Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 2 (1): 43-47, 2008
ISSN 1819-3455

The Effectiveness of *Nigella sativa* Against Liver Damage in Rats

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Abstract: The role of *Nigella sativa* (*N. sativa*) in the prevention of liver damage induced by carbon tetrachloride (CCl_4) was investigated. Twenty five Wister albino rats were allocated into 5 groups named as A, B, C, D and E. Group (A) was given paraffin oil, group (B) was given dimethylsulfoxide, group (C) was given CCl_4 to induced hepatotoxicity, group (D) and (E) were administered with CCl_4 together with 250 and 500 mg kg^{-1} body weight (b.wt.) methanolic extract of *N. sativa* which was dissolved in dimethylsulfoxide, respectively. Rats were scarified after 10 days. There was an increase in the body weights of the control groups A and B at a rate of 2%. However, the body weights in group C, D and E were reduced by 10.3, 9.3 and 10.3%, respectively. There were no significant changes in the blood picture between the control groups and the treated ones on day 10. The mean plasma ALT, AST and ALP were found to be significantly higher in both CCl_4 and *N. sativa* treated groups compared to the controls, but the increase was less in the groups which were treated with *N. sativa* methanolic extract with CCl_4. The bilibrubin concentration was raised from 0.2 to 0.7 in the group treated with CCl_4 and to 0.6 and 0.4 in those treated with 250 and 500 mg kg^{-1} b.wt. of *N. sativa* methanolic extract. The histopathological changes in the livers of the group treated with CCl_4 exhibited severe centrilobular vacuolation and congestion but in the groups treated with 250 and 500 mg kg^{-1} b.wt., these changes were to a lesser extent.

Key words: *Nigella sativa*, carbon tetrachloride, liver damage

INTRODUCTION

Nigella sativa seeds, commonly known as black seed or black cumin, belongs to the family Ranunculaceae, has been traditionally used in Arabian countries (Ali and Blunden, 2003) and Europe (Lautenbacher, 1997) for culinary and medicinal purposes as a natural remedy for a number of diseases and conditions.

There is a common Islamic belief that the black seed is a remedy for all aliments except aging or death.

The seeds are believed to have a carminative, stimulatory and diaphoretic property and are used in the treatment of allergic diseases (Kalus *et al*., 2003).

N. sativa seeds contain fixed oils, proteins, alkaloids, saponin and essential oils. Most of *N. sativa* activities have been attributed to volatile oils mainly thymoquinone and dithymoquione (Lautenbacher, 1997).

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Thymoquinone was found to protect against biochemical and histological markers of liver damage (AL Gharably *et al.*, 1997). El-Dakhakhny *et al.* (2000) found that pretreatment of rats with *N. sativa* for 4 weeks was effective in protecting against CCl_4 and D-galactosamine induced hepatic damage.

Today in the ethanomedicinal practice there is search for treatment that stems from natural substances for curing liver disorders to replace the currently used drugs.

The present study aimed to evaluate the hepatoprotective role of *N. sativa* methanolic extract against a potent hepatotoxin, CCl_4, induced liver damage in Wister albino rats.

MATERIALS AND METHODS

Preparation of *Nigella sativa* Methanolic Extract

Nigella sativa seeds were purchased from local market at Omdurman, Sudan and purified. The seeds were dried and extracted according to Harborne method (1976). Seventy gram of granulated seeds were inserted in soxhlet apparatus (Quick Fit, Ex 5183). The oil have been separated using 100 mL of petroleum ether (40-60%) overnight. The sample was unpacked and left to dry, then repacked again with adding methanol (99.9%) as a solvent to get the polar constituents of the plant. The extract was evaporated till dryness using a rotavapor.

Animals and Treatment

Twenty five healthy adult Wister albino rats of both sex were used in the study in 2005. They were maintained in cages within the Department of Biochemistry, Faculty of Veterinary Medicine, University of Khartoum. Water and feed were provided *ad libitum*. They were kept for 7 days, as an adaptation period.

The rats were divided randomly into 5 groups, 5 rats each. They were treated daily for 10 days. Group A received liquid paraffin at dose rate of 0.2 mL kg^{-1} b.wt. intaperitoneally (i/p). Group B received dimethylsulfoxide I/p at a dose rate of 0.2 mL kg^{-1} b.wt. Group C was injected i/p with 0.2 mL kg^{-1} b.wt. of CCl_4 dissolved in liquid paraffin at a concentration of one volume CCl_4 to 9th volume liquid paraffin. Groups D and E were injected with CCl_4 together with 250 and 500 mg kg^{-1} b.wt. of the *N. sativa* methanolic extract dissolved in dimethylsulfoxide per/os, respectively.

Clinical signs and mortality were recorded. Blood samples were collected from the orbital plexus by means of heparinized capillary glass tubes according to the method of Waynforth (1980). The blood was collected either in EDTA tubes for hematological studies, or in centrifuge tubes to separate serum. After 5 min of centrifugation of the blood at 3000 rpm, supernatant sera were immediately separated for biochemical analysis.

The hematological parameters were determined by sysmex automated hematology analyzer KX-21. The biochemical analysis was carried out on blood sera using Roche Diagnostic Hitachi 902 Analyzer. Serum Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) activities were determined according to Reitman and Frankel (1957). Alkaline phosphatase (ALP) was measured according to Chemie (1972). Total bilirubin concentration was determined according to Jendrassik and Grof (1938).

At the end of the experimental period, animals were necropsed. Livers and kidneys were excised immediately and weighed. Then small pieces of organs were fixed in neutral formalin, embedded in paraffin wax, sectioned at 5 μm and stained with hemotoxylin and eosin (Drury and Wallington, 1980). Data was analyzed using complete randomized design in the experiment. General Linear Model's procedure of SAS (1998) software was used to run the data analysis at 0.05 level probabilities. Body weights and organ body weight ratio were compared using t-test procedure according to Mendenhall (1971).

RESULTS AND DISCUSSION

There were no clinical signs observed in rats of all groups except that two rats which received 250 mg kg^{-1} b.wt. of *N. sativa* methanolic extract looked dull after 5 days.

The control groups showed an increase in the body weight gain by 3.2%. However, the treated groups with CCl_4, 250 and 500 mg kg^{-1} b.wt. of *N. sativa* methanolic extract showed loss in body weight by 10.3, 9.3 and 10.3%, respectively, which was not statistically significant compared to the control. On the other hand no significant changes in the relative liver and kidney weights (Table 1).

There were no significant differences between the control and the treated groups in WBC, RBC, Hb, PCV, MCV and MCHC on day 10 (Table 2).

There was a statistically significant increase in ALT, AST and ALP on day 5 and 10 in the groups received CCl_4 and *N. sativa* methanolic extract compared to control groups A and B. But the groups (D and E) which were given both CCl_4 and *N. sativa* methanolic extract showed significant inhibition ($p>0.05$) of increased plasma levels compared to the group which given only CCl_4 (Table 3). There were no significant differences in the total bilirubin concentration between the treated and control groups. However, the total bilirubin concentration raised from 0.2 on day 5 to 0.7 mg dL^{-1} on day 10 in the group received CCl_4 compared to 0.6 and 0.4 in those treated with 250 and 500 mg kg^{-1} b.wt. of *N. sativa* methanolic extract, respectively (Table 3).

Table 1: Effects of *Nigella sativa* methanolic extract on body weight and relative organ weight of Wister albino rats intoxicated with CCl_4

	Body weight (g)		Relative organ weight (%)	
Groups	Day 0	Day 10	Liver	Kidney
A	147.5±27^a	152.2±26^a	2.6±01^a	0.4±0.1^a
B	137.6±37^a	142.0±34^a	2.7±03^a	0.3±0.0^a
C	147.7±27^a	132.5±19^a	3.1±0.3^a	0.4±0.0^a
D	156.3±19^a	141.7±22^a	3.5±0.5^a	0.4±0.1^a
E	138.9±10^a	124.6±7.5^a	3.7±0.3^a	0.4±0.0^a

Means (±SD) within the same column having the same superscript letter(s) are not significantly different at ($p>0.05$) based on t-test

Table 2: Effect of *Nigella sativa* methanolic extract on hematological values of Wister albino rats intoxicated with CCl_4 on day 10 of the experiment

Groups	WBC (10^6 μL^{-1})	RBC (10^6 μL^{-1})	Hb (g dL^{-1})	PCV (%)	MCV (fl)	MCHC (g dL^{-1})
A	13.9±1.9^a	7.2±0.8^a	14.4±1.1^a	47.5±3.7^a	64.6±2.0^a	30.5±2.0^a
B	11.8±2.9^a	6.7±0.5^a	13.7±0.7^a	43.4±4.8^a	62.0±4.2^a	31.7±2.2^a
C	10.9±3.2^a	6.4±1.2^a	12.5±1.0^a	40.4±8.8^a	62.7±3.9^a	30.9±3.7^a
D	12.9±3.5^a	6.5±1.5^a	12.9±2.5^a	41.5±2.2^a	63.8±0.6^a	29.9±1.8^a
E	12.9±3.9^a	6.7±0.5^a	12.8±0.5^a	42.4±4.9^a	63.6±4.9^a	30.6±3.1^a

Means (±SD) within the same column having the same superscript letter(s) are not significantly different at ($p>0.05$) level based on SAS software method

Table 3: Effect of *Nigella sativa* methanolic extract on some biochemical parameters of Wister albino rats intoxicated with CCl_4 on day 5 and 10

Days	Groups	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})	Bilribuin (mg dL^{-1})
5	A	69.6±10.6^c	152.2±19.1^c	167.1±50.0.2^c	0.3±0.03^a
	B	58.0±9.7^c	163.8±24.5^c	160.0±55.2^c	0.1±0.04^a
	C	1185.2±430.4^a	1626.8±737.6^a	241.2±33.2^a	0.2±0.1^a
	D	944.0±455.5^a	896.8±403.5^b	242.8±65.5^a	0.2±0.8^a
	E	581.3±34.8^b	713.8±187.2^b	224.0±12.8^a	0.2±0.03^a
10	A	85.4±9.6^c	177.8±17.8^c	167.1±21.8^c	0.1±0.02^a
	B	59.4±11.0^c	176.6±21.5^c	129.2±24.8^c	0.2±0.01^a
	C	1160.3±141.1^a	1961.0±311.3^a	453.5±176.5^a	0.7±0.8^a
	D	810.6±246.2^b	1425.4±732.4^b	298.0±65.4^b	0.6±0.3^a
	E	572.0±283.6^b	849.8±262.5^b	293.5±130.5^b	0.4±0.2^a

Means (±SD) within the same column having different small superscript letter(s) are significantly different at ($p>0.05$) level based on SAS software method

Histologically the livers of rats received CCl_4, showed central vein congestion, centrilobular vacuolation and inflammatory cells infiltration compared to the normal livers of the control groups. These changes were less noticed in the treated groups.

The present study demonstrated a decrease in body weights in rats treated with CCl_4 and *N. sativa* methanolic extract. This is in harmony with the previous findings of Zaoui *et al.* (2002) who stated a significant slow down of the body weights occurred in animals treated with *N. sativa*.

Meral and Kanter (2003) showed that *N. sativa* induced a significant increase for the reduced RBC, WBC, PCV and Hb levels. However, El-Sarha *et al.* (1997) reported a significant reduction in RBC and PCV in goats which were treated with *N. sativa* seeds while WBC was significantly increased. In our findings the WBC, RBC, PCV and Hb were unaffected but are in the lower range compared to the control. This indicates that CCl_4 has no inhibitory effect in the synthesis of blood parameters as well as *N. sativa* do not affect the haempoietic system.

Ali and Blunden (2003) studied pharmacological and toxicological properties of *Nigella sativa*. They found that crude extracts of the seeds caused protection against nephrotoxicity and hepatotoxicity induced by either disease or chemicals. They stated that the seeds were characterized by a very low degree of toxicity.

In the present study, ALT, AST and ALP were significantly increased in rats received CCl_4. This increase was improved when using *N. sativa* extract as evidenced by a decrease of the elevated serum levels of ALT, AST and ALP. These results are in line with that reported by Al Gmadi (2003) and Nevin and Dilara (2005) who stated a marked inhibition of increased enzyme plasma level in animals treated with *Nigella sativa* compared to those received CCl_4. Mansour *et al.* (2001) stated that thymoquinone at a doe rate of 12.5 mg kg^{-1} b.wt. given intraperitoneally to mice caused significant reduction of elevated serum enzymes. In contrast, El Dakhakhny *et al.* (2000) and Zaoui *et al.* (2002) found that *Nigella sativa* fixed oil induced no change in hepatic enzymes level and serum bilirubin in treated rats after 4 and 12 weeks, respectively. Daba and Abdel Rahman (1998) tested thymoquinone and slybin in isolated hepatocytes against tetrabutylhydroxide induced liver toxicity and found less degree of protection by thymoquinone.

Histopathological studies provide evidence for the hepatoprotective effect of the plant as hepatic cells have potentiation of regeneration of cells and minimum changes were observed. The biochemical mechanisms involved in the development of CCl_4 hepatotoxicity have long been investigated (Lee *et al*., 2003). It is generally believed that its toxicity is due to lipid peroxidation caused by carbon trichloromethyl radical. Inhibition of lipid peroxidation by *Nigella sativa* a meliorates CCl_4 induced toxicity (Mansour *et al*., 2001). El Dakhakhny *et al.* (2000) claimed that hepatoprotective effect of thymoquinone is due to the presentation of intracellular glutathione.

In conclusion, based on the experimental findings it was suggested that administration of *Nigella sativa* play a role in protection of liver damage caused by CCl_4. Thus it may be valuable as a co-therapeutic agent against conditions associated with liver injury. More detailed studies using different doses and covering longer period of observation are needed before reaching a clear cut conclusion about the future of liver damage treatment by *Nigella sativa*.

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