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Ziziphus mauritiana Fruit Extract Inhibits Carbon Tetrachloride-induced Hepatotoxicity in Male Rats

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Abstract: The aqueous extract of *Ziziphus mauritiana* fruit (Zm) was evaluated for its protective activity against CCl_4-induced liver damage. 250, 500 mg/kg bw of Zm fruit extract or 100 mg/kg silymarin (standard) were administered to different groups of rats prior to CCl_4 administration. Both 250 and 500 mg/kg bw of Zm fruit extract significantly ($p<0.05$) reduced (dose dependently) the levels of enzymes and non-enzymes markers of tissue damage when compared to rats given CCl_4 only. These findings were supported by liver histology and suggest that Zm fruit possessed reach hepatoprotective principles that inhibited the toxicity of CCl_4 against the liver.

Key words: *Ziziphus mauritiana* fruit, CCl_4, hepatotoxicity, protection

INTRODUCTION

CCl_4 is an injury agent for animal experiment, which induced reactive oxygen formation and depletion of glutathione. It may reduce antioxidant enzymes and antioxidant substrates to induce oxidative stress. CCl_4 requires bioactivation by cytochrome p450 system of phase I in liver and yields the reactive metabolic trichloromethyl radical ($CCl_3^{\bullet}$) and proxy trichloromethyl radical ($^{\bullet}OOCCl_3$). These free radicals can bind with Polyunsaturated Fatty Acid (PUFA), forming alkoxy ($R^{\bullet}$) and peroxy radicals ($ROO^{\bullet}$), that can generate lipid peroxidation, cause damage in cell membrane, change enzyme activity and finally induce hepatic injury or necrosis (Weber *et al.*, 2003). Lipid peroxidation is considered to be of fundamental importance in cell ageing and damage (Popovic *et al.*, 2006). At present, in spite of an increasing need for agents to protect the liver from damage, modern medicine lacks a reliable liver protective drug. Therefore a number of natural substances have been studied to evaluate their protective activities (Willet, 1994).

Ziziphus mauritiana Lam belongs to the family *Rhamnaceae* (Michel, 2002). The fruit of *Ziziphus mauritiana* fruits (Indian jujube) is rich in vitamin C, phenolic compounds, with good mineral contents. This study was designed to evaluate the effect of administering aqueous fruits extract of *Ziziphus mauritiana* in carbon tetrachloride induced liver damage.

MATERIALS AND METHODS

Plant: *Zizphus mauritiana* fruits were purchased from Yola market Adamawa State in April, 2007 and authenticated in Botany Department of Federal University of Technology, Yola with voucher specimen number BC/DD07/01. The fruit was further dried at room temperature and made into powder using mortar and pestle.

Animals: Thirty male albino rats weighing 120-150 g were purchased from the animal house of Biochemistry Department University of Jos, Plateau State. The animals were housed in stainless steel cages and kept at room temperature $28\pm2^{\circ}C$ under 12/12 h light/dark and were fed with pelleted standard laboratory feed (Vital Feed; Grand Cereal and Oil Mills, Jos) and water *ad libitum*.

Experimental design: Each group consisted of six (6) animals treated as follows:

Group I (control): Rats were given isotonic solution p.o. (0.5 ml of saline/animal)

Group II: Single dose of CCl_4 + diet/water

Group III treated (250 mg/kg bw): Zm fruit + CCl_4 + diet/water

Group IV treated (500 mg/kg bw): Zm fruit + CCl_4 + diet/water

Group V Silymarin (100 mg/kg bw): Silymarin + CCl_4 + diet/water

Group III, IV and V were pre-treated with aqueous extract of *Ziziphus mauritiana* fruit or silymarin for seven days prior to CCl_4 administration. CCl_4 was administered in olive oil (1:1) 2 ml/kg bw to induce liver damage.

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Preparation of aqueous extract: The fleshy part of the fruit was dried and pulverized in to fine powder with laboratory mortar and pestle. The powder was then sieved using 0.33 mm Endicott test sieve (Endicott, London). 100 g of the powder was mixed with 600 ml of distilled water and allowed to stand for 6 h with continuous shaking. The mixture was then filtered using Whatman No. 4 filter paper. The filtrate was evaporated using rotary evaporator at reduced temperature >50°C to obtain 22.54±2.1 g/100 g.

Collection of serum/liver for analysis: Rats from all groups were sacrificed 48 h after CCl_4 administration.
Blood was collected via the ocular vein and allowed to stand for 10 min before been centrifuged at 3000 rpm for 15 min to collect the serum. Serum was separated for the estimation Alanine Aminotransferase (AST), Aspartate Aminotransferase (ALT) and Alkaline Phosphatase (ALP), Bilirubin and cholesterol.
Liver tissues were collected for histopathological analysis; a portion of the liver was fixed in 10% formalin, processed using routine histology procedures, embedded in paraffin, cut in 5 micro mole sections and mounted on a slide. The samples were stained with hematoxylin and eosin for histopathological examination.

Statistical analysis: Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS). Student's 't' test was used to determined statistical difference between two means when $p<0.05$.

RESULTS

Table 1 represents the effect of pretreatment with *Ziziphus mauritiana* aqueous fruit extract on Aspartate Amino Transaminase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) levels in CCl_4 induced liver damage. All the level of AST, ALT and ALP significantly ($p<0.05$) increased in group II (i.e. CCl_4 alone) compared to control (group I). However, pretreatment with 250 and 500 mg/kg of *Ziziphus mauritiana* aqueous fruit extract for seven days prior to CCl_4 administration significantly ($p<0.05$) decreased the levels AST, ALT and ALP (groups III and IV) compared to

group administered CCl_4 alone (group II). Pretreatment with 500 mg/kg significantly ($p<0.05$) decreased indices of tissue injury compared to group treated with 250 mg/kg i.e. group III). Pretreatment with silymarin also significantly ($p<0.05$) reduced the level of AST, ALT and ALP compared to CCl_4 group alone. Pretreatment with extracts alone without CCl_4 did not significantly ($p<0.05$) change the levels of markers of tissue injury as analyzed compared to normal group. This shows that at this dose level its consumption was safe.
The levels of both bilirubin and cholesterol were significantly ($p<0.05$) increased in group II compared to group I. Pretreatment with 250 and 500 mg/kg of *Ziziphus mauritiana* aqueous fruit extract prior to CCl_4 administration resulted in significantly ($p<0.05$) decreased levels of both bilirubin and cholesterol compared to group administered CCl_4 alone. Pretreatment with the extract alone without CCl_4 did not result to any significant change in the levels of both bilirubin and cholesterol compared to control.
CCl_4 administration produced general liver morphological changes and necroses, severe centrilobular vacuolar degeneration and mononuclear cell aggregation as shown in Fig. 2 compared to normal liver morphology in Fig. 1. Pretreatment of rats with *Ziziphus mauritiana* revealed moderate and apparently normal organ with very few hepatocytes with tiny cytoplasmic vacuoles (Fig. 3 and 4). Thus *Ziziphus mauritiana* pretreatment greatly inhibited liver morphological changes and necrosis due to CCl_4 hepatotoxicity.

DISCUSSION

CCl_4-induced hepatic injury is an experimental model widely used for hepatoprotective drugs screening. CCl_4 undergoes a biotransformation by hepatic microsomal cytochrome p450, to produce trichloromethyl free radicals. These hepatotoxic metabolites can react with protein and lipid in the membrane of cells or organelles leading to necrosis of hepatocytes (Brent and Rumack, 1993). As a result of hepatic injury, the altered permeability of the membrane causes the enzymes

Table 1: Effect of CCl_4 toxicity and *Ziziphus mauritiana* aqueous fruit extract on enzyme markers and non-enzyme markers of liver damage

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TB (mg/dl)	CHOL (mg/dl)
Control	25.20±2.28	13.30±1.67	576.81±10.7	10.46±2.54	1.72±0.22
CCl_4	93.60±3.51*	21.90±1.74*	1043.40±6.5*	26.56±2.90*	3.66±0.13*
Zm 250 mg/kg + CCl_4	43.92±2.89**	15.90±2.70	626.23±14.1**	17.70±3.49**	3.10±0.10
Zm 500 mg/kg + CCl_4	38.90±4.74	17.90±11.74	499.26±30.6	17.26±2.56	2.22±0.08**
Silymarin 100 mg/kg + CCl_4	23.83±2.50	11.90±2.10	558.50±29.4	10.15±1.25	1.70±0.24

Results are Mean±SD, (n = 6). *Significantly higher than normal group ($p<0.05$). **Significantly lower than group administered CCl_4 only. Significantly lower than group pre-treated with 200 mg/kg aqueous extract of *Ziziphus mauritiana* ($p<0.05$)
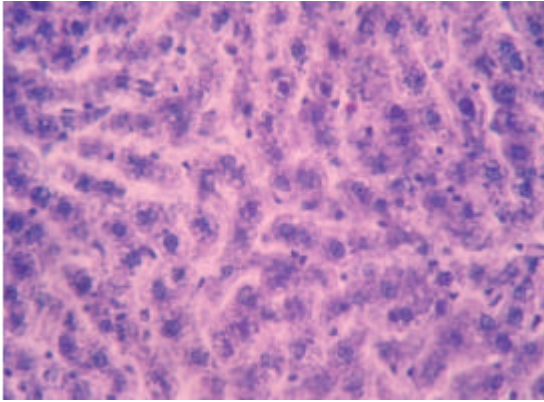
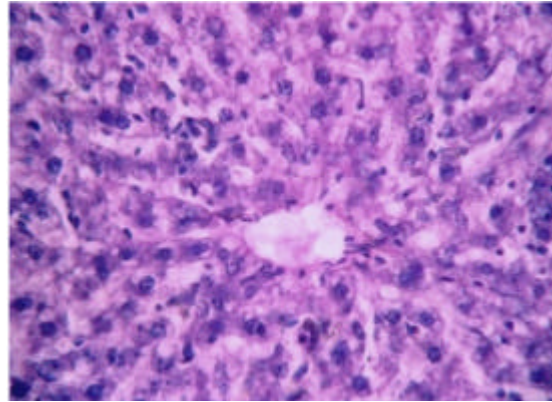Fig. 1: Histology of normal liver tissue from control rat liver (H and E; x650)
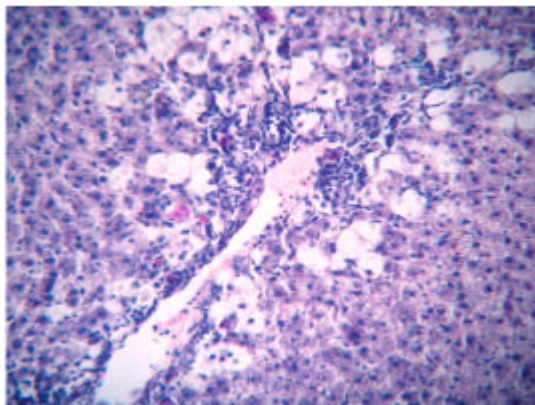

Fig. 2: CCl₄ alone showing severe centrilobular vacuolar degeneration, hepatocellular necrosis and mononuclear cell aggregations (H and E; x650)

Fig. 3: Liver tissue of rat pre-treated 250 mg/kg Zm plus CCl₄, showing an apparently normal organ with very few hepatocytes with tiny cytoplasmic vacuoles (arrows) (H and E; x650)

from the cells to be released into the circulation (Drotman and Lawhorn, 1978). The magnitude of

Fig. 4: 500 mg/kg Zm plus CCl₄, showing an apparently normal organ with very few hepatocytes with tiny cytoplasmic vacuoles (H and E; x650)
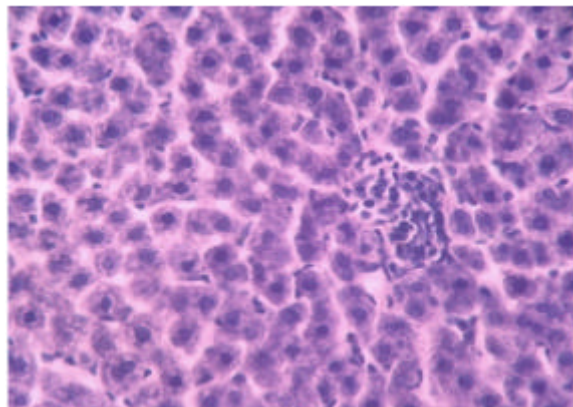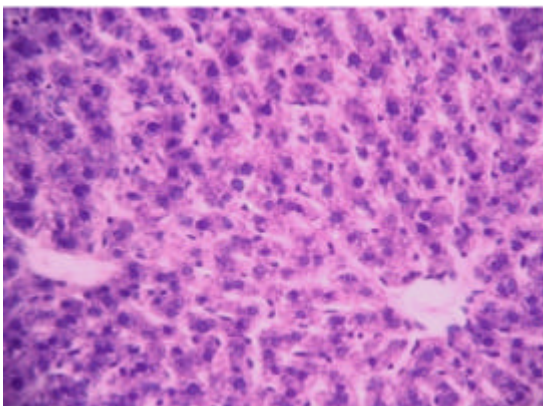

Fig. 5: Liver tissue of rat pre-treated with 100 mg/kg bw silymarin showing an apparently normal liver with a focal area of mild mononuclear periportal cell aggregate (H and E; x650)

hepatic damage is usually assessed by measuring the level of released cytosolic transaminases including ALT and AST in the circulation (Guti'errez and Solis, 2009).

The rise in the serum levels of ALP, AST and ALT as observed in the present study could be attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and are released into circulation after cellular damage (Recknagel *et al.*, 1989), other researchers had reported increased level of AST, ALT, ALP and bilirubin due to CCl₄ hepatotoxicity (Dahiru *et al.*, 2005; Galati *et al.*, 2005; Guti'errez and Solis, 2009). The increase in the level of serum bilirubin is an index of the degree of jaundice. This could possibly be as a result of increased production, decreased uptake by the liver, decreased conjugation, decreased secretion from the liver or blockage of bile ducts (Bun *et al.*, 2006). Increase cholesterol level due to CCl₄ could be as a result of its peroxidative degradation in the

adipose tissues resulting in fatty in filtration in to the circulation. The result of this study demonstrated that pretreatment of rats with *Ziziphus mauritiana* aqueous fruit extract effectively protected rats against CCl₄ induced hepatotoxicity in a dose dependent manner as evidenced by the decreased serum ALT, AST, ALP, bilirubin and cholesterol.

The histopathological findings in this study agrees with earlier reports that CCl₄ causes necrosis, mononuclear cell infiltration, steatosis foamy degeneration of hepatocytes (Nan *et al.*, 2003; Hung *et al.*, 2006; Liu *et al.*, 2006). *Ziziphus mauritiana* fruit extract protected the liver against CCl₄ induced liver morphological changes, fatty liver development and cellular degeneration. It is possible that the extract might have blocked adipogenesis. Reduced serum cholesterol as observed in the result due to pretreatment with *Ziziphus mauritiana* was an indication of anti-atherosclerotic activity, hence possible modulation of lipid metabolism.

Conclusion: The results of this study were a clear demonstration that *Ziziphus mauritiana* aqueous fruit extract possessed potent hepatoprotective action against CCl₄ induced liver damage in rats. The protective effects observed could be as a result of the rich phenolic compounds; caffeic acid, p-hydroxybenzoic acid and vitamin C (Muchuweti *et al.*, 2005).

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