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Short Communication

Protective effect of *Ziziphus mauritiana* leaf extract on carbon tetrachloride -induced liver injury

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Protective effect of ethanol extract of *Ziziphus mauritiana* leaf was studied on carbon tetrachloride-induced liver damage. Pretreatment of rats with 200 and 300 mg/kg body wt of *Z. mauritiana* leaf extract protected rats against carbon tetrachloride liver injury by significantly lowering aspartate aminotransaminase (AST), alanine aminotransamine (ALT), alkaline phosphatase (ALP), total bilirubin (TB), and lipid peroxide levels compared to control. The extract at both doses also significantly restored depleted levels of glutathione and vitamin E compared to control. Reduction in lipid peroxidation, restoration of glutathione and vitamin E levels indicate strong antioxidant property of the leaf. Phytochemical screening of the leaf extract of *Z. mauritiana* indicates probable presence of flavonoids, phenolic compounds, tannins and saponins.

Key words: *Ziziphus mauritiana*, carbon tetrachloride, non-enzyme antioxidants, lipid peroxidation.

INTRODUCTION

Medicinal plants play a key role in the human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material (WHO, 1993). Scientific studies available on a good number of medicinal plants indicate that promising phytochemicals can be developed for many health problems (Gupta, 1994). *Ziziphus mauritiana* Lam. belongs to the family *Ramnaceae*. It is called jujube tree or Indian jujube (Morton, 1987; Michel, 2002). The plant is commonly known as magarya in Hause and whuya in Kilba (Nigeria). The leaves of the plant are used in the treatment of diarrhoea, wounds, abscesses, swelling and gonorrhoea (Michel, 2002). They are also used in the treatment of liver diseases, asthma and fever (Morton, 1987). This study aimed to evaluate the efficacy of the leaves of *Z. mauritiana* extract on carbon tetrachloride-induced liver damage. Experimental liver damage produced by carbon tetrachloride (CCl_4) has been extensively studied and the profile of damage even after the single administration of this hepatotoxin has been well established (Anand et al., 1992). Cell damage by free radicals has been reported as the predominant mechanism of hepatotoxicity (Gregus and Klaassen, 1995) . The critical process underlying CCl_4 hepatotoxicity is the combining effect of both lipid peroxidation and the covalent binding of CCl_4 reactive metabolites to lipids and proteins (Masuda and Nakamura, 1990). It has been shown that $CC1_4$ induced lipid peroxidation can be obstructed by natural antioxidants (Subramanian et al., 1999; Wang et al., 2000). The identification of naturally occurring inhibitors of peroxidation resulting in cell damage could therefore lead to important new strategies for disease prevention (Subramanian et al., 1999).

MATERIALS AND METHODS

Preparation of Plant Extract

Leaves of *Z. mauritiana* were collected from Dugwaba district, Hong Local Government Area of Adamawa State in the month of March

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Table 1. Effect of ethanol extract of *Zizyphus mauritiana* leaf on CCl_4-induced liver injury.

Treatment	AST (IU)	ALT (IU)	ALP (IU)	TB (mol/L)
Vehicle	138 ± 3.25	79 ± 2.09	128 ± 4.61	1.22 ± 0.01
CCl_4 only	497 ± 5.68^a	321 ± 5.21^a	298 ± 6.18^a	4.01 ± 0.17^a
Zm (200mg/kg bw) + CCl_4	296 ± 7.34^b	163 ± 3.12^b	195 ± 7.32^b	2.10 ± 0.02^b
Zm (300mg/kg bw) + CCl_4	243 ± 8.13^b	102 ± 5.42^b	168 ± 5.44^b	1.82 ± 0.03^b
Silymarin (100 mg/kg bw) + CCl_4	181 ± 4.01	92 ± 3.41	108 ± 3.87	1.06 ± 0.04

AST, aspartate aminotransaminase; ALT, alanine aminotransaminase; ALKP, alkaline phosphatase; TB, total bilirubin.
Values are mean ± S.E.M; n = 6; ^{a}p<0.01 compared to control; ^{b}p< 0.01 compared to CCl_4 alone.

Table 2. Effect of *Zizyphus mauritiana* leaf extract on non-enzyme antioxidants and lipid peroxide levels on CCl_4-induced liver injury.

Treatment	Vitimain E (mg/mg protein)	GSH (mg/100g tissue)	Lipid peroxidation (nmol MDA/mg protein)
Vehicle	5.23 ± 0.28	93.18 ± 2.09	3.50 ± 0.21
CCl_4 only	2.06 ± 0.25^a	49.32 ± 5.87^a	8.63 ± 0.44^a
Zm (200mg/kg bw) + CCl_4	3.42 ± 0.22^b	76.34 ± 7.62^b	6.89 ± 0.21^c
Zm (300mg/kg bw) + CCl_4	3.81 ± 0.41^b	83.61 ± 3.94^b	5.81 ± 0.23^b
Silymarin (100 mg/kg bw) + CCl_4	3.89 ± 0.33	87.61 ± 3.18	6.03 ± 0.38^c

Values are mean ± S.E.M; n = 6; ^{a}p<0.01 compared to control; ^{c}p<0.05 compared to control; ^{b}p< 0.01 compared to CCl_4 alone.

2005 and authenticated by the Botany Department of Federal University of Technology, Yola. A voucher specimen of the plant has been deposited (BCDD-03) in the Department of Biochemistry, Federal University of Technology, Yola. The leaves were dried under room temperature (35 ± 2^{o}C) and made into powder using mortar and piston. The extract was obtained from the powder (100 g) through soxhlet extraction with 80% ethanol. The extract was concentrated into a semi-solid material using rotary evaporator at < 50^{o}C. The extract was dissolved in distilled water to 200 or 300 mg/ml and administered to rats orally at a dose of 200 or 300 mg/kg body wt.

Animals

Male Wister rats weighing 180 – 220 g were obtained from the National Veterinary Research Institute Vom, Nigeria. The animals were housed in stainless steel cages and kept in well ventilated room under 12 h light/dark cycle and fed with standard diet, Vital Feed (Grand Cereals and Oil Mills, Jos) and water *ad-libitum*. The study was carried out based on the guidelines for the use and care for laboratory animals.

Carbon tetrachloride-induced hepatotoxicity

Hepatic injury was induced in four groups of six rats through oral administration of CCl_4 diluted with liquid paraffin oil (1:1) in a dose of 1 ml/kg body wt for 2 days to all the animals except for control (group I) (Rao and Mishra, 1998) after pretreatment with extract or Silymarin.

Hepatoprotective activity

Ethanol extract of *Z. mauritiana* leaves at doses of 200 and 300 mg/kg body wt were administered to group III and IV, respectively, and Silymarin 100 mg/kg body wt to group V six times at 12 h intervals prior to carbon tetrachloride treatment. Group II animals received CCl_4 only. After 36 h of CCl_4 treatment blood was drawn from the rats by puncturing the retro-orbital flexus and animals sacrificed. Liver was excised after dissecting the animals and washed with cold normal saline and sucking up the moisture. The homogenate of the liver (10%) was prepared in ice-cold KCl solution (1.15%, w/v) using Teflon homogenizer. A portion of the homogenate was used to assay for glutathione while the remaining homogenate was centrifuged at 4000 g for 10 min. The supernatant was used for the estimation of lipid peroxidation.

Assessment of liver functions

Blood collected from all groups was separated into serum for the analysis of some biochemical parameters of liver tissue damage. Levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin were assayed using clinical test kits (Randox, Randox Laboratories Ltd., UK) . Total glutathione (Ellman, 1957) and lipid peroxidation (Wills, 1987) were also measured.

Statistical analysis

All data are expressed as mean ± S.E.M. Results were statistically analysed by Student's 't' test for significant difference between group means.

RESULTS AND DISCUSSION

Extraction of plant material with 80% ethanol gave a yield of 19.35 g/100 g representing 19.35% of plant material. The levels of aspartate aminotransaminase, alanine aminotransaminase, alkaline phosphatase (Table1), bilirubin and lipid peroxidation (Table 2) were elevated in

animals administered CCl_4 only when compared with control. Groups pretreated with *Z. mauritiana* extract (200 and 300 mg/kg body wt) or Silymarin had their AST, ALT, ALP, bilirubin and lipid peroxidation levels significantly lowered than group administered CCl_4 alone. The levels of glutathione and vitamin E were significantly reduced in rats given CCl_4 compared to control (Table 2). Ethanol extract of *Z. mauritiana* leaves (200 and 300 mg/kg body bw) significantly restored the levels of glutathione and vitamin E compared to group treated with CCl_4 alone. Pretreatment with 300 mg/kg body wt of the extract had better lowering effect on the enzyme makers and other parameters of liver tissue damage compared to group pretreated with 200 mg/kg body wt of the extract.

Elevation of serum AST, ALT, and ALKP is a known effect of CCl_4 toxicity which specifically affect the liver (Anand et al., 1992) and activities of AST, ALT are most commonly used biochemical makers of liver damage (Sturgill and Lambert, 1997). The hepatotoxic effect of CCl_4 has been attributed to its metabolism by Cytochrome P_{450} to yield toxic trichloromethyl radicals that act as free radical initiators (Kim et al., 2003). Action of this free radicals increase hepatic lipid peroxidation level in CCl_4 totoxicity. This finding is in accordance with the known hepatotoxic effect of CCl_4 that causes oxidative damage in the liver (Farber and Gerson, 1984). Pretreatment with the extract of *Z. mauritiana* clearly protected against the rise of the enzyme makers of tissue damage by CCl_4 probably due to its interference with cytochrome P_{450}, thereby preventing the formation of hepatotoxic free radicals (Nadeem et al., 1997) or/and promotion of its glucuronidation (Gilman et al., 1992). Glutathione and Vitamin E protect cells against CCl_4 induced toxicity by detoxifying electrophiles, preventing oxidation of –SH groups of proteins and by scavenging free radicals (Kim et al., 2003; Saravanan et al., 2003). Increase in both levels of glutathione and vitamin E in groups pretreated with the extract shows that the extract is rich in principles with antioxidant properties. Restoration of glutathione content by *Z. mauritiana* extract could be due to its inhibitory effect on glutathione peroxidase activity and stimulatory effect on glutathione reductase. Reduction in lipid peroxidation in hepatocytes exposed to CCl_4 after pretreatment with *Z. mauritiana* extract may be due to increased levels of glutathione. Glutathione is important in quenching the reactive intermediates and radical species generated during oxidative stress (Kim et al., 2003). The action of the extract exhibited a dose dependant protection of the hepatocyte against CCl_4-induced hepatotoxicity. Reduction in levels of lipid peroxidation, restoration of glutathione and vitamin E levels indicates a strong antioxidant property. Phytochemical screening of the plant extract revealed the probable presence of tannins, flavonoids and saponins. Further studies are in progress to ascertain the possible active phytochemical principle responsible for the observed effect.

In conclusion, pretreatment of rats with ethanol extract of *Z. mauritiana* leaves (200 and 300 mg/kg body wt) protected against CCl_4-induced hepatic injury by maintaining the levels of glutathione, vitamin E and decrease in lipid peroxidation. It is possible that the extract contains high levels of phytochemicals with antioxidant properties, which brought about the observed effects.

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