

PROTECTIVE EFFECT OF NIGELLA SATIVA SEEDS ON CCL₄-INDUCED HEPATOTOXICITY

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ABSTRACT

The role of *Nigella Sativa* (NS) was investigated in the prevention of carbon tetrachloride (CCl₄) induced liver fibrosis. Twenty seven adult wistar-albino rats were allocated into three groups (control, CCl₄-induced hepatotoxicity and hepatotoxicity and NS-treated) and after the treatment, rats were sacrificed, blood samples were collected for determination of plasma aminotransferases, malondialdehyde (MDA) levels and erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity. Mean plasma AST, ALT and MDA levels were found to be significantly higher in CCl₄ induced hepatotoxic rats group than in the control group (p<0.05). Mean erythrocyte GSH-Px and SOD levels were found to be significantly lower in CCl₄ induced group compared with the control group and levels of these parameters were found significantly increases after NS treatment. NS may be used in CCl₄-induced hepatotoxicity in rats to prevent lipid peroxidation, increase anti-oxidant defence system activity and also prevent liver damage.

Key Words: *Nigella Sativa*, Carbon tetrachloride, Liver fibrosis.

ÖZET

CCl₄'ün Sebep Olduğu Karaciğer Hasarı Üzerine *Nigella Sativa* Tohumunun Koruyucu Etkileri

Karbon tetrakloridin (CCl₄) indüklediği karaciğer fibrozisinin engellenmesinde *Nigella Sativa*'nın rolü araştırılmıştır. 27 erişkin wistar-albino rat, 3 grup halinde ayrılmıştır (kontrol, CCl₄'ün indüklediği hepatotoksisite ve hepatotoksisite+NS tedavisi) ve tedaviden sonra ratlar sakrifiye edilerek plazma aminotransferaz, malondialdehit (MDA) düzeyleri ve eritrosit süperoksit dismutaz (SOD) ve glutatyon peroksidaz (GSH-Px) aktivitesi saptanmak üzere kan örnekleri toplandı. Ortalama plazma AST, ALT ve MDA düzeyleri CCl₄'ün indüklediği hepatotoksik ratlarda kontrol grubu ile karşılaştırıldığında anlamlı şekilde yüksek bulundu (p<0.05). Ortalama eritrosit GSH-Px ve SOD düzeyleri CCl₄'ün indüklediği grupta kontrol grubu ile karşılaştırıldığında anlamlı şekilde düşük bulundu ve bu parametrelerin düzeyleri NS tedavisinden sonra anlamlı şekilde arttığı bulundu. NS, ratlarda CCl₄'ün indüklediği hepatotoksisitede lipid peroksidasyonunu önlemek, antioksidan savunma sistemini aktivitesini arttırmak ve karaciğer hasarını önlemek için kullanılabilir.

Anahtar Kelimeler: *Nigella Sativa*, Karbon tetrakloridin, Karaciğer fibrozisi.

INTRODUCTION

Chronic liver diseases commonly result in liver fibrosis. Carbon tetrachloride (CCl₄) is widely used for experimental induction of liver fibrosis. CCl₄ is a potent hepatotoxin producing centrilobular necrosis which cause liver injury. It has been widely accepted that liver injury produced by CCl₄ depends on its metabolism to highly reactive trichloromethyl (CCl₃) radical which initiate lipid peroxidation. These substances lead to CCl₄ hepatotoxicity by starting lipid peroxidation in the membranes (1). Reactive oxygen metabolites may play an important role in the inflammation process after intoxication by CCl₄ (2, 3). The cellular generation of reactive oxygen species (ROS) has been associated with, or contributes to, human disease states such as inflammatory diseases, neurodegenerative aging. It has also been found that metabolism CCl₄ involves the production of free radicals through its activation by drug metabolizing enzymes located in the endoplasmic reticulum (4). It has also been associated with tissue re-oxygenation

following hypoxia or anoxia, and cytotoxicity induced by endobiotics and xenobiotics. Cellular sources of ROS production include plasma membrane NADPH oxidase and intracellular cytosolic xanthine oxidase, peroxisomal oxidases, endoplasmic reticular oxidases, and mitochondrial electron transport components.

The seeds of *Nigella sativa* L. (Ranunculaceae), sometimes known as black seed, black cumin or habatul Barakah have long been used in the Middle East as a traditional medicine for a variety of complaints, headache, cough, flatulence, as a choleric, antispasmodic and uricosuric (5-11). In recent years, the seeds have been subjected to a range of pharmacological investigations. NS is presently used in traditional medicine and for culinary preparations in many countries. *N. Sativa* seeds contain 36%–38% fixed oils, proteins, alkaloids, saponin and 0.4%–2.5% essential oil (6). The fixed

oil is composed mainly of unsaturated fatty acids, including the unusual C20:2 arachidic and eicosadienoic acids (12). The essential oil was analysed by Burits and Bucar (13) using GC-MS. Many components were characterized, but the major ones were thymoquinone (27.8%–57.0%), ρ -cymene (7.1%–15.5%), carvacrol (5.8%–11.6%), t-anethole (0.25%–2.3%), 4-terpineol (2.0%–6.6%) and longifoline (1.0%–8.0%). Thymoquinone readily dimerizes to form dithymoquinone (14). Four alkaloids have been reported as constituents of *N. sativa* seeds. Two, nigellicine (15) and nigellidine (16) have an indazole nucleus, whereas nigellimine and its N-oxide (17) are isoquinolines.

This experiment was carried out to investigate the role of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and *Nigella sativa* (NS) on the prevention of carbon tetrachloride (CCl₄)-induced liver fibrosis in rats.

MATERIAL and METHODS

The present study was undertaken to evaluate the effect of NS seeds on carbon tetrachloride (CCl₄)-induced hepatotoxicity. Male Sprague Dawley rats (280–300 g), fed a standard chow diet and given water ad libitum were used in all experiments. Twenty seven adult Wistar-albino rats were used in this study and were divided into three experimental groups: control, CCl₄-induced hepatotoxicity and hepatotoxicity and *N. Sativa*-treated. The control group of rats received only tap water. The CCl₄-induced hepatotoxicity was injected of 0,15ml/100 g body weight CCl₄ subcutaneously for 3 days/week to the rats in both groups. The CCl₄-induced hepatotoxicity + *N. Sativa*-treated group was given extract of *N. Sativa* seeds 800 mg/kg orally every day for 4 weeks. After the 4 week treatment, rats were sacrificed; blood samples were collected for determination of plasma aminotransferases, malondialdehyde (MDA) levels and erythrocyte SOD and GSH-Px activities.

Plasma aspartat transaminase (AST) and alanine transaminase (ALT) levels were measured according to Olympus Kits in Olympus AU600 otoanalyzer.

Erythrocyte GSH-Px assay; GSH-Px enzymes activity was determined using the method previously described by Paglia and Valentine (19).

Erythrocytes SOD activity was measured (using the Sun Y. method) at 560 nm and is expressed as U/g Hb (20).

Thiobarbituric acid reactive substances (TBARS), measured as MDA, were determined in plasma. In the present study, tissue MDA which is the last product of lipid peroxidation was determined spectrophotometrically according to the method described by modified Satoh and Yagi (21, 22).

The statistical significance of differences between the control and treatment groups in these studies was determined by ANOVA (one-way). Data are expressed as mean \pm standard deviation (mean \pm SD). The level of significance chosen was $p < 0.05$.

RESULTS

The present study was undertaken to evaluate the antihepatotoxic effects of *Nigella sativa* seeds against carbon tetrachloride. The carbon tetrachloride injected into rat produced hepatotoxicity manifested as a significant rise in plasma AST, ALT and MDA levels. Administration of *Nigella sativa* showed marked inhibition of increased this increased plasma levels. Pretreatment with NS seeds significant protected the liver against CCl₄ induced hepatotoxicity.

Mean plasma AST, ALT and MDA levels were found to be significantly higher in CCl₄ induced hepatotoxic rats group than in the control group ($p < 0.05$). Mean erythrocyte GSH-Px and SOD levels were found to be significantly lower in CCl₄ induced group compared with the control group, and levels of these parameters were found significantly increases after NS treatment.

Table 1. Effect of NS on plasma AST, ALT, MDA and Erythrocyte GSH-Px and SOD levels in rats intoxicated with CCl₄.

	Control	CCl ₄ intoxicated	CCl ₄ + <i>N. Sativa</i> -treated
AST (U/L)	136.4 \pm 13,8	1043.7 \pm 65.7*	287.3 \pm 34.5*
ALT (U/L)	47.5 \pm 8.9	247.9 \pm 12.6*	48.4 \pm 9.6
MDA (nmol/ml)	3.40 \pm 0.8	7.42 \pm 2.1*	4.46 \pm 0.92
GSH-Px (U/g Hb)	52.5 \pm 6.1	31.6 \pm 5.7*	48.36 \pm 8.2
SOD (U/g Hb)	2546.20 \pm 369.4	1688.4 \pm 320.9*	1912.9 \pm 403.6

* $p < 0.05$, compared to control

DISCUSSION

The mechanism of CCl₄-induced liver damage is considered to be due to the enzymatic activation (cytochrome P450) of CCl₄ into the trichloromethyl free radical (CCl₃) 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 (23). These processes are known as lipid peroxidation, leading to its functional and structural disruption (24). It has been shown that both the fixed oil of *N. Sativa*, as well as thymoquinone (the main compound of the essential oil), inhibit non-enzymatic lipid peroxidation in liposomes (25).

It has also been shown that compounds isolated from *N. Sativa* (including thymoquinone, carvacol, *t*-anethole and 4-terpineol) have appreciable free radical scavenging properties (13). Generation of free radicals may be, at least partially, the basis of many human diseases and conditions. Therefore, the antioxidant action of *N. sativa* may explain its claimed usefulness in folk medicine. This antioxidant property would explain its action against CCl₄ hepatotoxicity (26), liver fibrosis and cirrhosis (27), and hepatic damage induced by *Schistosoma Mansoni* infection (28).

In some countries *N. Sativa* seeds are sold to treat conditions that include liver diseases (29). The mechanism of the hepatoprotective action of thymoquinone is not certain, but may be related to the preservation of intracellular glutathione (the depletion of which by oxidative stress is known to increase the susceptibility of cells to irreversible injury). It has been shown that pretreatment of rats with *N. Sativa* oil for 4 weeks was effective in protecting against carbon tetrachloride and D-galactosamine-induced hepatic damage (30).

The protection against the former hepatotoxicity was partial, while that of the latter was complete. In rabbits, experimental liver cirrhosis and fibrosis (induced by carbon tetrachloride) was shown to be prevented by the prior administration of *N. sativa*. The seed extract improved the histological picture and the indices of oxidative status of the liver (27). In mice, thymoquinone (8 mg/ kg/day for 5 days before and 1 day after carbon tetrachloride was administered with the drinking water) was also found to protect against the biochemical and histological markers of liver damage (31). The protection was suggested to be related to the ability of thymoquinone to inhibit lipid peroxidation.

In the present study, plasma liver enzymes and MDA levels were found to be significantly higher in CCl₄ induced group as compared to the other groups whereas, mean these parameter levels in treated groups were also found decreases. Superoxide dismutase (SOD) acts as a cellular defence element against potentially harmful effects of superoxide ions by catalyzing the dismutation of these ions. We found that CCl₄ induced group had significantly lower mean erythrocyte SOD and GSH-PX activities than the control and NS treatment groups. These results indicate that SOD and GSH-PX which have an antioxidative effect as a defensive role are intracellular enzymes in erythrocytes in CCl₄-induced hepatotoxic rats. CCl₄ induced hepatotoxicity produced in rats leading to hepatic injury, triggers the peroxidation cascade of membrane lipids and results in the generation of toxic radicals. Although both injuries occur due to oxidative stress, it is indispensable to use a correct antioxidant in adequate amount. But, studies in mice and rats have shown that treatment with NS extract significantly protects from cisplatin-induced falls in leukocytes counts, hemoglobin levels, mean osmotic fragility and haematocrit increase, influences leukocytes activities and causes the death of mice lymphocytes in vitro (32). The slowdown of body weight evolution in NS treated rats might be related to the serum lipids and glucose levels decrease as a consequence of a possible reduction in food intake by the drug administration. Other explanations are also possible, like a toxic effect. In conclusion, these results support the traditional use of NS and its derived products as a treatment for the dyslipidemia and the hyperglycemia, and related abnormalities; however, indicate a relative toxicity of this plant extract. Acute and chronic toxicity and the mode of the action of the ns fixed oil must be studied.

This protection was evident from the significant increase in SOD and GSH-Px levels and the significant decrease in serum aminotransferases and MDA. Treatment with *N. sativa* decreased the elevated aminotransferases and MDA concentrations, increased the lowered GSH-Px and SOD concentrations, and prevented lipid-peroxidation-induced liver damage in hepatotoxic rats.

It is concluded that NS decrease the liver enzymes and increase the antioxidant defence system activity in the CCl₄-treated rats. *N. sativa* may be used in CCl₄-induced hepatotoxicity rats to prevent lipid peroxidation, increase anti-oxidant defence system activity and also prevent liver damage.

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