

A Protective Role Of Nigella Sativa Oil Against The Harmful Effect Of CCl₄ On The Liver Cells

K Al-Kubaisy, M Al-Noaemi

Citation

K Al-Kubaisy, M Al-Noaemi. *A Protective Role Of Nigella Sativa Oil Against The Harmful Effect Of CCl₄ On The Liver Cells*. The Internet Journal of Nutrition and Wellness. 2006 Volume 3 Number 1.

Abstract

The effects of the essential oil Nigella sativa was studied for its antihepatotoxic effects in rats against carbon tetrachloride (CCl₄) which induced hepatocytic damage.

The increased levels of serum enzymes (SGOT, SGPT and SAP) and bilirubin observed in rats treated with CCl₄ were greatly reduced in the animals treated with N.sativa oil and CCl₄. The decreased levels of proteins and albumin observed in rats after treatment with CCl₄ were found to increase in rats treated with N. sativa oil and CCl₄. These biochemical observations were supported by histopathological test of liver sections.

INTRODUCTION

A number of plants used in the traditional medicine were shown to possess antioxidant activity (Kiso et al., 1985; Hikino et al., 1986; Halliwell, 1991; Udem et al., 1997; Aniya et al., 2000 ; Suboh et al., 2004). Nigella sativa, L- a widespread medicinal plant of the Ranunculaceae family has multiple application in many countries, because of its insecticidal action (Dashpande et al., 1974). It improved the T-cells activity (El- kadi and Kandil, 1987), prevents the decrease in hemoglobin level and leukocytes counts caused by cisplatin (Nair et al., 1990). Antimicrobial effect of Nigella sativa have been reported (Hanafy and hatem, 1991; Osfor et al., 1995). It has also been shown to affect smooth muscles of rats and guinea pig (Aqal and shaheen, 1996).

The main constituents of Nigella sativa reported to be present in the oil are thymoquinone, dithymoquinone carvacol and anethole 4-terpinole (Worthen et al., 1998; Bruits and Bucar, 2000).

This study was aimed to produce additional antihepatotoxic information on the effect of N.sativa oil in rats.

MATERIAL AND METHODS

The Nigella sativa oil was obtained from pharm, Saudia Arabia.

Test animals: Wistar rat of either sex weighing (100-150g)

were used.

The animals were housed at 25C and 60-70% RH, they were given balanced diet and water ad libitum.

The 24 rats were divided in four groups:

1. The first group received single dose of carbon tetrachloride 2 ml / kg i.p. on day 3,4 and 5.
2. The second group was treated with 20ml/ kg i.p. of Nigella sativa oil on all 5 days and carbon tetrachloride 2 ml/ kg on day 2 and day 3 about one hour after the administration of thoil.
3. The third group was treated with 30ml/ kg i.p. of Nigella sativa. oil on all 5 days and carbon tetrachloride 2 ml/kg i.p. on day 2 and 3 about one hour after administration of oil
4. The forth group serving as control and received single daily dose of normal saline every day up to 5 days.

After day five, blood sample were obtained by cardiac puncture. The blood samples were allowed to clot for 30 min. The serum samples obtained by centrifugation at room temperature, were used for biochemical estimation.

Serum levels of glutamate oxaloacetate transaminase (GOT),

glutamate pyruvate transaminase (GPT) and Alkaline phosphatase (AP) were measured by standard method using (Boehringer-mannhiem) diagnostic kits. Total protein and albumin determination were carried out using Boehringer diagnostic kits.

The animals were then sacrificed and liver were removed and fixed in 10 % formalin.

The paraffin sections, 6 micron thickness were cut and stained with hematoxylin and eosin (H&E) and observed under the light microscope.

STATISTICAL ANALYSES

Results were expressed as mean \pm S.E. statistical evaluation was performed using (ANOVA) for comparison between groups.

RESULTS

Administration of carbon tetrachloride (CCl₄) caused a significant ($P < 0.001$) increase in the serum levels of hepatospecific enzymes as compared to control group (table 1). Serum bilirubin level was also significantly

($P < 0.001$) increased by CCl₄ treated rats, whereas, total protein and albumin levels were significantly ($P < 0.001$) decreased by CCl₄ treated rats than the control level (table 1).

Treatment of animals with Nigella sativa oil and CCl₄ (i.p.) resulted in reduction of (SGPT), (SGOT), (SAP) and T. bilirubin levels as compared to CCl₄ treated group. While the levels of total protein and albumin were increased as compared with CCl₄ treated group.

Histopathological studies demonstrated that CCL₄ induced sever and generalized degeneration necrosis of hepatocytes, postal infiltration and Kupffer cell hyperplasia as compared to normal group. Pretreatment with Nigella sativa oil markedly reduced the extent of necrosis.

Figure 1

Table 1: Effect of Nigella Sativa oil on biochemical parameters in rats intoxicated with CCl₄ (2ml/ kg), (each value is the mean \pm S.E).

Groups parameters	(I) Control	(II) CCl ₄	(III) CCl ₄ +20 ml oil	(IV) CCl ₄ + 30ml oil
GOT (u/ml)	76.83 \pm 5.50	161.87 \pm 12.73	129.49 \pm 13.02**	102.15 \pm 4.58**
GPT (u/ml)	31.56 \pm 5.11	67.04 \pm 3.01	40.97 \pm 1.53**	36.15 \pm 1.72**
AP(u/ml)	43.40 \pm 1.83	174.38 \pm 4.19	40.98 \pm 1.53**	36.15 \pm 1.73**
T bilirubin (mg%)	1.36 \pm 0.15	8.55 \pm 0.28	2.52 \pm 0.16**	2.07 \pm 0.20**
T Protein (mg%)	8.45 \pm 0.30	5.35 \pm 0.24	6.72 \pm 0.41**	6.57 \pm 0.29**
Albumin (mg%)	3.53 \pm 0.17	0.74 \pm	3.66 \pm 0.15**	2.62 \pm 0.25**

** The mean difference is significant at the 0.001 level
Groups (II) and III are compared with group I. $P < 0.001$
Groups II and IV are compared with group I. $P < 0.001$

Figure 2

Table 2: Histopathological changes in hepatocytes of rats

Microscopic observation	Groups			
	I	II	III	IV
Necrosis:	absent	sever	moderate	moderate
Portal infiltration:	absent	sever	moderate	moderate
Kupffer cell hyperplasia:	absent	sever	moderate	moderate

DISCUSSION

Measurement of the enzyme activity in serum is of important value because it helps to assess the state of the liver and other organs. Normally serum GPT, GOT and AP levels are low, but these enzymes are released into circulation after cellular damage and increased because they are cytoplasmic in location.

It is well known that CCl₄ is converted into the CCl₃ free radicals and this reacts rapidly with oxygen which may contribute to hepatotoxicity and subsequent increase in hepatic enzymes (Cheesmen et al., 1985). Hepatotoxicity action of CCl₄ and leakage of liver enzymes into blood were recorded by several investigators (Hikino et al., 1986; Gadgoli and Mishra, 1995; 1999).

In the present study N-sativa oil was used as protector against harmful effect of carbon tetrachloride. When it was given before CCl₄ treatment it produced protective effects against CCl₄- induced hepatotoxicity. These results were evident from the significant reduction in serum (SGOT), (SGPT) , (SAP) and in total bilirubin. Furthermore, there were an increase in the level of total protein and albumin levels in the treated animals with N. sativa oil. The above results were supported by the histopathological results.

Our observations are consistent with the results of Burits and Bucer (2000) who found that the constituents of the essential oil of N.sativa possessed variable antioxidant activity and

were also found to have an effective free radical scavenging activity, when tested for lipid peroxidation in liposomes.

The anti-protein oxidant activity of N.sativa oil observed in the this study, seems to be due to the richness of this oil with many radical scavenging compounds (Houghton et al., 1995; Ghosheh et al., 1999; Enomoto et al., 2001).

Worthen et al. (1998) reported that thymoquinone and dithymoquinone constituent of N.sativa have an antitumor activity.

Also, Badary et al. (2002) found that thymoquinone has strong antioxidant potential due to the scavenging activity towards free radicals.

Meanwhile, Swamy and Tan (2000) concluded that N.sativa possesses a cytotoxic effect as well as a potent effect on the cellular immune response.

From the results obtained in this study, it is concluded that N.sativa oil may have affinity to repair liver tissue from free radicals and can be used as adjuvant therapy.

References

1. Aniya Y., Shimabukuro H., Shimoji M et al., (2000) Antioxidant and hepatoprotective action of the medical herb *Artemisia campestris* from the Okinawa islands *Boil pharm Bull* 23, 309-312
2. Aqel M. and Shaheen R.(1996) Effects of the volatile oil of *Nigella sativa* seeds on the uterine smooth muscle of rat and guinea pig. *J.Ethenopharmacol.* 52, 23-26.
3. Badary O.A., Taha R.A., Gamel El Din A.M. and Abdel Wahab M.H. (2002) The in vitro antioxidant and pro-oxidant action of thymoquinone and tert butylhydroquinone. *The Egypt J. Biochem. and Mol. Biol.* 20, 1-16.
4. Burits M. and Bucar F. (2000) Antioxidant activity of *Nigella sativa* essential oil- *Phytother.Res.* 14, 323-328
5. Cheesmen, K.H., Albano E.F., Thomasi A. and Stater T. (1985) Biochemical studies on the metabolic activation of halogenated alkenes. *Environment Health perspect* 64, 85-101.
6. Deshpande R.A., Adhikary P. and Tipnis H. (1974) Stored grain pest control agent from *Nigella sativa* and *pogostemon heyneanus*. *Bulletin of Grain Tech.* 12, 232-234.
7. El-Kadi A.and Kandil O.I. (1987) The black seed (*N.sativa*) and immunity, its effect on human T.cell subset Federation proceedings 46(4), 1222.
8. Enomoto S. Asano R. Iwahori Y. et al., (2001) Hematological studies on black cumin oil from the seeds of *Nigella sativa* L. *Boil. pharm Bull.* 24, 307-310.
9. Gadgoli C.H. and Mishra S.H. (1995) Preliminary screening of *Achilla millefolium*, *chochorium intybus* and *capparis spinosa* for antihepatotoxic activity. *Fitoterapia* 66(4), 319-323.
10. Gadgoli C.H. and Mishra S.H. (1999) Antihepatotoxic activity of P-methoxy benzoic acid from *capparies spinosa* *J.Ethenopharmacol.* 66, 187-192.
11. Ghosheh O.A., houdi A.A and Croocs P.A.(1999) High performance liquid chromatographic analysis of the pharmacologically active quinone and related compounds in the oil of the black seed (*Nigella satival.*) *J.pharm. Biomed anal.* 19, 757-762.
12. Halliwell B. (1991) Introduction to free radicals in human disease. *Saudi Med. J.* 12, 13-19.
13. Hanafy M.S.M. and Hatem M.E. (1991) Studies on the antimicrobial activity of *Nigella sativa* seed. *J-Ethenopharmacol.* 34: 275-278.
14. Hikino H., Tohkin M., Kiso Y., et al. (1986) Antiohepatotoxic action of *allium sativum* bulbs. *Planta Medica* 163-168.
15. Houghton P. Zarka R., Heras B., Hoult J. (1995) Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Medica* 61, 33-36.
16. Kiso Y.,Tohkin M.and Hikino H. (1985) Mechanism of antihepatotoxic activity of *atractylon*. *Planta Medica* 50, 97-100.
17. Nair S.C., Salomi M.J., Panikkar B.and Panikkar K.R. (1991) Modulatory effects of *crocus sativus* and *Nigella sativa* extracts on cisplatin-induced toxicity in mice. *Jethenopharm col.* 31, 75-83.
18. Osfor M.M.H., El-khayat Z.A. and El-Naggar M. (1995) Antibacterial effect of *Nigella sativa* oil administration in albino rat.*J.Egypt-Grem. Soc. Zool.* 18 (A) 149-162.
19. Suboh S.M., Bilito Y. and Abuarjai T.A. (2004) Protective Effects of selected Medicinal plants against protein degradation, lipid peroxidation and Deformability loss of oxidatively stressed human erythrocytes. *Phytotherapy Research.* 18, 280-284.
20. Swamy S.H. and Tan B.K. (2000) Cytotoxic and immuno-potentiating effects of ethanolic extract of *Nigella Sativa* seeds. *J.Ethenopharmacol.* 70: 1-7.
21. Udem S.C., Madubunyi I.I., Okoye J.O.A and Anika S.M. (1997) Antihepatotoxic effects of the ethanolic extracts of *combretum dolichopetalum* root bark and *Morinda lucida* leaf. *Fitoterapia LXV III No. 1.* 21-25.
22. Worthen D.R.,Ghosheh O.A. and Crooks P.A. (1998) The in vitro anti-tumor activity of some crude and purified components of black seeds, *Nigella sativa*. *Anticancer Res.* 18, 1527-1532.

Author Information

Khaled Al-Kubaisy

Department of Medical Sciences, Al-Balqa Applied University, Al-Zarka University College

Mohammed Al-Noaemi

Head, Pharmacy Department, Al-Zaytoonah University