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HEPATOPROTECTIVE EFFECTS OF THE LEAF EXTRACTS OF CASSIA OCCIDENTALIS AGAINST CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN ALBINO RATS

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Abstract

*Cassia occidentalis,*commonly known as Fedegoso, is used in traditional medicine for the treatment of liver ailments and other various diseases. The objective of this study was to investigate the hepatoprotective effect of the aqueous and methanol (AE and ME) leaf extracts of *Cassia occidentalis* against carbon tetrachloride (CCl_4)-induced hepatotoxicity on the serum biochemistry and histomorphology of the liver of albinorats. Twenty adult rats were used for this study. They were weighed and divided into five groups (n=4). Pre-treatments with oral doses of 800mg/kg body weight of AE and ME for two weeks via oral gavage preceded liver injury induction using subcutaneous administration of 3ml/kg b.wt. of CCl_4mixed in equal parts with olive oil. Blood samples were collected from the rats and sera obtained were used for the determination of serum levels of Alanine transaminase (ALT), and Aspartate transaminase (AST), alkaline phosphatase (ALP). Upon sacrifice, under anaesthesia, liver tissues were excisedfor histological processing and microscopy. Results obtained showed increased serum activities of ALT, AST, and ALP in CCl_4-treated rats when compared with normal control ($p<0.05$). However, pre-treatments with AE and ME markedly reduced the serum levels of the liver marker enzymes ($p<0.05$). Microscopical examination of the liver showed centrilobular necrosis, ballooning degenerated and vacuolatedhepatocytes in CCl_4-treated rats but amelioration of the liver damage was observed in rats pre-treated with *C. occidentalis* extracts. In conclusion, aqueous and methanol leaf extracts of *Cassia occidentalis* possess significant hepatoprotective activities against CCl_4-induced hepatotoxicity in rats.

Keywords: *Cassia occidentalis, liver, CCl_4, hepatoprotection*

Introduction

The use of plants as traditional medicine and pharmacopoeial drugs has been in existence from time past. Majority of the global population depend on plants due to their medicinal benefits (1).*Cassia occidentalis* is an annual or perennial Ayurvedic plant and the roots, leaves and seeds are used in several traditional medicines to cure various diseases(2).The phytochemicals present in the plant include saponins, flavonoids, terpenoids, anthraquinones and glycosides (3).Its anti-inflammatory, anti-oxidant, wound healing, antibacterial, antifungal, anticancerous, antimutagenic, antipyretic, analgesic and antidiabetic properties have been documented (1,2,4,5).

The liver is a vital organ that functions principally for the maintenance of the body's metabolic homeostasis. Liver disorders have far-reaching consequences since it is the firstorganto encounter a wide variety of toxic, metabolic, neoplastic and microbial insults. Acute and chronic exposure to toxicants detrimentally alter the major functions of the liver. Since it is the major drug metabolizing and detoxifying organ in the body, it is hence subject to potential damage from the enormous array of pharmaceutical and environmental chemicals which may lead to life threatening conditions (6).

In experimental studies to assess the hepatoprotective efficacy of natural products, drug or chemical hepatotoxicants are used. Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxic agent in experimental model of liver diseases. CCl_4 exerts its hepatotoxic effects largely due to its active metabolite, trichloromethyl radical. In rats, CCl_4 administration enhances hepatic protein oxidation and results in oxidized proteins accumulation in the liver (7). Many natural products with hepatoprotective effects against CCl_4 and other hepatotoxic agents have been reviewed (8,9). The leaves of *Cassia occidentalis* are used in folklore-based practices for the treatment of liver ailments; however,there is paucity of information in documented scientific research on its effect on CCl_4-induced liver injury.Hence, the present study was designed to investigate the hepatoprotective effects of the aqueous (AE) and methanol(ME) leaf extracts of*C. occidentalis* against carbon tetrachloride (CCl_4) induced hepatotoxicity in Albino rats.

Methods

Plant collection and authentication

The leaves of *Cassia occidentalis* were collected from various sites within Enugu metropolis, Enugu State in Nigeria. The plant material was authenticated by a botanist at the hebarium section of Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The fresh leaves were dried in an airy room for two (2) weeks after which they were crushed into fine powder using a gasoline powered grinding machine.

Plant extractions

Crude Aqueous Extraction: About three hundred (300) grams of the powdered leaves of *C. occidentalis* was soaked and homogenized in 1.2litres of distilled water and left for 12 hours. The homogenate was sieved using a muslin cloth and the filtrate obtained was stored at 4°C in a refrigerator until required. The concentration of the filtrate (aqueous extract) was determined by evaporating 2ml of the extract in an evaporating dish of known weight in an oven (Gallen kamp UK) to dryness. The dish containing the residue was obtained by subtracting the weight of the dish and the average weight taken. The extractive value of the aqueous extract (AE) was 120mg/ml.

Crude Methanol Extraction: One thousand (1000) grams powdered *C. occidentalis* leaves was homogenized with 6 litres of 80% methanol in a well-stoppered container. The homogenate was shaken intermittently for a period of 3 days (72hrs). The extract was filtered using Whatmann No. 2 filter paper and the resultant filtrate was evaporated to dryness on a rotary evaporator. The yielded residue was stored in the refrigerator (4±2°C) until required. Reconstitution of the methanol extract (ME) was done using 3% Tween 80 to get a concentration of 180mg/ml prior to use.

Animal housing

Twenty (20) albino rats (~180-200g) were obtained from Animal House of Department of Physiology, University of Nigeria Enugu Campus. They were housed under standard condition of temperature (27±2°C) with twelve-hour light/dark periodicity. The animals were weighed and divided into five (5) groups (I - V) of four (4) animals each. These animals

were housed in clean gauzed cages in groups and fed on standard feed pellets (Guinea feed® Nigeria Plc) and clean water *ad libitum*. Acclimatization was for two weeks. Animals were handled in this study in accordance with protocols approved by Institutional guidelines on Animal Care and Use Committee and conform to established guidelines set by National Institutes of Health on experiments involving the use of animals.

Experimental design and conduct

After acclimatization, animals in groups I and II were orally administered 800mg/kg body weight (b.wt.) of ME and AE of *Cassia occidentalis* leaves respectively. Rats in groups III and V were given water while those in group IV received 2ml/kg b.wt. of 3% tween 80. All treatments were given once daily via oral gavage for two (2) weeks.

Experimental induction of hepatotoxicity

Liver damage was induced in animals in groups I, II and III, one (1) hour after the oral administration of extracts on the last day. This was performed by subcutaneous administration of the rats with 3ml/kg b.wt. of CCl4 dissolved in equal parts with olive oil.

Sample collection and biochemical assays

After twenty (20) hours of liver injury induction, the experiment was terminated. Blood samples were collected by retro-orbital puncture of the medial canthus of the eye under anesthesia using capillary tube into plain tubes. After clotting for about 45 mins, the blood samples were centrifuged at 3000rpm for 15 mins and sera were separated from each sample for biochemical analysis. Serum levels of Alkaline Phosphatase (ALP), Aspartate Transaminase (AST) and Alanine transaminase (ALT) were estimated using standard commercial reagents kits.

Tissue processing and microscopy

The liver tissues of the animals were excised under ether anesthesia and were further fixed in 10% formal saline prior to histological processing using paraffin wax embedding technique for light microscopical examination. The tissues were taken through processes of dehydration, clearing and wax impregnation using an Automatic tissue processor. The wax impregnated tissues were "blocked out" with paraffin wax prior to cutting of sections with the rotary microtome (Hertz 150, Cambridge model). The tissue sections of 5µm were produced and further stained according to the Haematoxylin and Eosin [H and E] staining technique as described by Baker *et al.* (10). The sections were examined using Olympus binocular microscope with in-built lighting system.

Statistical analysis

The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20.0. Data obtained were expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) and unpaired two-tailed Student's t-test were used to determine the differences among the groups. The level of significance was considered at $p<0.05$.

Results

Biochemical parameters

CCl4 administration resulted in significant increase ($p<0.05$) in activities of serum AST, ALT and ALP in CCl4-treated rats when compared to the normal control groups (I and II) as shown in Table 1. Rats pre-treated with oral administration of 800mg/kg of ME and AE of *C. occidentalis* prior to liver injury induction using CCl4 showed marked and significant reduction of serum AST, ALT and ALP activities compared to the CCl4 group ($p<0.05$).

Histological findings

The photomicrographs of the hepatic lobules of control rats (Water and 3% Tween 80) revealed normal hepatocytes, central veins, sinusoidal spaces and portal tracts (Figure 1a & 1b respectively), while that of the CCl4-only treated rats (negative control) showed marked centrilobular necrosis, ballooning degeneration and vacuolation of hepatocytes (Figure 1c). Treatments with ME and AE revealed moderate to marked reduction of lesions caused by CCl4 (Figures 1d & 1e respectively). Additionally, presence of inflammatory cellular infiltrates was observed in the extracts-treated groups.

Discussion

Medicinal plants have provided numerous plant-derived therapeutic agents for the treatment of ailment since ancient times(1**).** This study investigated the hepatoprotective effect of leaf extractof *Cassia occidentalis* against carbon tetrachloride (CCl4) induced hepatotoxicity by estimating the liver enzymes and examining the liver histology of Albino Wistar rats.

In the present study, treatment with carbon tetrachloride caused ballooning degeneration and

vacuolation of hepatocytes around the central veins suggesting its potency in inducing hepatotoxicity in experimental animals. Previous studies have documented similar histopathological changes following treatment with CCl_4 (7,11,12). The mechanism for CCl_4-induced hepatotoxicity is via its reductive dehalogenation caused by cytochrome P450 enzymes in the endoplasmic reticulum of hepatocytes leading to lipid accumulation and ultimately cell death (13). The fatty degeneration of the hepatocytes following treatment with CCl_4 is due to lipid accumulation because the liver transport of triglyceride-rich low density lipoproteins into the plasma is impaired (14). Thus, the vacuolations observed in the present study are the sites where the lipids accumulated but cleared from the tissues during histological processing with the ante-medium (Xylene). However, pre-treatments with the methanol and aqueous extracts of *C. occidentalis* leaves showed moderately preserved liver tissue parenchyma of the rats. Extracts of *C. occidentalis* are rich in phytochemicals such as saponins and flavonoids which are well-known plant principles responsible for the hepatoprotection of various plants (15-17). The tissue preservation observed in the present study may be due to a single or combined effect of the phytochemicals present in the plant material.

The degree of liver injury is widely evaluated by measuring its markers including serum Alanine Transaminase (ALT) and Aspartate Transaminase (AST) (18); ALT being a more specific enzyme for liver dysfunction. Increased levels of these serum liver markers were observed upon treatment with CCl_4 in the present study and this finding correlates with the histopathological report suggesting liver injury. However, decreased levels were observed in rats pre-treated with methanol and aqueous extracts when compared with the CCl_4-only treated rats. This shows evidence of alleviation of the injury caused by the hepatotoxicant (CCl_4). Previous studies have shown similar hepatoprotection of the aqueous and ethanolic extracts of roots and leaves of *C. occidentalis* following injuries caused by drug and chemical hepatotoxicants (7,19-21).

In conclusion, the biochemical and histopathological results obtained from this study confirm the hepatoprotective effect of the leaf extracts of *Cassia occidetalis* against carbon tetrachloride-induced liver damage in experimental rat model.

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Table 1: Effect of pre-treatment with crude methanol and aqueous extracts of *Cassia occidentalis* leaves on some serum biochemical parameters [ALT, AST and ALP] of albino rats with CCl4-induced hepatotoxicity

Treatment	Biochemical Parameters		
	ALT(iu/l)	AST(iu/l)	ALP(iu/l)
ME + CCl_4	21.25±6.16*	20.00±5.23*	48.50±4.99*
AE + CCl_4	20.00±5.23*	13.00±4.80*	102.50±14.71*
3%Tween 80	27.75±2.29*	40.25±0.95	64.75±11.83*
Water	31.75±3.64*	18.00±2.04*	67.50±1.94*
CCl_4	48.50±4.99	31.75±4.39	718.25±33.20

Values are expressed as Mean±Standard error of mean. * $p<0.05$ when compared with CCl_4-only treated group (Negative control). ALT – Alanine transaminase; AST – Aspartate transaminase; ALP – Alkaline phosphatase; ME – Methanol extract; AE – Aqueous extract

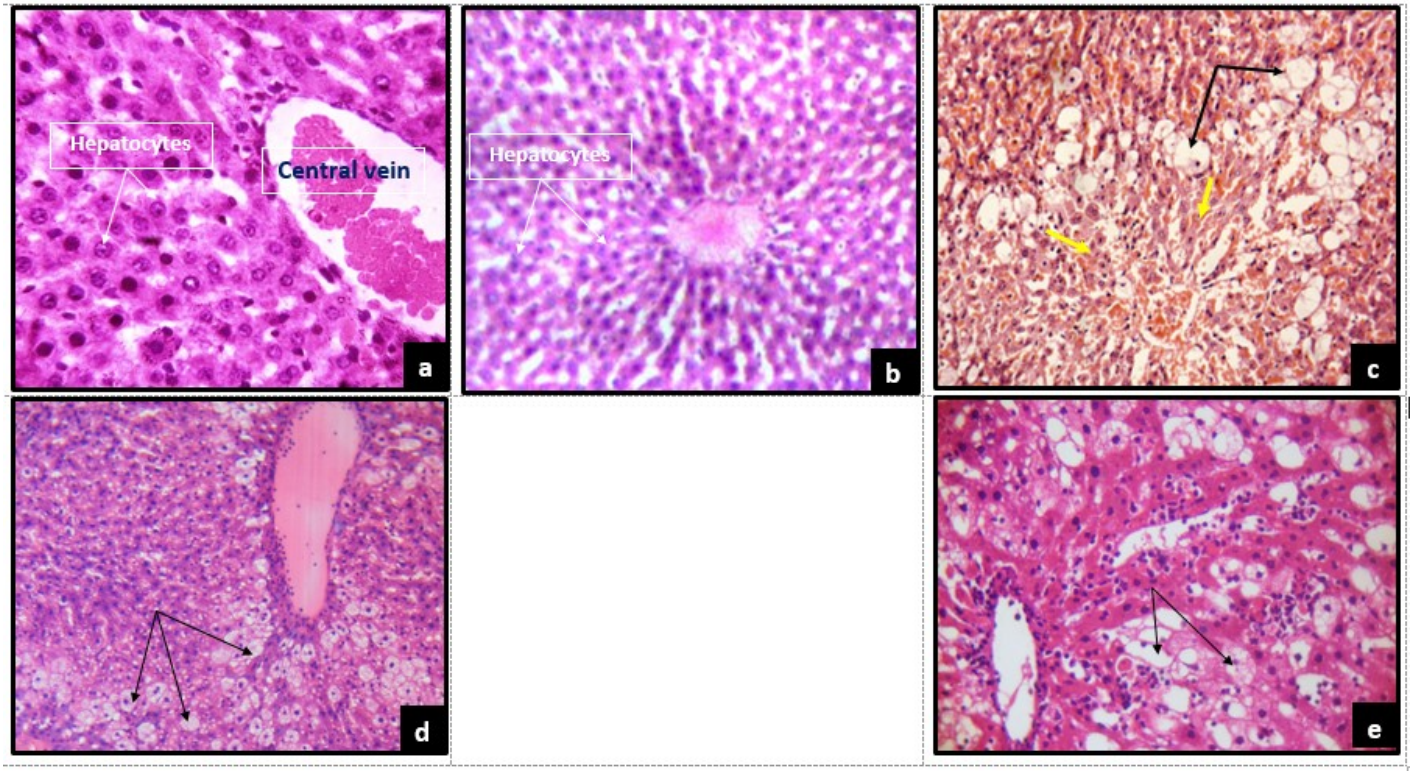

Figure 1: Photomicrographs of liver sections from control rats (water-treated [a] and 3% Tween-treated [b]), CCl4-only treated [c], ME pre-treated rats [d] and AE pre-treated rats [e]. **[a and b]**: Normal histomorphology of the hepatic tissues revealing nornal central veins, sinusoidal spaces and hepatocytes. **[c]:** Liver tissue revealing centrilobular necrosis (yellow arrows), ballooning degeneration of hepatocytes (black arrows) and vacuolations. **[d and e]**: Liver tissues showing markedly reduced centrilobular necrosis; few ballooned hepatocytes (black arrows), vacuolationsand inflammatory cellular infiltration are however observed. [Stain: H&E; Mag. a&e- X400; b,c&d – X100]