

Protective effect of *Nigella sativa* oil against thioacetamide-induced liver injury in rats

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Abstract

The aim of this study was to investigate the possible protective effect of *Nigella sativa* oil (NSO) and compared with the N-acetylcysteine (NAC), an antioxidant which is known to have liver protective effects, against thioacetamide (TAA)-induced liver injury. Accordingly, we aimed to investigate the toxicity of a hepatotoxic agent, thioacetamide, compared the NSO and NAC liver preventive effects in experimental animal model. Wistar-albino rats were randomly allocated to five groups, each consisting of eight rats, and were subjected to different treatment regimens for 6 days. Alanine aminotransferase (ALT), malondialdehyde (MDA), and protein carbonyl (PC) levels decreased significantly by NSO treatment ($P<0.05$). Besides total bilirubin (T-Bil), cholesterol, superoxide dismutase (SOD), and catalase (CAT) activities increased significantly by NSO treatment ($P<0.05$). NSO treatment did not produce a significant effect on myeloperoxidase (MPO), hydroxyproline (OH-P), and glutathione peroxidase (GSH-Px) activities. These results indicated that NSO improved the liver cell damage caused by TAA, protected against oxidative tissue-damage, contributed to the oxygen radical scavenging activity, increased antioxidant activity and decreased lipid peroxidation. Thus, these results indicate that NSO has protective and antioxidant effects as in the treatment of NAC on the liver injury in rats.

Keywords: Liver, N-acetylcysteine (NAC), *Nigella sativa* oil (NSO), Thioacetamide (TAA), Oxidative Stress

Introduction

Nigella sativa is a dicotyledonous medicinal plant, belonging to the family Ranunculaceae, which is native to Southern Europe, North Africa, and Asia Minor and is widely cultivated in Pakistan and India. The plant reproduces asexually and forms capsulated fruits containing numerous white trigonal seeds, when being exposed to air it turns black thus commonly known as Black Seed [1,2]. Many reports are focused to investigate the pharmacological and therapeutic effects of different parts of this plant. Studies have reported the occurrence of fats, fatty acids, essential oils, enzymes, proteins, peptides, alkaloids, saponins, phenols and polyols in seeds of *N. sativa* and its oil [3-5].

Reviews have reported *N. sativa* as having antioxidant and neuroprotective effects in addition to many other therapeutic effects, such as antitumor, immunopotential, anti-inflammatory, antimicrobial, and hepatoprotective actions [2,6-9]. Recently, most of the biological effects of

N. sativa have been attributed to the active constituent, thymoquinone (TQ), which is present in the volatile oil extract [10,11]. It is used in ethnomedicine to treat ailments and symptoms including, asthma, bronchitis, inflammation, eczema, fever, influenza, hypertension, cough, headache, dizziness, diabetes, kidney and liver dysfunctions, nervous disorders, rheumatism, cancer and related inflammatory diseases, gastrointestinal problems, and overall for general well-being [12-14].

TAA is a hepatotoxin and frequently used for experimental purposes. Acute TAA application results in hepatitis, chronic TAA application have been shown to cause liver cirrhosis [15-16]. Among the different models TAA is a commonly used hepatocarcinogen for induction of experimental liver fibrosis. Based on the TAA-induced rat liver cirrhosis resemble to the human liver disease as it shares a number of metabolic and histological alterations [17,18]. Thus TAA administration 300 mg/kg/day ip. for 3 day to rats in this study [16].

NAC has antioxidant, anticytotoxic and antiapoptotic properties and may therefore be useful in counteracting damaging events of TAA-induced liver injury. This drug has a diversity of applications, because of the chemical properties of the thiol moiety present in its structure. The

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ability of the reduced thiol moiety to sweep reactive oxygen species (ROS) is well-established by NAC [19].

Although the protective effect of *N. Sativa* products on liver injury has been demonstrated by some experimental studies [8,9]. There is not available data in the literature concerning the possible protective effect of NSO, which is a potent antioxidant and free radical scavenger, against TAA-induced liver injury in rats. Therefore, the aim of this study was to investigate the possible preventive action of the NSO and compared with the NAC on the oxidative stress and liver injury induced by TAA in rats.

Materials and Methods

Chemicals

NSO, TAA, 5,5'-dithiobis (2-nitrobenzoic acid; Ellman's reagent), thiobarbituric acid (TBA), trichloroacetic acid (TCA), hypochlorous acid (HOCl), and 2,4-dinitrophenylhydrazine (DNPH) were from Sigma Aldrich GmbH, Germany. NAC (Fluimucil, 10% solution) was purchased from Zambon Group (Italy). All other chemicals and solvents were of the highest grade commercially available.

Animals

The animals involved in this study were produced, maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Inonu University, Animal Ethical Committee. The experiments were performed according to the standards of animal research institute (Reference Number: 2009/51).

Experimental protocol

40 male *Wistar Albino* rats (12 weeks old) weighing around 200-250 g were used. Rats were divided into five groups. Group 1 (n=8), control group, rats were not treated with intraperitoneal injections on the 6 consecutive days of treatment. In group 2 (n=8), TAA group, the rats were given intraperitoneal injections of TAA (300 mg/kg/day) on the 4th, 5th, 6th days of experiment at intervals of 24 hours. In group 3 (n=8), NSO-1+TAA group, the rats were given 1 ml/kg/day NSO by gavage on the 1th, 2nd, 3rd days of treatment and received 1ml/kg/day NSO by gavage together with intraperitoneal injection of TAA (300 mg/kg/day) on the 4th, 5th, 6th days of treatment at intervals of 24 hours. In group 4 (n=8), NSO-2+TAA group, the rats were given 10 ml/kg/day NSO by gavage on the 1th, 2nd, 3rd days of treatment and received 10 ml/kg/day NSO by gavage together with intraperitoneal injection of TAA (300 mg/kg/day) on the 4th, 5th, 6th days of treatment at intervals of 24 hours. In group 5 (n=8), NAC+TAA group, the rats were given intraperitoneal injections of NAC (50ml/kg/day) on the 1th, 2nd, 3rd days of treatment and received daily intraperitoneal injection of NAC (50ml/kg/day) together with intraperitoneal injection of TAA (300 mg/kg/day) on the 4th, 5th, 6th days of

treatment at intervals of 24 hours. Treatment to the whole groups was carried out synchronously. On treatment day 6, all rats were humanely killed, then blood and liver tissues were removed.

Induction of Hepatotoxicity

Hepatotoxicity was induced by intraperitoneal injection of TAA (300 mg/kg/day) on the 4th, 5th, 6th days of experiment at intervals of 24 hours [20]. 30 minutes after TAA injection supportive therapy in all rats by subcutaneous administration of 5% dextrose (10 ml/kg) and NaCl 0.9% with potassium (20 mEq/L) was given every 6 hours to avoid weight loss, hypoglycemia, and renal failure, as previously described [21]. The doses of NSO was selected on the basis of previous works [22].

Histopathological examination

For all histopathological examinations, liver tissues were fixed overnight in 10% formaldehyde solution (dissolved in phosphate buffer, pH 7.4), and then the incubation solution was altered successively from 70% alcohol to 100% alcohol. The incubating solution was further changed from 100% alcohol to xylene, and the tissues were then embedded in paraffin wax, sectioned in 5 μ m slices and stained with H&E.

Biochemical Determination

Liver was washed, finely minced, and submerged in ice-cold 0.2 mmol/L Tris-HCl (pH 7.4) and mechanically homogenized (Ultra Turrax T 25 basic; IKA, Wilmington, NC, United States) at 16000 g for 2 min at 4-8 °C. In order to evaluate the prooxidant-antioxidant balance, we determined the free radicals production by measuring level of MDA and PC, activity of SOD, CAT, and GSH-Px.

Determination of CAT, SOD and GSH-Px Activity

Catalase (CAT) activity was determined according to Aebi's method [23]. The principle of the assay is based on the determination of the rate constant (s^{-1} , k) or the H_2O_2 decomposition rate at 240 nm.

Superoxide dismutase (SOD) activity was determined according to the method of Sun et al. [24] with a slight modification [25]. The principle of the method is based on the inhibition of NBT reduction by the xanthine-xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the sample after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate.

Glutathione peroxidase (GSH-Px) activity was measured by the method of Paglia and Valentine [26]. The enzymatic reaction in the tube, which is containing following items: NADPH, reduced glutathione (GSH), sodium azide, and glutathione reductase, which was initiated by addition of H_2O_2 and the change in absorbance at 340 nm was monitored by a spectrophotometer.

Detection of liver MDA level

The formation of thiobarbituric acid reactive substances (TBARS) during an acid-heating reaction was measured as an index of oxidative stress as previously described [27]. Briefly, the samples were mixed with 1 ml of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67% (Sigma Chemical) and then heated in a boiling water bath for 15 mins. Malondialdehyde (MDA) equivalents were determined by the absorbance at 535 nm using 1,1,3,3-tetramethoxypropane (Sigma Chemical) as an external standard. The results were expressed as nmol/g protein (Lowry assay).

Determination of PC level

The oxidative damage to proteins was assessed by the determination of Protein carbonyl (PC) groups. PC concentration was determined according to the procedure described by Levine et al [28]. Protein carbonyl content was determined spectrophotometrically at 360 nm by the method based on the reaction of the carbonyl group with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone. Protein was determined by the method of Lowry et al [29]. The results were expressed as The calculation of carbonyl content was done using a molar absorption coefficient of 22,000. The results were expressed as nmol/mg prot.

Determination of MPO level

Myeloperoxidase (MPO) (EC 1.11.2.2) activity was determined according to the method of Renlund et al. at this method using a 4-aminoantipyrine/phenol solution as the substrate for MPO mediated oxidation by H₂O₂ and changes in absorbance at 510 nm were recorded. All samples were assayed in duplicate [30]. Protein concentrations in the supernatants were measured using the Bradford's method [31]. Data are presented as U/g protein.

Determination of ALT, AST, ALP, GGT, T-Bil and Cholesterol levels

Total bilirubin (T-Bil) and cholesterol levels were examined using an automated, analyzing system according to the manufacturer's protocol. Plasma concentrations of Alanine transaminase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT) activities were measured using a kinetic UV method defined by the International Federation of Clinical Chemistry, with pyridoxal phosphate and NADH as the cofactors. An Olympus autoanalyzer and commercial kits of the same brand were used for the two

analyses in the routine biochemistry laboratories of Turgut Ozal Medical Center (Malatya, Turkey).

Determination of Hydroxyproline Level in liver tissue

Hydroxyproline (OH-P) levels were determined spectrophotometrically with Woessner's method [32]. The liver tissue were used for hydroxyproline content determination with spectrophotometrically methods as an index of collagen accumulation. Samples were incubated with Chloramine T solution for 20 min at room temperature, and with Ehrlich's solution at 65 °C for 15 min. The absorbance of reaction solutions at 550 nm were measured and amount of hydroxyproline was obtained as microgram.

Statistical Analysis

The suitability of the data to the normal distribution were analyzed with the Shapiro Wilk test. The comparison between groups of normally distributed variables were analyzed by one-way ANOVA. Following in multiple comparisons, the homogeneous variances were analyzed with Tukey test and non-homogeneous variances were analyzed with Tamhane T2 test. These data were expressed as mean $\pm$ SD. The comparison between groups of non-normally distributed variables were analyzed with Kruskal-Wallis H test. Conover test was performed for multiple comparison. These data were expressed as median (min-max). The significance level for all analyses was set at probability of less than or equal to 0.05.

Results

Liver injury produced by TAA was evaluated by biochemical and histopathological examinations.

Hepatic antioxidant enzyme activities

Liver SOD activity in the control and NSO-1+TAA groups were significantly ($P < 0.05$) higher than TAA group. The decrease of SOD induced by TAA intoxication was significantly ($P < 0.05$) inhibited by the treatment with 1 ml/kg/day NSO. SOD activity in the NAC+TAA group was significantly ($P < 0.05$) higher than NSO-2+TAA groups (Table 4).

Liver CAT activity in the NSO-2+TAA group was significantly ($P < 0.05$) higher than TAA group. The decrease of CAT induced by TAA intoxication was significantly ($P < 0.05$) inhibited by the treatment with 10 ml/kg/day NSO (Table 1).

Table 1. Liver cholesterol, OH-P levels and CAT activity in TAA intoxicated rats.

	Cholesterol (mg/dL)	OH-P (mg/g dry tissue)	CAT (k/g protein)
Control	42,29 $\pm$ 5,28	3,83 $\pm$ 0,98 ^b	0,48 $\pm$ 0,36
TAA	4,80 $\pm$ 2,33	2,79 $\pm$ 0,44 ^a	0,47 $\pm$ 0,26
NSO-1+TAA	6,50 $\pm$ 1,00 ^c	3,14 $\pm$ 0,79	0,45 $\pm$ 0,11
NSO-2+TAA	13,00 $\pm$ 4,30 ^b	2,85 $\pm$ 0,47	0,55 $\pm$ 0,15 ^b
NAC+TAA	16,43 $\pm$ 6,86 ^b	3,00 $\pm$ 0,58	0,65 $\pm$ 0,28

Values are given as mean $\pm$ SD. ^a $p < 0.05$ vs control group; ^b $p < 0.05$ vs TAA group; ^c $p < 0.05$ vs NAC+TAA group (n=8).

Table 2. Liver T-Bil, MDA, PC levels in TAA intoxicated rats.

	TB (mg/dL)	MDA(nmol/g wet weight)	PC(nmol/mg prot)
Control	0,1(0,1-0,1)	5,41(2,12-11,7)	0.10(0.10-0.16)
TAA	1,3(0,3-3) ^a	7.33(5.54-18.5) ^c	0.60(0.14-1.51) ^a
NSO-1+TAA	1,5(0,5-7,9) ^b	6.35(3.27-12.28) ^{b,c}	1.16(0.15-1.15) ^{a,c}
NSO-2+TAA	2,0(0,1-3,4) ^b	9.69(5.78-26.1) ^a	0.17(0.09-1.33) ^{b,c}
NAC+TAA	2,5(0,6-3,6) ^b	13.48(6.4-20.34) ^a	0.14(0.12-0.15) ^{a,b}

Values are given as median (min-max). ^ap < 0.05 vs control group; ^bp < 0.05 vs TAA group; ^cp < 0.05 vs NAC+TAA group (n=8).

No significant differences were observed in liver GSH-Px enzymatic activity between all groups. (Table 4).

Lipid peroxidation

Liver MDA level in the NSO-1+TAA groups was significantly (P<0.05) lower than TAA group. The elevation of MDA induced by TAA intoxication was significantly (P<0.05) inhibited by the treatment with 1 ml/kg/day NSO (Table 2).

PC

Liver PC level in the TAA group was significantly (P<0.05) higher than control, NSO-2+TAA and NAC+TAA groups. The elevation of PC induced by TAA was significantly (P<0.05) inhibited by the treatment with 10 ml/kg/day NSO and NAC (Table 2).

MPO

Liver MPO activity in the TAA group was significantly (P<0.05) higher than control and NAC+TAA groups. The elevation of MPO activity induced by TAA was significantly (P<0.05) inhibited by the treatment with NAC. (Table 4).

OH-P

Liver OH-P level in the TAA group was significantly (P<0.05) lower than the control group (Table 1).

Cholesterol and T-Bil. Levels

Cholesterol level in the TAA group was significantly (P<0.05) lower than NSO-2+TAA and NSO+NAC groups. The reduction of cholesterol level induced by TAA was significantly (P<0.05) inhibited by the treatment with 10 ml/kg/day NSO and NAC (Table 1).

T-Bil. level in the TAA group was significantly (P<0.05) lower than NSO-1+TAA, NSO-2+TAA and NSO+NAC groups. The reduction of T-Bil. level induced by TAA was inhibited by the treatment with 1 ml/kg/day NSO, 10 ml/kg/day NSO and NAC (Table 2).

Plasma ALT, AST, ALP, GGT activities

ALT level in the control group was significantly (P<0.05) lower than TAA, NSO-1+TAA, NSO-2+TAA and NSO+NAC groups. Liver ALT level in the NSO-1+TAA groups was significantly (P<0.05) lower than TAA group. The increase of ALT induced by TAA intoxication was significantly (P<0.05) inhibited by the treatment with 1 ml/kg/day NSO. (Table 3). AST level in the NAC+TAA group was significantly (P<0.05) higher than the control group (Table 3).

ALP level in the control group was significantly (P<0.05) lower than TAA, NSO-1+TAA and NSO-2+TAA groups. ALP level in the TAA group was significantly (P<0.05) lower than NSO-1+TAA, NSO-2+TAA group (Table 3).

Table 3. Liver ALT, AST, ALP activities in TAA intoxicated rats.

	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	46,29±7,74	77,86±17,27 ^c	223,14±43,26
TAA	5339,29±1886,62 ^a	10836,14±7269,22	342,29±46,48
NSO-1+TAA	4984,67±2295,74 ^{a,b}	8138,17±5322,17	552,50±97,85 ^{a,c}
NSO-2+TAA	3586,63±2138,95 ^a	5730,00±4340,52	506,00±92,02 ^a
NAC+TAA	4569,38±770,45 ^a	5576,13±1990,34 ^a	304,00±29,73

Values are given as mean ± SD. ^ap < 0.05 vs control group; ^bp < 0.05 vs TAA group; ^cp < 0.05 vs NAC+TAA group (n=8).

Table 4. Liver GGT, GSH-Px, MPO and SOD activities in TAA intoxicated rats.

	GGT(U/L)	GSH-Px(u/g protein)	MPO (U/g/prot)	SOD (u/mg protein)
Control	1(1-3)	1.01(0.99-1.18)	119,7(18,4-143,4) ^b	0.31(0.24-0.39) ^b
TAA	6(4-10) ^{a,c}	1.02(0.75-2.40)	190(145-239) ^{a,c}	0.21(0.12-0.32) ^{a,c}
NSO-1+TAA	3,5(1-20) ^a	1.09(0.68-1.2)	156(97-236) ^a	0.26(0.16-0.33) ^b
NSO-2+TAA	2(1-5) ^c	0.96(0.60-1.15)	167(91-255) ^a	0.20(0.10-0.24) ^c
NAC+TAA	2(1-3) ^a	0.76(0.23-1.26)	93(65-148) ^b	0.20(0.17-0.41)

Values are given as median (min-max). ^ap < 0.05 vs control group; ^bp < 0.05 vs TAA group; ^cp < 0.05 vs NAC+TAA group (n=8).

GGT level in the control group was significantly ($P<0.05$) lower than NSO-1+TAA and NSO-2+TAA groups. Likewise GGT level in the NSO-1+TAA group was significantly ($P<0.05$) higher than TAA, NSO-2+TAA, NSO+NAC groups (Table 4).

Results of liver histopathology

In the TAA group, liver necrosis and inflammation were significantly higher than in the control and TAA+NAC

groups. In the control group, liver necrosis and inflammation were significantly lower than in all groups (Fig 1). As can be seen in the figures, necrosis and inflammation in the liver significantly increased in the TAA, NAC+TAA, NSO-1+TAA and NSO-2+TAA groups, respectively.

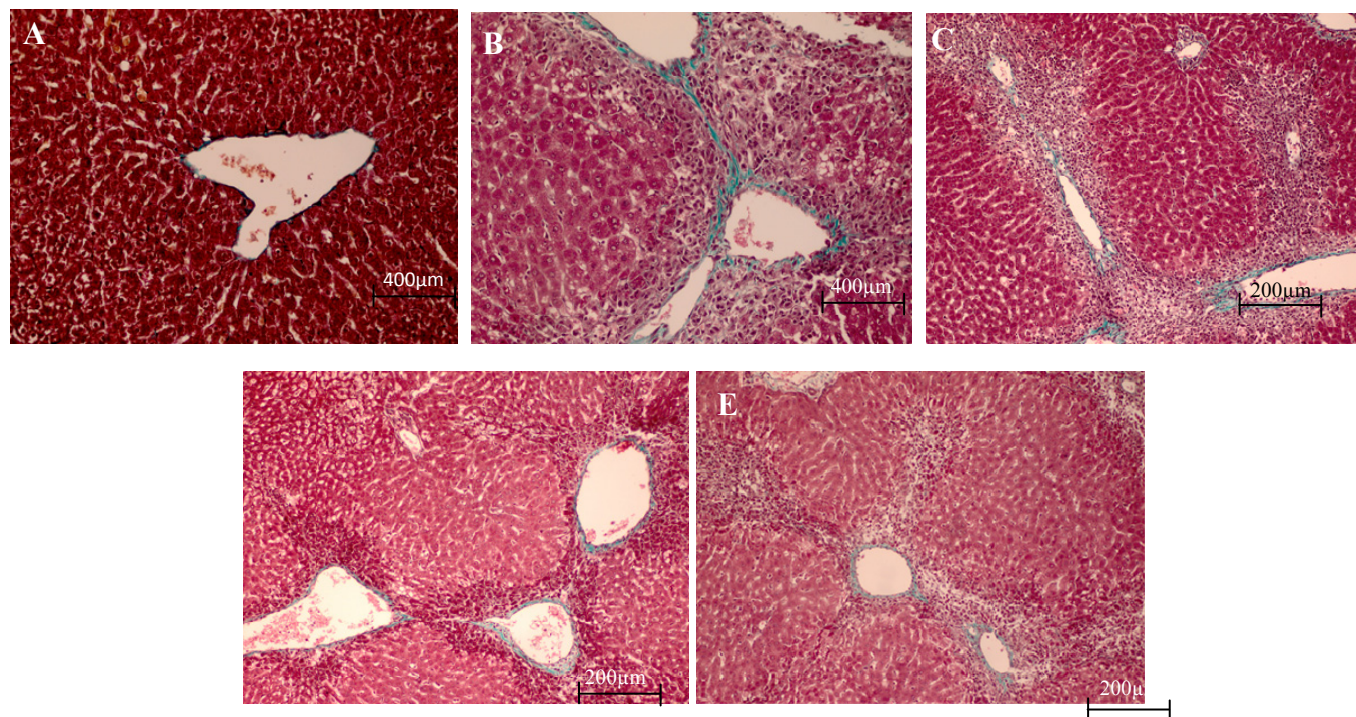


Figure 1. Histopathological examination of liver tissue of rats under various treatment conditions. Livers were sectioned and stained with Trichrome by standart techniques. (A) Normal liver histology in the control group (400x). Showing normal hepatic architecture. (B) Severe liver necroinflammation in the TAA group (400x). Showing numerous vacuolation, many fibroblasts and inflammation. (C) Severe liver necroinflammation in the NSO-1+TAA group (200x). (D) Severe liver necroinflammation in the NSO-2+TAA group (200x). Showing a reduction in the histological injury as a few hepatic nodules. (E) Severe liver necroinflammation in the NAC+TAA group (200x). Showing a few reduction in the histological injury.

Discussion

The liver plays a crucial role in the metabolic elimination of the drugs and other foreign compounds, thus making it an important target for toxicity. Chemical toxins (including TAA) are often used as the model substances causing experimental hepatotoxic injury in both *in vivo* and *in vitro* conditions [33]. TAA is a potent hepatotoxic agent. The toxicity of TAA results from its bioactivation in the liver to reactive metabolites, causing the production of ROS responsible for oxidative stress [34]. These events are followed by glutathione depletion, a reduction in SH-thiol groups and oxidation of cell macromolecules, including lipids.

NS seed is one of the most promising medicinal plant with many therapeutic effects. One of its most important effects is its antioxidant activity. It acts as an anion scavenger that neutralizes oxygen radicals.

NAC has antioxidant, anticytotoxic and antiapoptotic properties and may therefore be useful in counteracting

liver injuries induced by TAA and treatment of liver. For comparison, we used NAC, which has known therapeutic effects linked to the antioxidant and free radical scavenging action, and is commonly used as an antidote against drug-induced hepatopathies.

In the present study, SOD, CAT and GSH-Px were measured as an index of the antioxidant status of tissues. In this study, significant reduction of antioxidant enzymes (SOD, CAT) activity showed oxidative stress of the liver. We reported that NSO markedly elevated the levels of SOD and CAT, and protected the liver from TAA, indicating that inhibition of the oxidative cascading stress was one of the main mechanisms in TAA-induced liver injury in the rats. Our observations also showed that daily treatment with NSO significantly increased antioxidant enzymes activities. These results are similar to previous studies. Previous studies were reported that, NSO enhanced synthesis of the endogenous antioxidant enzymes [35]. These results denoted that NSO proved to have oxygen radical scavenging and antioxidant properties. In

our study, decreased activities of hepatic SOD and CAT of TAA-intoxicated rats may be due to oxidative stress induced inactivation and/or exhaustion. NSO treatment may contribute to the antioxidant system in the liver following TAA administration. These agents may protect against TAA-induced toxicity by overcoming the inactivation of antioxidant enzyme systems and upregulating of SOD and CAT activities in the liver.

TBARS are indicators of the oxidative stress, since they are generated from the breakdown of lipid peroxyl radicals. Our findings indicated that MDA content was enhanced by TAA administration and free radicals being released in the liver. Treatment of NSO decreased the MDA in the liver of rats. NSO has an ameliorating effect on TAA-induced liver injury, according to earlier studies [36]. Similarly, an earlier study showed that MDA level increased in TAA-induced hepatic cirrhosis [37]. In this study, NSO may protect the liver from histological damages induced by TAA through antioxidative effect. The protective effect of NSO could be attributed to the inhibition of lipid peroxidation which leads to stabilizing plasma membranes and preventing the release of hepatic enzymes. Thus, using NSO may be useful for liver injury positively.

In the current study, we measured PC formation as an index of oxidative damage in the liver. Treatment with NSO and NAC were significantly decreased the increased liver PC content level. These results are similar to previous studies [38]. This finding indicated that the increase of PC formation might be a result of the oxidative stress and the decrease of detoxification process. NSO and NAC protected against the induction of TAA toxicity due to possible oxidative stress as well as the imbalance between the production of ROS and endogenous antioxidant defence systems.

The MPO is enzymatically active in cases of acute liver injury and cirrhosis. In the present study, treatment with NAC was significantly decreased the increased liver MPO level. These results are similar to previous studies [39]. The increased MPO activity indicated that the entrance of neutrophils in the liver could play a central role in liver injury induced by TAA. Nevertheless, our data showed that the activity of MPO in the liver was reduced with the NAC treatment. Consequently, the results obtained suggest that NAC possesses anti-inflammatory properties and prevented the activation of neutrophils in addition to producing antioxidant effects in rat. Thus, using NAC may be useful for liver injury positively.

Serum enzymes, including AST, ALT and ALP are mainly monitored for the evaluation of liver damage. It is reported that TAA may cause increase in AST, ALT and ALP activities [40]. These alterations in enzyme levels may depend on exposure time and doses. These results suggest that increased serum enzyme activities are associated with hepatic degeneration and it is likely that TAA induced biochemical changes in liver. In the present study, the

elevated activities of ALT and ALP may indicate the loss of structural integrity of cell membranes in the liver and are also associated with massive necrosis of the liver. Treatment with NSO caused a decrease in the activity of the ALT enzyme, which may be a consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by TAA.

The histological observations basically supported the results obtained from the plasma enzyme assays. It has been reported that TAA increased inflammatory cells infiltration, fatty changes, accumulation of collagenous fibers, and necrotic damage [41]. Treatment with both NSO showed the normal architecture of the liver, suggesting the reversal of the damage to a large extent. It is suggested that NSO acts by its stabilizing effect on the plasma membrane. NSO attenuates tissue degeneration, thus acting as an effective antioxidant.

In conclusion, our data obtained show some different results according to the applied TAA and NSO doses, and investigating time used in the experiments. NSO has a potent hepatoprotective action upon TAA-induced hepatic damage in rats. The TAA-induced hepatotoxicity may be related with oxidative damage. NSO treatment found to have an ameliorating effect on TAA-induced liver injury, according to histopathological and biochemical findings. The hepatoprotective effects of NSO may be due to its ability to block the bioactivation of TAA. Treatment with NSO decreased the harmful effects of TAA both by inhibiting free-radical formation and by restoration of the antioxidant systems. NSO improved the liver cell damage caused by TAA, protected against oxidative tissue-damage, contributed to the oxygen radical scavenging activity and increased antioxidant activity. The effects of NSO were similar to that of NAC. Thus, NSO has protective and antioxidant effects as in the treatment of NAC on the liver injury in rats. Consequently, treatment with NSO reduced the subsequent TAA injury in rat liver, demonstrated by normalized liver histopathology, decrease in lipid peroxidation, improvement the antioxidant enzyme status and cellular protein oxidation.

In summary, our data indicated that NSO has protective effects against TAA-induced hepatotoxicity through attenuating lipid peroxidation, improving the antioxidant enzyme status and reducing histopathological damages. Further investigations on the molecular mechanism of effect of the NSO with genetics and immunochemistry studies are required and may have a considerable impact on future clinical treatments of patients with liver injury.

References

1. Khan MA. Chemical composition and medicinal properties of *Nigella sativa* Linn. *Inflammopharmacology*. 1999;7(1):15-35.
2. Salem ML. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. *International Immunopharmacology*. 2005;5(13-14):1749-70.

3. Mohamadin AM, Sheikh B, Abd El-Aal AA, Elberry AA, Al Abbasi FA. Protective effects of *Nigella sativa* oil on propoxur induced toxicity. *Pest Biochem Physiol.* 2010;98:128-34.
4. Cheikh-Rouhou S, Besbes S, Blecker C, Deroanne C, Attia H. *Nigella sativa* L. Chemical composition and physicochemical characteristics of lipid fraction. *Food Chem.* 2007;101:673-81.
5. Bourguou S, Ksouri R, Bellila A, Skandran I, Falleh H, Marzouk B. Phenolic composition and biological activities of Tunisian *Nigella sativa* L. shots and roots. *C Biol.* 2008;331(1):48-55.
6. Ashraf SS, Rao MV, Kaneez FS. *Nigella sativa* extract as a potent antioxidant for petrochemical-induced oxidative stress. *J Chromatogr Sci.* 2011;49(4):321-6.
7. Kanter M. *Nigella sativa* and derived thymoquinone prevents hippocampal neurodegeneration after chronic toluene exposure in rats. *Neurochem Res.* 2008;33(3):579-88.
8. Al-Ghamdi MS. Protective effect of *Nigella sativa* seeds against carbontetrachloride-induced liver damage. *Am J Chin Med.* 2003;31(5): 721-8.
9. Mahmoud MR, El-Abhar HS, Saleh S. The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. *J Ethnopharmacol.* 2002;79(1):1-11.
10. Gali-Muhtasib H, El-Najjar N, Schneider-Stock R. The medicinal potential of black seed (*Nigella sativa*) and its components. In: Khan MTH, Ather A, eds, *Lead Molecules from Natural Products: Discovery and New Trends.* Elsevier BV, 2006;133-53.
11. Salem ML. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. *Internet Immunopharmacol.* 2005;5(13-14):1749-70.
12. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. *Phytother Res.* 2003;17(4):299-305.
13. ML Salem. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. *Int. Immunopharmacol.* 2005;5:1749-70.
14. MA Khan. Chemical composition and medicinal properties of *Nigella sativa* Linn. *Inflammopharmacology.* 1999;7(1):15-35.
15. Doğru-Abbasoğlu S, Kanbağlı Ö, Balkan J, Çevikbaş U, Aykaç-Toker G, Uysal M. The protective effect of taurine against thioacetamide hepatotoxicity of rats. *Human Exp Toxicol.* 2001;20(1):23-7.
16. Balkan J, Doğru-Abbasoğlu S, Kanbağlı Ö, Çevikbaş U, Aykaç-Toker G, Uysal M. Taurine has protective effect against thioacetamide-induced liver cirrhosis by decreasing oxidative stress. *Human Exp Toxicol.* 2001;20(5):251-4.
17. H Dashti, B Jeppsson, I Hagerstrand. Thioacetamide- and carbontetrachloride-induced liver cirrhosis. *Eur Surg Res.* 1989;21(2):83-91.
18. Muller A, Machnik F, Zimmermann T, Schubert H. Thioacetamide-induced cirrhosis-like liver lesions in rats-usefulness and reliability of this animal model. *Exp Pathol.* 1988;34(4):229-36.
19. Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide and hypochlorous acid. *Free Radic Biol Med.* 1989;6(6):593-7.
20. Shapuro H, Ashkenazi M, Weizman N, Shahmurov M, Aeed H, Bruck R. Curcumin ameliorates acute thioacetamide induced hepatotoxicity. *J Gastroenterol Hepatol.* 2006;21(2):358-66.
21. Bruck R, Aeed H, Avni Y, Shirin H, Matas Z, Shahmurov M, Avinoach I, Zozulya G, Weizman N, Hochman A. Melatonin inhibits nuclear factor kappa B activation and oxidative stress and protects against thioacetamide induced liver damage in rats. *J Hepatol.* 2004;40(1):86-93.
22. Kokdil G, Tamer L, Ercan B, Ilcim A, Aras N, Atik U. Effects of *Nigella unguicularis* fixed oil on blood biochemistry and oxidant/antioxidant balance in rats. *J Ethnopharmacol.* 2005;99(1):131-5.
23. Aebi H. Catalase. In: Bergmeyer HU ed. *Methods of Enzymatic Analysis.* New York: Academic Press. 1974;673-7.
24. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem.* 1988;34(3):497-500.
25. Durak I, Yurtaslan Z, Canbolat O, Akyol O. A methodological approach to superoxide dismutase (SOD) activity assay based on inhibition of nitroblue tetrazolium (NBT) reduction. *Clin Chim Acta.* 1993;214(1):103-4.
26. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med.* 1967;70(1):158-69.
27. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol.* 1990;186:421-31.
28. Levine RL, Garland D, Oliver CN. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol.* 1990;186:464-78.
29. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. *J Biol Chem.* 1951;193(1):265-75.
30. Renlund DG, Macfarlane JL, Christensen RD, Lynch RE, et al. Quantitative and sensitive method for measurement of myeloperoxidase. *Clin Res.* 1980;28:75A.
31. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976;72:248-54.
32. Woessner JB. The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. *Arch Biochem Biophys.* 1961;93:440-7.
33. Domenicali M, Caraceni P, Giannone F, Baldassarre M, Lucchetti G, Quarta C, Parti C, Catani L, Nanni C, Lemoli RM, Bernardi M. A novel model of CCl₄-induced cirrhosis with ascites in the mouse. *Journal of Hepatology.* 2009;51(6):991-9.
34. Pallottini V, Martini C, Bassi AM, Romano P, Nanni G, Trentalancia A. Rat HMGCoA reductase activation in thioacetamide-induced liver injury is related to an increased reactive oxygen species content. *J Hepatol.* 2006;44(2):368-74.
35. Zaoui A, Cherrah Y, Mahassine N, Alaoui K, Amarouch H, Hassar M. Acute and chronic toxicity of *Nigella sativa* fixed oil. *Phytomedicine.* 2002;9(1):69-74.
36. Burits M, Bucar F. Antioxidant activity of *Nigella sativa* essential oil. *Phytother Res* 2000;14(5):323-8.
37. Brown KE, Brunt EM and Heinecke JW. Immunohistochemical detection of myeloperoxidase and its oxidation products in Kupffer cells of human liver. *Am J Pathol.* 2001;159(6):2081-8.
38. Uz E, Uz B, Selcoki Y, Bayrak R, Kaya A, Turgut H.F, Mete E, Karanfil A, İlhan A, Sahin S, Erdemli K. Cardioprotective Effects of *Nigella sativa* Oil on Cyclosporine A-Induced Cardiotoxicity in Rats. *Basic Clin Pharmacol Toxicol.* 2008;103(6):574-80.
39. Tayman, C. Cekmez, F. Kafa, I.M. Canpolat, F.E. Cetinkaya, M. Tonbul, A. Uysal, S. Tunc, T. Sarici, S.U. Arch Bronconeumol. 2013;49(1):15-21.

40. Anbarasu C, Raj Kapoor B, Bhat KS, John Giridharan, A Arul Amuthan, Satish K. Protective effect of *Pisonia aculeata* on thioacetamide induced hepatotoxicity in rats. Asian Pac J Trop Biomed. 2012;2(7):511-5.
41. Nissar AU, Farrukh MR, Kaiser PJ, Rafiq RA, Afnan Q, Bhushan S, Adil HS, Subhash BC, Tasduq SA. Effect of N-acetyl cysteine (NAC), an organosulfur compound from *Allium* plants, on experimentally induced hepatic prefibrogenic events in Wistar rat. Phytomedicine. 2013;20(10):828-33.