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Therapeutic efficacy of *Nigella sativa* Linn. seed extract against CCl_4 induced hepatic injury in wistar rats

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Carbon tetrachloride (CCl_4) intake damages liver. We evaluated therapeutic potential of aqueous extract of *Nigella sativa* seeds against CCl_4 induced liver damage in rats. The hepatic damage induced by CCl_4 @ 1.5 mL/kg, ip was evidenced by a significant increase in the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, protein and urea lipid peroxidation (LPO) as well as reduction in hepatic antioxidant system e.g. reduced glutathione. Hepatic total protein and glucose-6-phosphatase activity were found decreased. Histological studies substantiated the above biochemical findings. However, after 48 h of administration of aqueous extract of *N. sativa* seeds (250, 500 and 750 mg/kg, po) it not only detoxified the toxicity but also reversed LPO, GSH, AST, ALT and serum protein changes at all the three doses. Both higher doses of extract were found effective in monitoring urea, albumin, total protein and G-6-Pase activity. However, on the basis of percent protection highest dose i.e., 750 mg/kg proved better. The result suggests that the aqueous extract of *N. sativa* seeds can be used as a hepatoprotective agent.

Keywords: Carbon tetrachloride, Fennel flower, Hepatoprotective agent, Liver damage

Liver diseases, a major cause of human mortality have become a global concern worldwide. In recent years jaundice and hepatitis have accounted for high death rate[1,2]. The rate of hepatotoxicity due to drugs has been reported to be much higher in developing countries like India (8-30%) compared to that in advanced countries (2-3%) with a similar dose schedule[3,4]. Many antihepatotoxic drugs have been assayed, but liver fibrosis, cirrhosis and its complications have not reduced in many clinical situations[5,6]. Drugs with plant origin received distinctive attention due to their potential to prevent vast array of diseases including hepatic disorders[7]. There are potent indigenous herbal medicines available for hepatic disorders in different parts of the world yet several of them need pharmacological validation[8].

Nigella sativa Linn. (Family Ranunculaceae), commonly called as kalonji and Fennel flower, is a widely used medicinal plant. Its major active principle is thymoquinone that accounts for majority of its pharmacodynamics actions[9]. Pharmacological properties of *N. sativa* such as isulinotropic[10], hypoglycemic[11], antibacterial and antifungal[12], antinociceptive, anti-inflammatory[13,14], neuroprotective[15], anti-histamine, genoprotective[16], anti-ulcer[17] and bronchodilator activities[18] have been reported. Thymoquinone is a pharmacologically active quinone, which possesses several properties including anti-inflammatory, analgesic, antioxidant[19,20], preventive effect on lipid peroxidation (LPO) level during global cerebral ischemia-reperfusion injury in rat's hippocampus[21], antineoplastic[22] and inhibition of eicosanoids generation[23,24]. Here, we studied the modulatory effect of *Nigella sativa* on liver injury caused by CCl_4.

Materials and Methods

Animals and Chemicals—Female Wistar rats (160±10 g, body wt), used in the present investigation, were housed under standard husbandry conditions (25±2 ºC, 60-70% RH and 14: 10 h L:D cycle). The rats were fed on standard pellet diet and water, *ad libitum*. The study was approved by Institutional Ethics Committee (CPCSEA/501/01/A) on the CPCSEA, India guidelines. All the chemicals used in this investigation were of analytical grade and were procured from Sigma Aldrich Co, USA, E-Merck (Germany), Loba, Ranbaxy and BDH, etc.

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Preparation of plant extract—*Nigella sativa* seeds were collected from Central Research Institute of Unani Medicine, Hyderabad. Collected seeds were shade dried, powdered (250 g) and soxhlet extracted with distilled water (4 L) for 18 h. Extract was filtered and lypholised to furnish aqueous extract. Obtained powder was given orally according to body weight.

Preparation of doses—The CCl_4 was given at the dose of 1.5 mL/kg, ip with vehicle (olive oil). Aqueous extract (250, 500 and 750 mg/kg) and silymarin (50 mg/kg) were administered to the animals according to their body weight.

Experimental design—Female rats were divided into following 7 groups of 6 animals each: Gr. 1 served as control and received vehicle only. Gr. 2 was administered seed extract *per se* at the dose of 750 mg/kg, orally. Grs. 3-7 were administered CCl_4 (1.5 mL/kg, ip) once only and gr. 3 was served as experimental control. After 48 h of CCl_4 administration, animals of groups. 4, 5 and 6 were administered with *N. sativa* seed extract @ 250, 500 and 750 mg/kg, po, once only, respectively. Gr. 7 was administered with silymarin (50 mg/kg) as positive control. Animals were sacrificed after 48 h of last treatment. Blood was collected just before the necropsy by puncturing the retro orbital venous sinus by the method of Riley[25].

Blood biochemical assays—Blood samples were allowed to stand at room temperature for 1 h and then centrifuged at 3000 rpm for 10 min and obtained serum was stored at –20 °C. Serum was used to determine the aspartate aminotransferase (AST)[26], alanine aminotransferase (ALT)[26] and protein[27]. Albumin and urea in serum were measured using the Merck kits.

Tissue biochemical assays—Fresh tissues of liver and kidney were immediately processed for the estimation of lipid peroxidation (LPO)[28]. Reduced glutathione (GSH)[29], total proteins[27] and the activity of hepatic glucose-6- phosphatse (G-6-Pase)[30] were measured according to standard methods.

Histopathological study—For histopathological study, liver was fixed immediately in Bouin's fixative and paraffin sections of 5 μm thickness were cut. Hematoxylin-eosin (HE) stained slides were observed under light microscope.

Statistical analysis—Data were subjected to statistical analysis through one-way analysis of variance (ANOVA) significant at 5% followed by Student's *t*-test considering $P \leq 0.05$[31]. Results are presented as mean±SE of 6 animals used in each group.

Results

Blood biochemical observations—The toxic effect of CCl_4 on various blood biochemical parameters were apparent (Table 1). Exposure to CCl_4 caused significant rise in liver marker enzymes i.e. AST and ALT as well as albumin, protein and urea contents in serum ($P \leq 0.05$). Therapy with aqueous extract showed recovery in a dose dependent manner. Lower doses (250 and 500 mg/kg) were also found to be effective, however 750 mg/kg was found to be most effective in recouping the activities of AST and ALT when analyzed statistically. However, in case of

Table 1—Effect of *N. sativa* seed extract on blood biochemistry
[Values are mean ± SE from 6 observations each. Figures in parentheses are % increase over CCl_4 group.]

Treatments	AST (IU/L)	ALT (IU/L)	Albumin (g/dL)	Urea (mg/dL)	S Protein (mg/100mL)
Gr. 1 Control (1.5 mL/kg, ip)	67.0±3.07	48.0±2.65	3.20±0.17	27.0±1.49	32.1±1.77
Gr. 2 NS *per se* (750 mg/kg)	69±3.84	48.6±2.68	3.24±0.17	28.2±1.55	31.9±1.76
Gr. 3 CCl_4	181±10.6#	409±22.6#	5.70±0.31#	64.6±3.57#	64.2±3.54#
Gr. 4 CCl_4+NS (250 mg/kg)	134±7.40*	226±12.4*	5.20±0.28	45.3±2.50*	39.2±2.16*
% protection	42	50	20	51	79
Gr. 5 CCl_4+NS (500 mg/kg)	127±7.02*	187±10.0*	4.60±0.25*	41.7 ±2.30*	36.1±1.99*
% protection	47	61	44	61	87
Gr. 6 CCl_4+NS (750 mg/kg)	120±6.63*	130±7.10*	4.30±0.23*	38.8±2.14*	34.8±1.92*
% protection	53	77	56	67	91
Gr. 7 CCl_4+S (50 mg/kg)	74.8±4.13*	53.6±2.96*	3.51±0.19*	30.9±1.70*	33.7±1.86*
% protection	93	98	88	90	95
F value (at 5% level)	51.4@	172@	20.1@	39.4@	31.7@

P values, ≤0.05; #CCl_4 *vs.* C, *CCl_4+therapy *vs*. CCl_4
NS, *Nigella sativa*; S, Silymarin; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase

Table 2—Effect of *N. sativa* seed extract on tissue LPO
[Values are mean ± SE from 6 observations each. Figures in parentheses are % increase over CCl_4 group.]

Treatments	LPO (n moles of TBARS/ mg protein)	
	Liver	Kidney
Gr. 1 Control (1.5 mL/kg, ip)	0.26±0.01	0.31±0.01
Gr. 2 NS *per se* (750 mg/kg)	0.30±0.01	0.35±0.01
Gr. 3 CCl_4	1.58±0.08#	1.89±0.10#
Gr. 4 CCl_4+NS (250 mg/kg)	0.73±0.04*	1.37±0.07*
% protection	64	33
Gr. 5 CCl_4+NS (500 mg/kg)	0.49±0.02*	1.12±0.06*
% protection	83	49
Gr. 6 CCl_4+NS (750 mg/kg)	0.43±0.02*	1.09±0.06*
Gr. 7 CCl_4+S (50 mg/kg)	0.30±0.01*	0.40±0.02*
% protection	97	94
F value (at 5% level)	165@	121@

P values, ≤0.05; #CCl_4 *vs.* C, *CCl_4+therapy *vs*. CCl_4
NS, *Nigella sativa*; S, Silymarin; LPO, Lipid peroxidation; TBARS, Thiobarbituric acid reactive substances

Table 3— Effect of *N. sativa* seed extract on tissue biochemistry
[Values are mean ± SE from 6 observations each. Figures in parentheses are % decrease over CCl_4 group.]

Treatments	GSH (µ mole/g)		T. Protein (mg protein)		G-6-Pase (mg Pi/100 g/min)
	Liver	Kidney	Liver	Kidney	Liver
Gr. 1 Control (1.5 mL/kg, ip)	7.30±0.40	7.80±0.43	15.2±0.84	14.7±0.81	5.50±0.30
Gr. 2 NS *per se* (750 mg/kg)	7.25±0.40	7.70±0.42	15.1±0.83	14.8±0.81	5.48±0.30
Gr. 3 CCl_4	4.10±0.22#	4.50± 0.24#	10.4±0.57#	9.30±0.51#	3.30±0.182#
Gr. 4 CCl_4+NS (250 mg/kg)	6.00±0.33*	6.10±0.33*	12.6±0.69*	10.8±0.59	4.00±0.22*
% protection	55	48	46	28	32
Gr. 5 CCl_4+NS (500 mg/kg)	6.70±0.37*	6.60±0.36*	14.1±0.77*	11.7±0.64*	4.70±0.25*
% protection	81	64	77	44	64
Gr. 6 CCl_4+NS (750 mg/kg)	6.90±0.38*	7.10±0.39*	14.2±0.78*	11.8±0.65*	4.80±0.26*
% protection	88	79	79	46	68
Gr. 7 CCl_4+S (50 mg/kg)	7.00±0.38*	7.40±0.40*	15.0±0.82*	14.6±0.80*	5.40±0.30*
% protection	91	88	96	98	95
F value (at 5% level)	11.6@	11.3@	6.21@	11.7@	11.9@

P values, ≤0.05; #CCl_4 *vs.* C, *CCl_4+therapy *vs*. CCl_4
NS, *Nigella sativa*; S, Silymarin; GSH, Reduced glutathione; G-6Pase, Glocose-6-Phosphatase

serum albumin and urea, the lowest dose failed to demonstrate any significant reversion, whereas both higher doses were found effective ($P \leq 0.05$). Serum protein was recovered by all three doses ($P \leq 0.05$). *Nigella sativa* seeds extract at the dose 750 mg/kg showed almost same recovery pattern when compared to positive control.

Tissue biochemical observations—Tables 2 and 3 demonstrate the LPO and GSH in liver and kidney as indicator of oxidative damage and antioxidant defense. Biochemical analysis reveals a significant rise in TBARS after CCl_4 exposure in liver kidney when compared to control group ($P \leq 0.05$). Therapy with seed extract at all three doses was found significantly effective in restoring the LPO and GSH in liver and kidney ($P \leq 0.05$). Hepatorenal protein contents and hepatic G-6-Pase activity decreased after CCl_4 administration (Table 3). Dose dependent recovery was found with extract therapy and maximum recovery was seen in higher dose. Highest dose (750 mg/kg) showed significant ($P \leq 0.05$) improvement in the renal protein whereas all three doses were found effective in maintaining activity of G-6-Pase and hepatic protein.

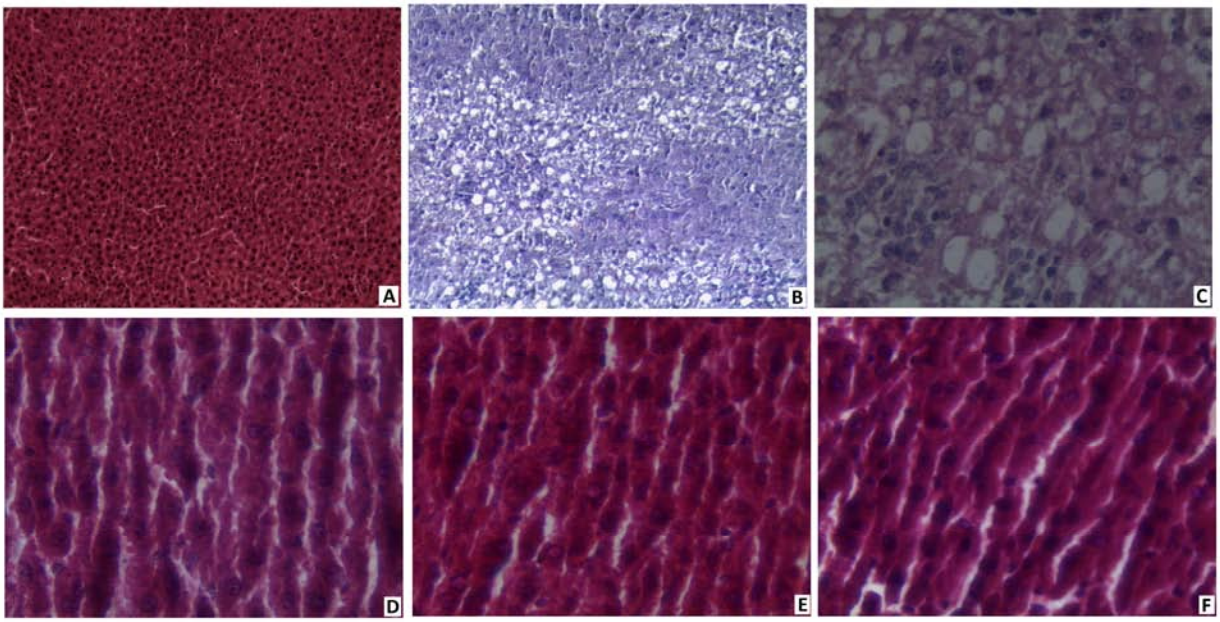

Fig. 1—(A) In histopathological studies of liver, the control group showed normal gross appearance [X 100; H & E]; (B and C) Liver section of CCl_4 treated rats showing massive fatty changes, ballooning degeneration, extensive vacuolization, increased number of pyknotic nuclei and broad infiltration of the lymphocytes with loss of cellular boundaries along with disturbed cord arrangement [X 100 and 400; H & E]; (D) With therapy of *N. sativa* seed extract (250 mg/kg) showing mild improvement in chord arrangement, degenerative changes were clearly visible and vacuoles were observed in the cytoplasm [X 400; H & E]; (E) *N. sativa* seed extract (500 mg/kg) showing marked protective effect as evident by absence of necrosis with better formed hepatocytes [X 400; H & E]; (F) Liver section hepatic of rats treated with 750 mg/kg *N. sativa* seed extract showed almost complete normalization of hepatic architecture, well preserved hepatic cells with clear sinusoidal space and central vein. Degenerative changes were minimum [X 400; H & E].

Histological observations—In histopathological studies of liver, the control group showed normal gross appearance (Fig. 1A). Administration to CCl_4 produced fatty degeneration, distended hepatocytes, compression of sinusoids and extensive vacuolation in cytoplasm. Severe hepatotoxicity was evidenced by distortion of the hepatic architecture, eosinophilic swelling along with hyperchromatic nuclei and necrosis accompanied by vacuolar fatty changes (Figs. 1B and C). Treatment with *N. sativa* seed extract protected the hepatic lesions caused by the toxicant. Cord arrangement was maintained along with maintained sinusoidal space. Leucocytic infiltration was seen only around portal triads. At 250 mg/kg dose it showed relatively less degree of recoupment (Fig. 1D). Presence of hepatic cords at some places indicated protection with extract treatment @ 500 mg/kg (Fig. 1E). The seed extract at 750 mg/kg dose showed reduced necrosis and inflammation with regenerated hepatocytes (Fig. 1F).

Discussion

CCl_4 is a known hepatotoxicant used to evaluate and screen hepatoprotective drugs. CCl_4 metabolism involves generation of free radicals through its activation by drug metabolizing enzymes located in the endoplasmic reticulum[32]. The reactive intermediate is believed to cause LPO and breakdown of cellular membranes[33]. The present results demonstrated that the seed extract treatment effectively recouped most of the serum and tissue biochemical alterations inflicted by the toxicant. Markers of hepatic damage and oxidative stress showed significant recoupment with the extract therapy in dose dependent manner.

The enzymes ALT and AST are important enzymes employed in assessing liver injury[34,35]. Cellular damage releases these enzymes into circulation and, hence it can be measured in the serum. Administration of CCl_4 resulted in significant hepatic damage as shown by the elevated levels of serum marker enzymes in the present study.

The increased levels of serum AST and ALT could be attributed to tissue breakdown, allowing the escape of intracellular enzymes from cytosol into the bloodstream. Therapy with the extract at all the three doses reversed the activity of transaminases and restored them towards normal values indicating maintenance of functional integrity of hepatic cell membrane. These findings are in support of the earlier works on *Momordica cymbalaria*[36], *Luffa acutangula*[37] and Sharbat-e Deenar[38].

In the present study administration of CCl_4 caused significant elevation in LPO level and depletion in the GSH concentration indicating production of lipid peroxidase and reduction of antioxidant potential. Treatment with NS extract significantly decreased the elevated level of LPO and improved the GSH status which may be due to the anti-oxidative property of the plant extract. Jain *et al*.[39] and Khan & Sultana[40] have also demonstrated similar findings with *Momordica dioica* and *Aegle marmelos correa* extracts.

Cellular damage due to LPO also leads to decreased activity of ER membrane bound enzyme such as G-6-Pase[41,42]. Acute administration of CCl_4 caused depletion in the activity of hepatic G-6-Pase in the present study which may be due to the membrane fragility and permeability of the organs. However, oral administration of therapeutic agent restored this enzyme activity as indicated by the improved physiological functions of the hepatic tissue.

Inhibition of protein synthesis indicates disruption and dissociation of polyribosomes from endoplasmic reticulum. We observed significant decrease in hepatic and renal protein content due to CCl_4 exposure. A reduction in protein synthesis during toxicity may also be due to LPO of cell membrane that reveals the severity of hepatotoxicity. Hence, decline in total protein content can be used as an index of the cellular dysfunction severity. Recovery against CCl_4 intoxication by *N. sativa* seed extract is in alignment with the earlier reports on *Vitex trifolia*[43], *Enicostema axillare*[44] *and* propolis[45] which also restored the protein level indicating the improved repair mechanism of liver/kidney due to extract supplementation.

The significant rise observed in albumin and urea concentration after CCl_4 exposure could be attributed to dysfunctional and dystrophic changes in liver and kidney. Oral administration of the extract showed significant protection against CCl_4 exposure, which might be due to the antioxidative property of the extract. All the biochemical effects of extract were comparable with those of silymarin, a proven hepatoprotective agent. These findings were further confirmed by the histopathological studies of liver.

The mechanism of CCl_4-induced liver damage could be attributed to the enzymatic activation (cytochrome P450) of CCl_4 into the trichloromethyl free radical (CCl_3) within the membrane of the endoplasmic reticulum. This is followed by chloromethylation, saturation, peroxidation and progressive destruction of the unsaturated fatty acid of the endoplasmic reticulum membrane phospholipids[46]. These processes are known as lipid peroxidation, leading to its functional and structural disruption[47]. It has been shown that both, *N. sativa* and thymoquinone, inhibit nonenzymatic lipid peroxidation in liposomes[23] and have appreciable free radical scavenging properties[48]. The biologically active compounds in *N. sativa* include thymoquinone, carvacrol, p-cymene, theymohydroquinone, anethole, terpineol, thymole and alpha pinene. The seeds also contain isoquinoline alkaloids called nigellicimine and pyrrazole alkaloids such as nigellidine and nigellicine. Moreover, *N. sativa* seeds also contain alpha-hederin, a water soluble pentacyclic triterpene and saponin, a potential anticancer agent[49,50]. Different components present in *N. sativa* may combine with free radicals and inactivate them thus, suppressing intracellular concentration of free radicals. In the present study, the *N. sativa* seed extract at 750 mg/kg dose level demonstrated significant protective effect in comparison with the lower doses (250 and 500 mg/kg). The possible mechanism of hepatoprotective action of the extract may be due to their antioxidant property as indicated by decrease in LPO and increase of GSH contents. Rest of the biochemical and histopathological parameters studies indicate the structural and functional integrity of the cells and provide further support to proposed mechanism of action. These data suggest that the aqueous extract of *N. sativa* seeds may act as a hepatoprotective agent.

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References

1 Pang S, Xin X & Stpierre M V, Determinants of metabolic disposition, *Pharmacol Toxicol*, 32 (1992) 625.

2 El-Toumy S A, Omara E A, Brouard I & Bermejo J, Evaluation of hepatoprotective effect of *Artimesia Monosperma* against carbon tetrachloride-induced hepatic damage rat, *Aust J Basic Appl Sci*, 5 (2011) 157.

3 Sharma S K, Antituberculosis drugs and hepatotoxicity, *Infect Genet Evol*, 4 (2004) 167.

4 Ramadoss S, Kannan K, Balamurugan K, Jeganathan N S & Manavalan R, Evaluation of hepato-protective activity in the ethanolic extract of *Sida rhombifolia* Linn. against paracetamol-induced hepatic injury in albino rats, *Res J Pharmaceut Biol Chem Sci*, 3 (2012) 497.

5 Mavier P & Mallat A, Perspectives in the treatment of liver fibrosis, *J Hepatol*, 22 (1995) 111.

6 Galisteo M, Sua´rez A, Montilla M P, Utrilla M P, ´Jime´nez J, Gil A, Faus M J & Navarro M C, Antihepatotoxic activity of *Rosmarinus tomentosus* in a model of acute hepatic damage induced by thioacetamide, *Phytother Res*, 14 (2000) 522.

7 Boudet A M, Evolution and current status of research in phenolic compounds, *Phytochemistry*, 68 (2007) 2722.

8 Suresh V & Asha V V, Preventive effect of ethanol extract of *Phyllanthus rheedii* on D-Galactosamine induced hepatic damage in wistar rats, *J Ethanopharmacol*, 116 (2008) 447.

9 Al-Ali A, Alkhawajah A A, Randhawa M A & Shaikh N A, Oral and intraperitoneal LD50 of thymoquinone, an active principle of *Nigella sativa*, in mice and rats, *J Ayub Med Coll Abbottabad*, 20 (2008) 25.

10 Farah K M, Atoji Y, Shimizu Y & Takewaki T, Isulinotropic properties of *Nigella sativa* oil in Streptozotocin plus Nicotinamide diabetic hamster, *Rec Veter Sci*, 73 (2002) 279.

11 El-Dakhakhny M, Mady N, Lembert N & Ammon H P, The hypoglycemic effect of *Nigella sativa* oil is mediated by extrapancreatic actions, *Planta Med*, 68 (2002) 465.

12 Haloci E, Manfredini S, Toska V, Vertuani S, Ziosi P & Kolani H, Antibacterial and antifungal activity assessment of *Nigella sativa* essential oils, *WAST*, 16 (2012) 1198.

13 Abdel-Fattah A M, Matsumoto K & Watanabe H, Antinociceptive effects of *Nigella sativa* oil and its major component, thymoquinone in mice, *Eur J Pharmacol*, 400 (2000) 89.

14 Ghannadi A, Hajhashemi V & Jafarabadi H, An investigation of the analgesic and anti-inflammatory effects of *Nigella sativa* seed polyphenols, *J Med Food*, 8 (2005) 488.

15 Kanter M, Coskun O, Kalayci M, Buyukbas S & Cagavi F, Neuroprotective effects of *Nigella sativa* on experimental spinal cord injury in rats, *Hum Exp Toxicol*, 25 (2006a) 127.

16 Babazadeh B, Sadeghnia H R, Kapurchal E S, Parsaee H, Nasri S & Tayarani-Najaran Z, Protective role of *Nigella sativa* and thymoquinone on serum/glucose deprivation-induced DNA damage in PC 12 cell, *Avicnna J Phytomed*, 2 (2012) 125.

17 Kanter M, Coskun O & Uysal H, The antioxidative and antihistaminic effect of *Nigella sativa* and its major constituent, thymoquinone on ethanol-induced gastric mucosal damage, *Arch Toxicol*, 80 (2006b) 217.

18 Gilani A H, Aziz N, Khurram I M, Chaudhary K S & Iqbal A, Bronchodilator, spasmolytic and calcium antagonist activities of *Nigella sativa* seeds (Kalonji): A traditional herbal product with multiple medicinal uses, *J Pak Med Assoc*, 51 (2001) 115.

19 Ali B H & Gerald B, Pharmacological and toxicological properties of *Nigella sativa* L, *Phytother Res*, 17 (2003) 299.

20 Valizadeh N, Zakeri H R , Amin ansafi G, Shafiee A, Sarkhail P, Heshmat R, Sereshti H & Larijani B, Impact of black seed (*Nigella sativa*) extract on bone turnover markers in postmenopausal women with osteoporosis, *DARU*, 1 (2010) 20.

21 Hosseinzadeh H, Bazzaz B S F & Haghi M M, Antibacterial activity of total extracts and essential oil of *Nigella Sativa* L. seeds in mice, *Pharmacolgyonline*, 2 (2007) 429.

22 Barron J, Benghuzzi H & Tucci M, Effects of thymoquinone and selenium on the proliferation of mg 63 cells in tissue culture, *Biomed Sci Instrum*, 44 (2008) 434.

23 Houghton P J, Zarka R, de las Heras B & Hoult J R, Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation, *Planta Med*, 61 (1995) 33.

24 Hossein H, Eskandari M & Ziaee T, Antitussive Effect of thymoquinone, A constituent of *Nigella Sativa* seeds in Guinea Pigs, *Pharmacologyonline*, 2 (2008) 480.

25 Riley V, Adaptation of orbital bleeding technique to rapid serial blood studies, *Proc Soc Exp Biol Med*, 104 (1960) 751.

26 Reitman S & Frankel S A, colorimetric method for the determination of serum glutamic oxalacetic and glutamic piruvic transaminases, *Am J Clin Pathol*, 28 (1957) 56.

27 Lowry O H, Rosenbrough N J, Farr A L & Randall R J, Protein measurement with Folin's phenol reagent, *J Biol Chem*, 193 (1951) 265.

28 Sharma S K & Krishna Murti C R, Production of lipid peroxides of brain, *J Neurochem*, 15 (1968) 147.

29 Brehe J E & Burch H B, Enzymatic assay for glutathione, *Anal Biochem*, 74 (1976) 189.

30 Banginski E S, Foa P P & Zak B, Glucose-6-Phoaphate, in: *Methods of enzymatic analysis*, 2nd ed. edited by HE Bergmeyer: (VCH, Weinhweim) 1974 115.

31 Snedecor G W & Cochran W G, *Statistical method*, 8th edition. (Affiliated East-West Press, Ames, Iowa), 1994, 217.

32 Ilhan N & Seçkin D, Protective effect of *Nigella sativa* seeds on CCl_4-induced hepatotoxicity, *FÜ Sağlık Bil Dergisi*, 19 (2005) 175.

33 De Groot H & Sies H, Cytochrome P-450, reductive metabolism, and cell injury, *Drug Metabolism*, 20 (1989) 275.

34 Ngaha E O, Akanji M A & Madusolumuo M A, Studies on correlation between chloroquine induced tissue damage and serum enzyme changes in the rat, *Experientia*, 45 (1989) 143.

35 Wegwu M O, Ayalogu & Sule O J, Anti-Oxidant Protective Effects of *Cassia alata* in rats exposed to carbon tetrachloride. *J Appl Sci Environ*, 9 (2005) 77.

36 Koneri R, Balaraman R, Firdous & Kumar V, Hepatoprotective Effects of *Momordica cymbalaria* Fenzl. against carbon tetrachloride induced hepatic injury in rats, *Pharmacologyonline*, 1 (2008) 365.

37 Jadhav V B, Thakre V N, Suralkar A A, Deshpande A D, & Naik A R, Hepatoprotective activity of *Luffa acutangula*

against CCl_4 and rifampicin induced liver toxicity in rats: A biochemical and histopathological evaluation, *Indian J Exp Biol*, 48 (2010) 822.

38 Shakya A K, Saxena M, Sharma N, Shrivastava S & Shukla S, Hepatoprotective efficacy of Sharbat-e-deenar against carbon tetrachloride induced liver damage, *J Environ Pathol Toxicol Oncol*, 28 (2012) 131.

39 Jain A, Soni M, Deb L, Jain A, Rout S P, Gupta V B & Krishna K L, Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica* Roxb. Leaves, *J Ethnopharmacol*, 115 (2008) 61.

40 Khan T H & Sultana S, Antioxidant and hepatoprotective potential of *Aegle marmelos* Correa. against CCl_4-induced oxidative stress and early tumor events, *J Enzyme Inhib Med Chem*, 24 (2009) 320.

41 Chandan B K, Saxena A K, Shukla S, Sharma N & Gupta D K, Hepatoprotective potential of *Aloe barbadensis* Mill. against carbon tetrachloride induced hepatotoxicity, *J Ethnopharmacol*, 111 (2007) 560.

42 Shakya A K & Shukla S, Evaluation of hepatoprotective efficacy of Sharbat-e-deenar against carbon tetrachloride induced liver damage: A unani herbal formulation, *Drug Dev Res*, (2011) 1.

43 Manjunatha B K & Vidya S M, Hepatoprotective activity of *Vitex trifolia* against carbon tetrachloride-induced hepatic damage. *Indian J Pharmacol Sci*, 70 (2008) 241.

44 Gite V N, Deshmukh R D, Sane R T, Takate S B & Pokharkar R D, Hepatoprotective activity of *Enicostema axillare* against CCl_4 induced hepatic injury in rats, *Pharmacologyonline*, 1 (2007) 25.

45 Bhadauria M, Propolis prevents hepatorenal injury induced by chronic exposure to carbon tetrachloride, *J Evid Based Complementary Altern med*, (2012) 1.

46 Reynolds E S & Moslen M T, Environmental liver injury: halogenated hydrocarbons, in: *Toxic Injury of the liver*. edited by E Farber and M Fisher (Marcel Dekker Inc, New York and Basel) 1979.

47 Recknagel R O, A new direction in the study of carbon tetrachloride hepatotoxicity, *Life Sci*, (1983) 401.

48 Burits M & Bucar F, Antioxidant activity of *Nigella sativa* essential oil, *Phytother Res*, (2000) 323.

49 Al-Jassir M S, Chemical composition and microflora of black cumin (*Nigella sativa* L.) seeds growing in Saudi Arabia, *Food Chem*, (1992) 239.

50 Atta-Ur-Rahman, Nigellidine-a new indazole alkaloid from the seed of *Nigella sativa*, *Tetrahedron Lett*, (1995) 1993.