibition
Ethylaceate fracrion	1.56	99.4
	3.13	98.0
	6.25	57.4
	12.50	100.0
	25.00	79.5
	50.00	100.0
Acetone fraction	1.56	79.0
	3.13	100.0
	6.25	99.5
	12.50	100.0
	25.00	84.1
	50.00	99.4
Ascorbic acid	0.50	93.3
	1.00	93.3
	1.50	96.9
	2.00	95.9
	2.50	92.8
	3.00	98.0

P-450 dependent mixed oxidase in the endoplasmic reticulum to form trichloromethyl free radical ($.CCl_3$) which combines with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation. These result in changes of structures of the endoplasmic reticulum and other membranes, loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver injury. These may explain what happened in the CCl_4-treated groups (Group II-VIII) in the present study.

The reduction of the elevated serum enzymes (especially ALT and total bilirubin) by the chloroform and ethyl acetate fractions may be due to the prevention of the leakage of intracellular enzymes by their membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of the marker enzymes return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes. Furthermore, bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hypatocyte[13-18]. Decrease in serum bilirubin after treatment with the fractions in liver damage induced by CCl_4, indicated the effectiveness of the extract in normal functional status of the liver. Therefore, reduction of ALT and bilirubin levels points towards an early improvement in the secretary mechanism of the hepatic cells. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by a hepatotoxin. Both silymarin and the fractions of the plant extract decreased CCl_4-induced elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells.

The antioxidant test was carried out using the DPPH model. DPPH is characterized as stable free radical by virtue of the delocalization of the spare electron over the molecule so that the molecule do no dimerise as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 520 nm. When a solution of DPPH was mixed with that of a substance that can donate a proton, this will give rise to the reduced form with the loss of the violet color. Representing the DPPH radical by R* and the donor molecule by AH, the primary reaction:

$$Z^{*}+AH \longleftrightarrow ZH+A^{*}$$

Here, RH is the reduced form of DPPH and A* is free radical produced in the first step. This later radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorized) by one molecule of the reductant. The reaction is therefore intended to provide a link with the reactions taking place in an oxidizing system, such as the autoxidation of a lipid or other unsaturated substances while the DPPH molecule R* is intended to represent the free radicals formed in the system, the activity of which is to be suppressed by the test substances. In the present study, the strong inhibition of the DPPH free radicals by the ethyl acetate and acetone fractions of the leaf extract of *A. cordifolia* which is comparable to that of standard antioxidant (ascorbic acid) indicates that the plant possesses strong antioxidant activity. It is suggestive from the present results that the antioxidant property of the plant reduced the formation of trichloromethyl peroxide radical thereby reducing tissue damage. Therefore the hepatoprotective activity of the plant may be due to its antioxidant potential.

Further, the preliminary phytochemical analysis gives a clue to the possible constituents responsible for the observed effects of the plant. Other workers have analyzed the chemical constituents of *A. cordifolia* leaves and identified: tannins, phenolic acids: gallic acid, ellagic acid, protocate-chic acid, flavonoids: quercetin, hyperin and guaijaverin and an alkaloid: triisopentenylguanidine[19]. The antioxidant effect demonstrated in the present study might probably be due in part to the presence of flavonoids and tannins. Perchellet *et al*[20] found out that foliage tannins have potent antioxidants and anti-inflammatory activities. Also flavonoids have been shown to possess antioxidant and anti-inflammatory activities[21]. Okuda *et al*[22] reported that tannins and related compounds may prevent the destructive

effects of lipid peroxide in liver cells by lowering the levels of lipid peroxide in liver cells. There are also reports that plants containing saponins possess antioxidant properties[23]. In addition, liver protective herbal drugs have been shown to contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthenes[23]. Thus it is possible that other secondary metabolites of the plant as observed in the present study and earlier studies may be responsible for the antioxidant and hence the hepatoprotective activity of the plant.

The present investigation indicates that *A. cordifolia* exerts significant protection against CCl_4-induced hepatotoxicity by its potential to ameliorate the lipid peroxidation through the free radicals scavenging activity, which enhanced the levels of antioxidant defense system. This effect may be attributed to the antioxidant principles present in the plant such as flavonoids, tannins and saponins. Further identification and the elucidation of the structures of the actual constituents responsible for this activity are underway.

Conflict of interest

We declare that we have no conflict of interest.

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