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Full Length Research Paper

Hepatoprotective activity of *Solanum nigrum* extracts on chemically induced liver damage in rats

R. A. M. Elhag[1], S. M. A. El Badwi[2], A. O. Bakhiet[3]* and M. Galal[1]

[1]Medicinal and Aromatic Plants Institute, National Centre for Research, Khartoum, Sudan.
[2]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Khartoum, P. O. Box 32, Khartoum-North, Sudan.
[3]Department of Pathology, Microbiology and Parasitology, College of Veterinary Medicine, Sudan University of Science and Technology P. O. Box 204 Khartoum-North, Sudan.

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The hepatoprotective effects of *Solanum nigrum* water and methanolic extracts were studied in rats injected with 0.2 ml/kg carbon tetrachloride (CCl_4) for 10 consecutive days. *S. nigrum* water extract (250 to 500 mg/kg) was administered to rats injected with CCl_4 for 10 days. The water extracts showed a hepatoprotective effect against CCl_4-induced liver damage, which was evident by the decrease in serum aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphates (ALP) activities bilirubin concentration and by mild histopathological lesions when compared with the group of rats injected with CCl_4 alone. The methanolic extracts of *S. nigrum* (250 to 500 mg/kg) also had hepatoprotective effects with levels of serum AST, ALT, ALP and bilirubin decreasing significantly in animals treated with *S. nigrum* methanolic extract compared to an untreated group.

Key words: *Solanum nigrum*, hepatoprotective, rats, carbon tetrachloride.

INTRODUCTION

Solanum nigrum (Solanaceae) is locally known as Enab Eldib and is widely distributed in various regions of Sudan. *S. nigrum* is used in folkloric medicine as a remedy for hepatomegaly, splenomegaly, edema, gonorrhea, (Chatterjee and Pakrashi, 1995) epilepsy, as a diuretic, emmenagogue and local application for painful swellings, abscess and ulcers, and for gastric ulcers (Akhtar and Munir, 1989). This plant species also has medicinal significance among Africans who include decoction or extract of the leaves in some ethnomedicinal preparations particularly for the treatment of dysentery (Ambasta et al., 1992), hemoptysis, haemorrhoids (Chatterjee and Pakrashi, 1995), wound sepsis, and laxatives (Ambasta et al., 1992).

Alpha ( ), beta (ß) and gamma (γ) carotenase, lutein, lycopene, crytoxanthine, vitamin C, glucose, fructose solasodine, tomatidenol, tigogenin, solamargine and solasonine are the main constituents in aerial parts of this plant.

Carbon tetrachloride (CCl_4) is used as a model to study hepatotoxic effects and causes liver damage through a number of mechanisms. Liver cell injury induced by carbon tetrachloride involves initially the metabolism of carbon tetrachloride to trichloromethyl free-radical by the mixed function oxidase system of the endoplasmic reticulum. It is postulated that secondary mechanisms link carbon tetrachloride metabolism to the widespread disturbances in hepatocyte function. These secondary mechanisms could involve the generation of toxic products arising directly from carbon tetrachloride metabolism or from peroxidative degeneration of membrane lipids. The possible involvement of radical species such as trichloromethyl (CCl_3), trichloromethylperoxy ($OOCCl_3$), and chlorine (Cl) free radicals, as well as phosgene and aldehydic products of lipid peroxidation, as toxic intermediates is discussed. Data do not support the view that an increase in cytosolic free calcium is important in the toxic action of carbon tetrachloride or bromotrichloromethane. In addition, carbon tetrachloride-induced inhibition of very low density lipoprotein secretion by hepatocytes is not a result of elevated levels of cytosolic free calcium (Brattin et al.,

*Corresponding author. E mail: amelbak@sustech.edu or amel33@gmail.com. Tel: +249 185380136 or +249 912347090. Fax: +249 185380136.

1985; Manibusan et al., 2007; Ismail et al., 2009) . Since information on the anti-hepatotoxic properties of *S. nigrum* is lacking, the present study evaluated the protective effect of this plant extract against CCl_4-induced hepatotoxicity in Wister rats.

MATERIALS AND METHODS

Plant materials

S. nigrum, whole plant, was collected from the vicinity of the River Nile bank in Khartoum, (January 2001) and authenticated by the botanists in the Medicinal and Aromatic Plants Research Institute (MARPI), National Centre for Research, Khartoum, Sudan.

Water extract

S. nigrum was shade dried and coarsely powdered. An infusion was made by adding boiling distilled water. The infusion was left for one hour at room temperature, filtered and the filtrate was kept at - 4°C until further use.

Methanolic extracts

The powdered plant tissue was Soxhlet extracted successfully with 70% Methanol following the complete removal of solvent and the final yield was 15% of the original.

Carbon tetrachloride (CCl_4)

Carbon tetrachloride (CCl_4) was obtained from El Gomhouria Company for Trading Pharmaceuticals Chemicals and Medical Appliances. Also known as: El Gomhouria Company, GOMAC, Cairo, Egypt.

Animals

Forty Wistar albino rats weighing between 80 and130 g, of either sex, were obtained from the animal house of the MARPI and maintained under standard environmental conditions and fed a pelleted diet, water was supplied *ad libitum* obtained from El-Gomhorya Company, Cairo, Egypt.

Experimental protocol

Water extracts

Twenty rats were divided into four groups of five animals each. Group 1 (the control) was interperitoneally (i.p.) injected with 0.2 ml/kg of liquid paraffin, each day for 10 days. In group 2 steatosis was produced by injecting i.p. CCl_4 (0.2 ml/kg/day) diluted (1:9) in liquid paraffin for 10 days. Groups 3 and 4 were treated in the same manner as Group 2 except that they were also given (orally) 250 and 500 mg/kg of water extract of *S. nigrum* respectively.

Methanolic extracts

Twenty rats were divided into four groups of five animals each. Group 5 (the control) and Group 6 were treated in an identical manner to Groups 1 and 2. Groups 7 and 8 were treated in the same manner as Group 6 except that they were also given (orally) 250 and 500 mg/kg of methanolic extract of *S. nigrum* respectively.

Blood was collected from the orbital plexus of anaesthetized rats for serum analysis and haematological examination before and after dosing with the plant extract. The serum was separated and kept at -20°C until further analysis.

Biochemical methods

Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) activities were measured by the method of Reitman and Frankel (1957) and Schmidt and Schmidt (1963). Serum alkaline phosphates (ALP) activity was estimated according to the method of Chemie (1972) and total bilirubin concentration was estimated by the method of Jendrassik and Grof (1938).

Haematological methods

Haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cell count (RBC), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) were measured by the method of Schalm et al. (1975).

Histopathological methods

Immediately following sacrifice of the rats, livers were collected and fixed in 10% formalin, embedded in paraffin wax, sectioned at 5u m and stained with haematoxylin and eosin (H&E).

Statistical analysis

The statistical significance of the data was calculated using the Student's t-test (Mendenhall, 1971).

RESULTS

Clinical observations

No clinical signs were seen in any of the groups except Groups 2 and 6 in which the rats showed loss of appetite. These groups also showed alterations in fat content and peticheal haemorrhage in the liver of the rats.

Serobiochemical changes

The activities of AST, ALT, ALP and serum bilirubin concentrations increased significantly ($P<0.05$ to 0.001) at day 5 and day 10 after CCl_4 administration and were significantly reduced ($P<0.05$ to 0.01) after administration of water extracts of *S. nigrum* at 250 mg/kg (Group 3) and 500 mg/kg (Group 4) (Table 1). For the methanolic extracts treatment at 250 and 500 mg/kg showed dose dependent reduction of the elevated AST, ALT, ALP and serum bilirubin concentration values (Table 3).

Haematological changes

The values of Hb, PCV and MCHC were significantly decreased ($P<0.05$ to 0.01) after CCl_4 administration and returned to normal following *S. nigrum* water extract administration (Groups 3 and 4). The PCV values did not

Table 1. Change in serum constituents of rats treated with *S. nigrum* water extract and CCl_4.

Groups	AST U/l (mean ± S.E)			ALT U/l (mean ± S.E)		
	Day 0	Day 5	Day 10	Day 0	Day 5	Day 10
Control group(G1)	19.30 ± 2.10	19.30 ±2.90	19.30 ± 1.30	12.30 ± 0.90	11.80 ± 0.90	11.00 ± 1.08
CCl_4 (G2)	20.30 ± 1.70	118.30 ± 9.30**	417.30 ± 12.20***	11.00 ± 1.20	52.00 ± 3.80**	71.50 ± 6.40**
CCl_4 + 250 mg/kg plants(G3)	19.80 ± 1.30	70.30 ± 4.40**º	73.00 ± 3.80**ººº	11.70 ± 0.90	37.30 ± 2.70**º	48.30 ± 4.40**º
CCl_4 + 500 mg/kg plants(G4)	19.00 ± 3.10	51.30 ± 3.90*ºº	62.00 ± 1.70**ººº	11.00 ± 12.00	26.00 ± 1.01***ºº	24.00 ± 2.20*ºº
	Bilirubin mg /di (mean ± S. E)			ALPU/l (Mean ± S.E)		
			Day 10	Day 0	Day 5	Day 10
Control group(G1)	0.19 ± 0.02	0.18 ± 0.01	0.16 ± 0.02	883.20± 83.60	989.30 ± 82.60	903.90 ± 71.60
CCl_4 (G2)	0.16 ± 0.01	00.48 ± 0.05**	1.21 ± 0.02*	811.70± 86.30	1974.70 ± 96.10**	1768.50 ± 75.50***
CCl_4 + 250 mg/kg plants(G3)	0.19 ± 0.04	0.30 ± 0.03*º	0.27 ± 0.40º	823.20± 72.50	984.40 ± 92.50ºº	993.00 ± 69.80ºº
CCl_4 + 500 mg/kg plants(G4)	0.18 ± 0.03	0.21 ± 0.03ºº	0.21 ± 0.02º	919.00± 67.90	944.00 ± 94.40ºº	886.90 ± 80.20ºº

The difference was found to be significant (* P<0.05 , ** P<0.01, *** P<0.001) when compared with group 1 (control) (ºP< 0.05, ºº P<0.01, ººº P< 0.001) when compared with group 2 (CCL_4)
S.E.= standard error.

Table 2. Haematological changes in rats treated with *S. nigrum* water extract and CCl_4.

Groups	Hb (g/dl) (Mean ± S. E.)		PCV (%) (Mean ± S. E)		MCV (fl) (Mean ± S. E.)	
	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10
Control group (G1)	13.70 ± 0.50	12.75 ± 0.85	35.50 ± 2.30	35.00 ± 2.40	85.10 ± 4.40	81.60 ± 3.50
CCl_4 (G2)	12.50 ± 0.60	7.50 ± 0.78**	37.50 ± 1.90	41.50 ± 0.65	89.20 ± 7.20	139.40 ± 3.60***
CCl_4 +250 mg/kg plants(G3)	12.00 ± 1.10	9.88 ± 0.60*	37.30 ± 0.85	39.10 ± 1.50º	82.29 ± 3.90	81.70 ± 4.30ººº
CCl_4 + 500 mg/kg plants(G4	11.80 ± 0.85	8.35 ± 0.70º	37.00 ±1.47	39.50 ± 1.5º	79.30 ± 4.10	84.10 ± 7.50ºº
	RBC ($\times 10^6$) (Mean ± S.E.)		MCHC (%) (Mean ± S. E.)			
Control group(G1)	14.16 ± 0.06	4.29 ± 0.24	39.40 ± 4.10	32.80 ± 3.10		
CCl_4(G2)	4.25 ± 0.18	2.99 ± 0.12**	34.40 ± 1.10	18.03 ± 1.70*		
CCl_4 +250 mg/kg plants(G3)	4.56 ± 0.22	4.00 ± 0.06ºº	32.50 ± 2.90	25.20 ± 2.80º		
CCl_4 + 500 mg/kg plants(G4	4.64 ± 0.21	4.48 ± 0.27º	31.90 ± 2.78	21.30 ± 3.10º		

The difference was found to be significant (* P<0.05, **P<0.01, *** P<0.001) when compared with group 1 (control) and (ºP< 0.05, ººP<0.01, ººº P< 0.001) when compared with group 2 (CCl_4), S.E. = standard error.

change in Groups 1 to 4 (Table 2). There were significant increases (P<0.05 to 0.01) in PCV and MCV and decreases (P<0.01 to 0.001) in Hb, RBC and MCHC in CCl_4 (Group 6). In Groups 7 and 8 some of these parameters returned to their normal values (Table 4).

Table 3. Changes in serum constituents of rats treated with *S. nigrum* methanolic extract and CCl_4.

Groups	AST U/l (Mean ± S. E.)			ALT U/l / (Mean ± S. E.)		
	Day 0	Day 5	Day 10	Day 0	Day 5	Day 10
Control group(G5)	24.00 ± 2.50	23.30±1.80	23.80 ±2.50	5.50 ±0.60	6.30 ± 0.90	9.00 ±1.30
CCl_4 (G6)	21.00 ± 3.90	410.00 ± 25.30*	470.00 ± 29.00**	5.80 ± 0.70	47.30 ± 2.60**	76.00 ± 4.90***
CCl_4 + 250 mg/kg plants(G7)	21.00 ± 3.90	109.70 ± 11.90*^{oo}	101.30 ± 9.50*^{oo}	5.50 ± 0.60	40.00 ± 3.40**	38.90 ± 49.00**^{oo}
CCl_4 + 500 mg/kg plants(G8)	19.50 ± 3.30	61.00 ± 9.90*^{oo}	50.70 ± 10.60^{oo}	5.50 ± 0.90	22.70 ± 3.90*^{oo}	27.00 ± 2.00*^{oo}
	Bilirubin mg/dl (mean ± S.E)			AP U/l (mean ± S.E)		
Control group(G5)	0.14 ± 0.02	0.14 ± 0.02	0.13 ± 0.01	853.40 ± 65.30	920.20 ± 39.01	901.00 ± 45.40
CCl_4 (G6)	0.12 ± 0.01	0.49 ± 0.03***	0.71 ± 0.06**	959.10 ± 68.90	1628.40 ± 48.10***	1675.60 ± 90.80**
CCl_4 + 250mg/kg plants(G7)	0.14 ± 0.02	0.40 ± 0.02***^{o}	0.19 ± 0.01*^{oo}	874.80 ± 72.10	1232.80 ± 72.20*^{o}	1076.40 ± 16.01*^{o}
CCl_4 + 500 mg/kg plants(G8)	0.13 ± 0.02	0.35 ± 0.04**^{o}	00.13 ± 0.01^{oo}	881.80 ± 69.30	1007.40 ± 36.50^{ooo}	929.50 ± 24.00^{o}

The difference was found to be significant (*P<0.05, ** P<0.01, *** P<0.001) when compared with group 5 (control) and (^{o} P< 0.05, ^{oo} P<0.01, ^{ooo} P< 0.001) when compared with group 6 (CCl_4), S.E. = standard error.

Table 4. Haematological changes in rats treated with *S. nigrum* methanolic extract and CCl_4.

Groups	Hb (g/dl) (Mean ± S. E.)		PCV (%) (Mean ± S. E)		MCV (fl) (Mean ± S. E.)	
	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10
Control group(G5)	12.95 ± 0.77	12.50 ± 0.37	37.50 ± 0.65	37.00 ± 1.10	91.98 ± 3.80	91.10 ± 3.60
CCl_4 (G6)	12.98 ± 0.76	8.30 ± 0.41**	38.40 ±1.40	41.50 ± 0.60*	88.30 ± 4.87	141.98 ± 6.70**
CCl_4 + 250 mg/kg plants(G7)	113.80 ± 0.68	9.70 ± 1.60	39.00 ± 1.20	30.70 ± 1.20*^{oo}	79.10 ± 7.60	83.24 ± 3.80^{ooo}
CCl_4 + 500 mg/kg plants(G8)	14.20 ± 0.83	11.58 ± 0.59^{oo}	37.30 ± 1.10	36.00 ± 0.70	80.40 ± 5.03	84.40 ± 6.12^{ooo}
	RBC (10^6) (Mean± S .E.)		MCH % (Mean± S .E.)			
	Day 0	Day 10	Day 0	Day 10		
Control group(G5)	4.10 ± 0.13	4.10 ± 0.10	33.80 ± 3.16	33.29 ± 0.97		
CCl_4(G6)	4.40 ± 0.18	2.90 ± 0.10***	34.04 ± 5.86	19.90 ± 1.28***		
CCl_4 + 250 mg/kg plants(G7)	5.01 ± 0.43	3.70 ± 00.29	34.09 ± 2.19	31.19 ± 4.60		
CCl_4 + 500 mg/kg plants(G8)	4.70 ± 0.40	4.30 ± 0.13^{ooo}	38.30 ± 5.05	30.28 ± 2.45^{o}		

The difference was found to be significant (* P<0.05, ** P<0.01,*** P<0.001) when compared with group 5 (control) and (^{o}P< 0.05, ^{oo} P<0.01, ^{ooo} P< 0.001) when compared with group 6 (CCl_4), S.E.= standard error.

Histopathological changes

In CCl_4 dosed rats (Groups 2 and 6), there was Necrosis and fatty vacuolation of centrilobular hepatocytes, congestion of hepatic veins and sinusoids, while in Groups 3 and 4 (250 and 500 mg/kg of *S. nigrum* water extract, respectively) only small vacuoles were seen in centrilobular hepatocytes. In Groups 7 and 8 the livers revealed

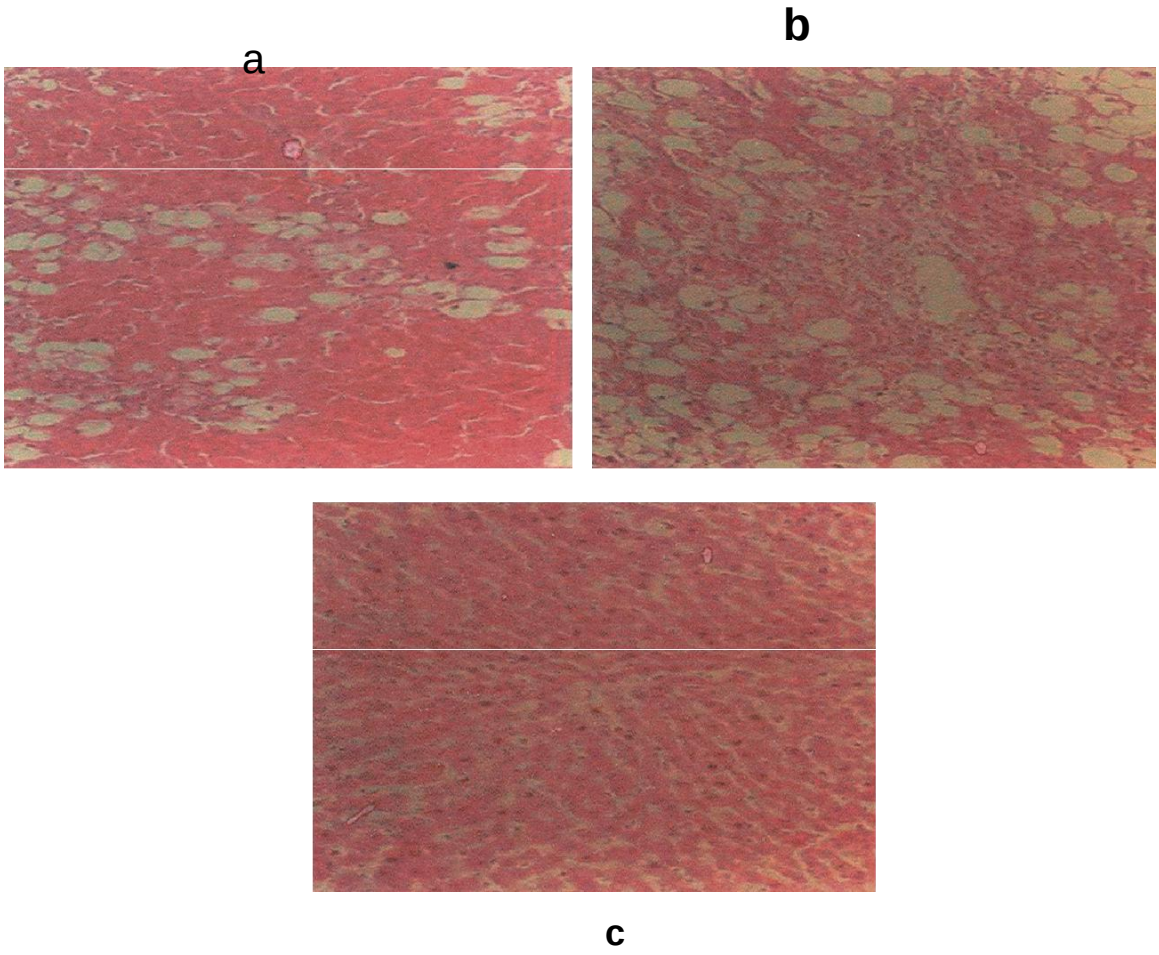

Figure 1. Liver cells of rats treated with methanolic extract of *S. nigrum* and CCl_4 (a) Section of liver treated with CCl_4 showing scattered areas of necrosis and massive vacuolation of hepatocytes involving a large part of the liver parenchyma, (b) Section of liver treated with 250 mg/kg/day of *S. nigrum* methanolic extract showing alterations in fat content, (c) Section of liver of the control group showing normal hepatocytes.

slight changes in the fat content of hepatocytes. The livers from the control Groups 1 and 5 showed no pathological changes Figure 1a, b and c.

DISCUSSION

The results of these investigations showed that water extracts of *S. nigrum* possess hepatoprotective activity against CCl_4 intoxication. Increases in AST, ALT, ALP and serum bilirubin as observed in the CCl_4 treated groups; indicated pathological conditions of the liver as reported by Gopalakrishnan et al. (1989) and Rana and Avadhoot (1992). In Groups 3 and 4 (the test groups) these values decreased especially in Group 4 (500 mg/kg) in a dose dependent manner indicating less damage to the liver.

In addition, the observed decrease in Hb, RBC and MCHC values in CCl_4 treated groups (Groups 2 and 6) is indicative of liver dysfunction. The histopathological images taken for the test groups (Groups 3, 4, 7 and 8) demonstrated less damage than in CCl_4 treated group indicating hepatoprotective effects of the water extract. Increased levels of AST, ALT, ALP and bilirubin were also observed by Sreepriya and Geetha (2005) in rats treated with N-nitrosodiethylamine (DEN) or phenobarbitone, indicating liver dysfunction. The same authors reported decreased levels of these enzymes in rats treated with *Cucurma lona* suggesting that it protected the liver against the carcinogenic effects of (DEN) or phenobarbitone.

The methanolic *S. nigrum* extract also appears to have hepatoprotective effects against CCl_4 induced liver damage. The increased activity of AST, ALT, ALP and bilirubin concentrations due to the CCl_4 group were somewhat counteracted by the methanolic extract tested in Groups 7 and 8. Indeed, it is of note that the enzyme levels in Groups 7 and 8 did not return to the level of the control, Group 5 thus indicating a lower t hepatoprotective effect than the water extract. Also, the histopathological

changes revealed less damage in the treated Groups 7 and 8 compared to the CCl_4 treated Group 6. This therefore indicates some protective effect of the methanolic extract.

In conclusion, the two extracts water and methanolic, of *S. nigrum* have hepatoprotective effect against CCl_4 intoxicated rats. The water extract appears to have a better hepatoprotective effect than the methanolic one which could be due to more polar phyto constituents.

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