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Protective Effect of *Raphanus sativus* Against Carbon Tetrachloride Induced Hepatotoxicity In Wistar Albino Rats

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Abstract: The present study aimed to investigate for a possible hepatoprotective activity of *Raphanus sativus* against carbon tetrachloride induced hepatotoxicity beside its toxicity and phytochemistry of the plant. Thirty albino rats were divided into 6 groups. The first served as a control, the second was injected with CCl_4 and the four other groups were injected with CCl_4 and treated orally and simultaneously with either methanolic or water extract at doses of 200 and 400 mg kg^{-1} (b.wt.). The animals were scarified after 10 days. The same doses were tested for toxicity. The phytochemical tests revealed presence of triterpenes, alkaloids, flavanoids, tannins, saponin and coumarins but negative for cyanogenic glycosides and anthraquinone glycosides. Biochemical results showed that CCl_4 induced hepatotoxicity which was reduced by the use of the plant as indicated by inhibition of the increased serum AST, ALT and ALP activities and bilirubin concentration beside histopathological changes. Toxicity study indicated that *Raphanus sativus* had no adverse effect on livers.

Key words: *Raphanus sativus*, carbon tetrachloride, hepatoprotective

INTRODUCTION

Liver damage remains one of the serious health problems. Numerous medicinal plants and their formulations are used in ethno-medical practices. *In vivo* and *in vitro* evaluation models have been developed for the ability of the plants to prevent or cure liver toxicity in laboratory animals induced by various hepatotoxins (Evans, 2002). Clinical research has confirmed the efficacy of several plants in the treatment of liver diseases (Luper, 1998).

Raphanus sativus, some times known as Radish or Alfgel belong, to the family Cruciferae. It is widely used to compact bacterial and viral infections, inflammation and cancer (Fant *et al*., 1998; Terras *et al*., 1993). The ethanomedicinal information of the plant describes the use of aqueous extract to have antiurolithiatic activity in rats (Vargas *et al*., 1999).

Kirtikar and Basu (1987) reported the use of the plant as anthelmintic and in diseases of heart. Ethanolic and aqueous extract have shown to possess hepatoprotective effect on rabbits (Zaman and Ahmad, 2004).

The present study was carried out to confirm the Sudanese folkloric use of *Raphanus sativus* as a hepatoprotective agent.

MATERIALS AND METHODS

Preparation of Plant Extract

Raphanus sativus seed was obtained from Omdurman General Market, Sudan and identified at Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum in 2005.

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The plant seeds were dried and extracted according to Harborne (1976) method. For aqueous extract the seeds were soaked in water for 2 h and filtered. The filtrate was frozen, dried till use. In methanolic extract 60 g granulated seeds were packed in a soxhlet apparatus and 100 mL chloroform was added to separate lipid and terpenoids, then unpacked and left to dry and repacked again with methanol to get the polar constituent.

Phytochemical screening of the plant was performed for sterols, triterpenes, alkaloids, flavonoids, tannins, saponins, cyanogenic glycosides, anthraquinone glycoside and coumarins. Ten grams of the powder plant kernel was refluxed with 100 mL of 80% ethanol for 4 h, cooled and filtered. The filtrate was screened using method of Harborne (1976).

Animals

Wister albino rats of both sexes were used. They were kept in cages and housed in standard enviroumental conditions of temperature, humidity and light. The rats were left for seven days adaptation and supplied with standard diet and water *ad libitum*.

Experimental Design

Two experiments were performed, one to study the hepatoprotective activity of the plant against carbon tetrachloride-induced liver damage in rats using water and methanolic extracts and the other about toxicity of the plant.

In hepatoprotective experiment thirty rats were used. They were divided randomly into 6 groups, 5 rats each and injected intra-peritoneally daily for 10 days. One group served as control and was injected with paraffin oil at a dose of 0.2 mL kg^{-1} b.wt. Liver damage was produced in the other 5 groups by injection of carbon tetrachloride (CCl_4), which was mixed with 9th volume of liquid paraffin oil at a rate of 0.2 mL kg^{-1} b.wt. Four groups out of these 5 groups received either methanolic or water extract of the plant at a dose of 200 and 400 mg kg^{-1} b.wt.

For toxicological study twelve rats were divided randomly into three groups, four rats in each. One group served as control. The other two groups received daily, for 21 days, methanolic extract orally at dose 200 and 400 mg kg^{-1} b.wt.

Blood samples were collected from the orbital plexus using halothane as an anesthetic (Waynforth, 1980). Blood was collected either on EDTA for haematology or in centrifuge tube to separate serum. Haematological studies measured according to Schalm (1965). Sera were analyzed for activities of serum tansaminasses (Asparate aminotransferase and alanine aminotransferase) according to Reitman and Frankel (1957) and alkaline phosphatase (ALP) according to Chemie method (1972). Total bilirubin concentration was measured as described by Jendrassik and Grof (1938).

Animals were necropsed after 10 and 21 days in the hepatoprotective and toxicological experiments respectively. Specimens from the liver, heart, kidney, lung and pancreas were collected immediately after slaughter, fixed in 10% formalin embedded in paraffin wax, sectioned at 5 μm and stained with hemtoxylin and eosin (Drury and Wallington, 1980).

Statistical Analysis

Data were analyzed using the student t-test according to procedures described by Mendenhall (1971).

RESULTS

Phytochemical screening showed that *Raphanus sativus* was positive for triterpenes, alkaloids, flavanoids, saponins and coumarins but negative for cyanogenic glycosides and anthraquinone glycosides.

There were no clinical signs observed in the controls and those received either methanolic or water extracts. However rats received CCl_4 exhibited depression, loss of appetite and reduced body.

At necropsy the control group showed no pathological changes, but in group which received CCl_4 the liver was severely pale and hemorrhages beside congestion of the lungs and hearts. In the groups received methanolic extract or *Raphanus sativus* there was moderate paleness of the liver. On the other hand livers of rats received 200 mg kg^{-1} b.wt. water extract were slightly pale with areas of congestion and congestion of the lungs. Livers of rats received 400 mg kg^{-1} b.wt. water extract were slightly congested.

In group which received CCl_4 there was a significant transient decrease ($p \leq 0.05$) in the Hb concentration at day 5 and a significant decrease ($p \leq 0.05$) in the values of PCV at day 10. However in group received methanolic extract at a dose of 200 and 400 mg kg^{-1}, there was no significant changes in blood pictures. The groups of animals received water extract exhibited no change in hematological values (Table 1).

In group received CCl_4 there were significant increase ($p \leq 0.05$) in the levels of AST and ALT at days 5 and 10. ALP and bilirubin concentration was significantly increased ($p \leq 0.05$) at day 10. The values of AST was not increased significantly in groups received 200 and 400 mg kg^{-1} b.wt. methanolic extract (Table 2).

Nevertheless there was significant increase ($p \leq 0.05$) in the levels of ALT at day 5 and 10 in groups received methanolic extract but these values were lowered at day 10. ALP and the bilirubin levels were unchanged.

In groups received water extract there were significant increase in the activities of AST at day 5 but ALT and ALP at day 5 and 10. The bilirubin concentration was unaffected in both groups.

There were no pathological changes seen in the control groups which had been injected with CCl_4. Increased vacuolation of hepatocytes, congestion of central vein, necrosis and inflammatory infiltration

Table 1: Effects of *Raphanus sativus* extracts on hematological values in rats injected with CCl_4

	Duration (days)					
	5			10		
Treatments	Hb (g dL^{-1})	RBC (10^bµL)	PCV (%)	Hb (g dL^{-1})	RBC (10^bµL)	PCV (%)
Control	13.0±1.2^{NS}	299.6±55^{NS}	43.0±3^{NS}	12.9±2.0^{NS}	308±28^{NS}	35.0±1.8^{NS}
CCl_4	10.8±1.2*	296.0±40^{NS}	34.2±4^{NS}	12.2±4.0^{NS}	299±8^{NS}	32.4±1.6*
200 mg kg^{-1} (methanolic extract)	13.1±0.5^{NS}	305.0±32^{NS}	35.2±2^{NS}	14.1±3.0^{NS}	318±17^{NS}	34.8±1.2^{NS}
400 mg kg^{-1} (methanolic extract)	12.4±0.5^{NS}	299.0±41^{NS}	35.6±3^{NS}	13.2±2.0^{NS}	310±21^{NS}	34.8±1.5^{NS}
200 mg kg^{-1} (water extract)	12.9±0.7^{NS}	301.0±51^{NS}	346.0±1.5^{NS}	12.5±0.9^{NS}	316±36^{NS}	35.7±2.0^{NS}
400 mg kg^{-1} (water extract)	13.2±1.0^{NS}	300.0±20^{NS}	33.9±0.8^{NS}	13.3±1.0^{NS}	309±28^{NS}	34.9±3.1^{NS}

*: $p \leq 0.05$; ^{NS}: Not Significant

Table 2: Effect of *Raphanus sativus* extracts on serum constituents in rats injected with CCl_4

Duratin	Serum constituents	Control	CCl_4	200 mg kg^{-1} Methanolic extract	400 mg kg^{-1} Methanolic extract	200 mg kg^{-1} Water extract	400 mg kg^{-1} Water extract
Day 5	AST	20.2±0.60^{NS}	35.40±2.50*	21.8±0.0^{NS}	19.2±7.90^{NS}	37.6±4.0*	33.6±2*
	ALT	21.4±0.70^{NS}	74.00±0.70*	68.0±0.0*	69.6±5.0*	68.4±0.0*	60.0±0.0*
	ALP	337.9±18^{NS}	397.00±16^{NS}	352.0±0.2^{NS}	362.0±1.4^{NS}	371.8±1.8*	393.0±17*
	BiL	0.8±0.30^{NS}	0.70±0.10^{NS}	0.7±0.0^{NS}	0.6±0.03^{NS}	0.7±0.1^{NS}	0.7±0.07^{NS}
Day 10	AST	25.0±1.80^{NS}	78.80±4.30*	27.8±3.2^{NS}	26.8±6.8^{NS}	26.4±18^{NS}	27.8±1.9^{NS}
	ALT	27.2±0.70^{NS}	115.00±1.3*	59.8±2.8*	57.0±0.3*	49.7±15.0^{NS}	45.0±7.0^{NS}
	ALP	318.9±25^{NS}	358.20±3.0*	322.0±3.0^{NS}	324.0±20^{NS}	343.0±35*	349.0±15^{NS}
	BiL	0.8±0.10^{NS}	1.06±0.20*	0.8±0.30^{NS}	0.7±0.07^{NS}	0.8±0.1^{NS}	0.8±0.05*

*: $p \leq 0.05$; ^{NS}: Not Significant

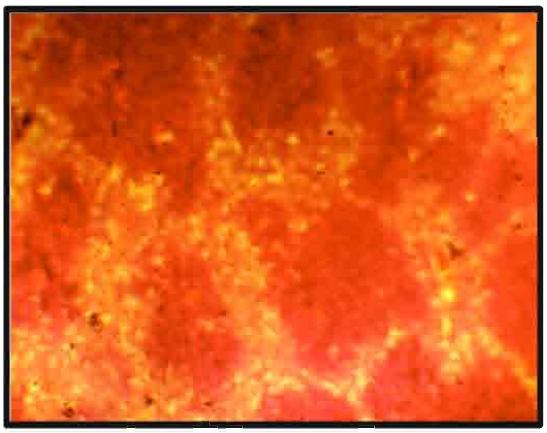

Fig. 1: Liver of animal received CCl_4. Notice the degree of vacuolation and Haemorrahge. H and Ex (200x0.96)

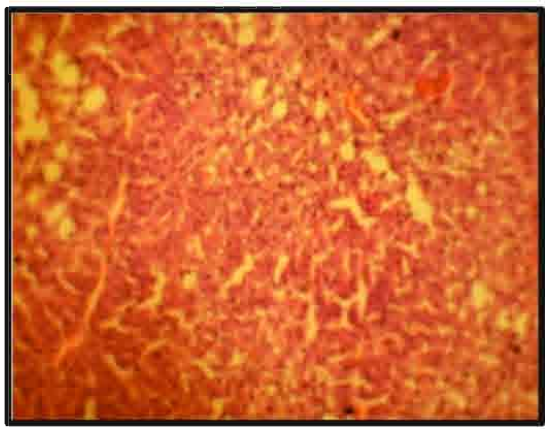

Fig. 2: Liver of animal treated with CCl_4 and 400 mg kg^{-1} methanolic extract of *Raphanus sativus* Notice the degree of vacuolation Haemorrahge. H and Ex (200x0.96)

of lymphocytes beside haemorrhage were evident in the group received CCl_4 (Fig. 1). In group treated with 200 mg kg^{-1} b.wt. methanolic extract the liver showed moderate vacuolation and congestion. In group treated with 400 mg kg^{-1} b.wt. methanolic extract, the liver showed mild vacuolation (Fig. 2).

In the group which had been treated with 200 mg kg^{-1} b.wt. water extract the liver showed vacuolation and congestion (Fig. 3), while the liver in the group which had been treated with 400 mg kg^{-1} b.wt. water extract showed mild vacuolation.

In toxicity experiment there were no clinical signs observed in the experimental rats. However, there were an increase in the body weight in group received the methanolic extract of *Raphanus sativus*. The livers of the control group were normal but in the group received 200 and 400 mg kg^{-1} b.wt. mild congestion in the liver was evident.

There were no significant changes in the hematological values between the groups. Also there were no significant changes in the activities of AST, ALT and ALP and bilirubin concentration.

However, histologically the liver showed slight congestion and dilation of the sinusoids in groups received *Raphanus sativus* (Fig. 4).

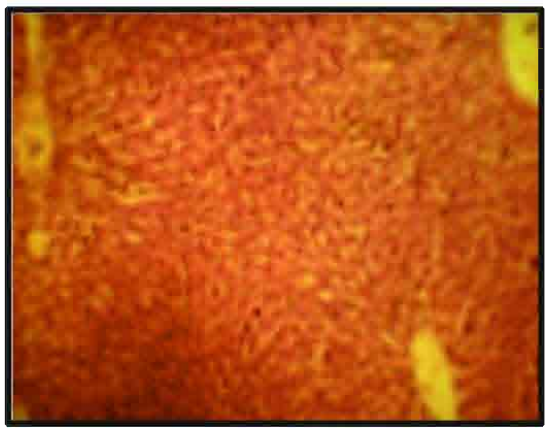

Fig. 3: Liver of animal treated with CCl_4 and 200 mg kg^{-1} water extract of *Raphanus sativus* Notice the degree of vacuolation. H and Ex (200x0.96)

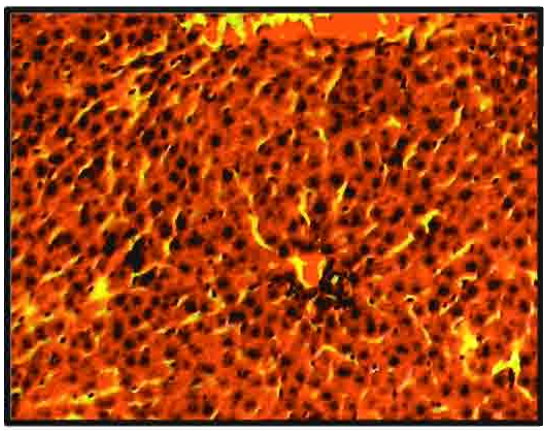

Fig. 4: Liver of animal treated with 200 mg kg^{-1} water extract of *Raphanus sativus* only. Notice the normal structures. H and Ex (200x0.96)

DISCUSSION

The results of the present study indicate that *Raphanus sativus* constitutes flavonoids saponins, alkaloids and coumarins. These findings were similar to those reported by Tawfik *et al*. (1978) but contrary to his finding cycanogycoside were absent. This may be due to parts of the plants used as he used fruits beside seeds. However, Songsak and Lockwood (2002) reported presence of sulforaphene sulforaphane, glucodelydroeurcin and gluconapin.

The present study demonstrated that clinical signs such as nervousness and weight loss were associated with administration of CCl_4. This might be attributed to the liver damage caused by CCl_4. These symptoms were masked by concurrent administration of the extract of *Raphanus sativus*.

CCl_4 has a transient effect on haemaglobin concentration which was counteracted by administration of *Raphanus sativus* since this reduction was unapparent. These results might indicate

that *Raphanus sativus* is free of active constituents that could have a haematolytic or inhibitory effect on blood synthesis (Onyeyilli *et al*., 1998). Experimental liver damage was induced in rats by administration of CCl_4, one of the most potent commouly used hepatotoxicity. CCl_4 is biotransformed under the action of cytochrome P450 into trichloro-pnethyl radical. This leads to the formation of lipid peroxides which playa role in its hepatotoxicity (Edwards *et al*., 1993).

In the present study, there were increased serum enzymes activities (AST, ALT and ALP) and concentration of bilirubin in rats treated with CCl_4 that might support the results obtained by Dobbs *et al*. (2003). This increase in serum enzymes activities were lowered when the plant extract was administered. Some plants have a hepatoprotectie activity due to presence of antioxidants (Takeoka and Dao, 2003; DeFeudis *et al*., 2003). However, the phytochemical analysis of the plant revealed the presence of antioxidants such as flavanoids and saponins. The plant may interfere with free radical formation and its constituents might be responsible for the observed protective role against liver damage; hence its a beneficial action against liver damage induced by CCl_4.

However, the mediated suppression of the increased activities of serum AST, ALT and ALP and concentration of bilirubin by *Raphanus sativus* extracts has probably given protection against CCl_4-induced liver injury as supported by the histopathological findings. Moreover it also provided evidence for the plant hepatoprotective effect as the hepatic cells were undergoing an accelerated regeneration.

Tests for the toxicity of the extracts using the biochemical analysis for liver functions indicated that they were safe. The parameters used to test these functions in treated animals did not differ significantly from those of control animals.

In conclusion, *Raphanus sativus* extract result in a decrease in liver enzymes activities hence it could be a useful and safe agent to act as hepatoprotective. Further work to clarify its mode of action should be investigated.

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