Original Research

Effect of Nigella sativa seed extract on carbon tetrachloride-induced hepatotoxicity in rats

Nandhini Krishnan, Suriyavathana Muthukrishnan*

Plant Therapeutics Laboratory, Department of Biochemistry, Periyar Palkalai Nagar, Periyar University, Salem 11, Tamilnadu, India

Received 29 June 2012; accepted 11 September 2012
Available online 20 November 2012

Abstract

Background: Nigella sativa is a spice consumed in India. It has been used in folk medicine for protection against liver diseases in Ayurveda, the Indian system of medicine.

Purpose: The objective of the study is to examine the pre- and post-treatment effects of N. sativa against carbon tetrachloride (CCl4)-induced liver damage in rats.

Methods: A 10% aqueous extract of N. sativa was administered to CCl4-treated male Wistar rats for 28 days. Hepatoprotective activity was assessed by using various enzymatic antioxidants and histopathological studies.

Results: The subsequently elevated enzymatic levels of aspartate transaminase, alanine transaminase, alkaline phosphatase, acid phosphatase, and lactate dehydrogenase were significantly restored toward normalization by the 10% aqueous extract of N. sativa. The extract shows significantly increased cellular glutathione level and antioxidant enzymes levels such as superoxide dismutase, catalase, peroxidase, glutathione peroxidase, and glutathione reductase, and inhibited malondialdehyde generation in CCl4-treated rats. Phytochemical analysis revealed the presence of phenols, flavonoids, and saponins, which are known for their hepatoprotective activities.

Conclusion: N. sativa possesses significant protective effects against hepatotoxicity induced by CCl4, which may be attributed to the combined action of phytoconstituents present in N. sativa.

Copyright © 2012, Taiwan Society of Emergency Medicine. Published by Elsevier Taiwan LLC. All rights reserved.

Keywords: Carbon tetrachloride; Hepatotoxicity; Nigella sativa

1. Introduction

Humans are continuously exposed to different kinds of chemicals such as food additives, industrial chemicals, pesticides, and other undesirable contaminants in the air, food, and soil. Carbon tetrachloride (CCl4) is a well-known hepatotoxicant and has been frequently used for generating liver injury in rat models. The liver is a vital organ of the human body which detoxifies exogenous xenobiotics, drugs, viral infections, and chronic alcoholism. While performing several detoxifications, the liver undergoes stress, leading to liver diseases ending in liver failure and serious health problems and death. Hepatotoxicity is a severe disease that can ultimately lead to liver cancer. This disease remains prevalent in industrial areas, despite the large amount of research that has been directed toward evaluating the hepatoprotective effects of natural plant preparations for the purpose of developing effective phytochemical agents. Studies have demonstrated that dietary intake of a variety of plant phytochemicals confers inhibitory effects against oxidative damage. Herbs have recently become attractive as health beneficial foods (physiologically functional foods) and as a source material for the development of drugs. Herbal medicines derived from plant extracts are being utilized increasingly to treat a wide variety of clinical diseases, with relatively little knowledge regarding their modes of action. The selected seed has undergone extensive phytochemical investigations, and the presence of a variety of chemical constituents such as phenolic acid,
Effects. Natural remedies from medicinal plants are considered to be effective and safe alternative treatments for hepatoxicity. In view of this, the aim of the present study was to explore the effects of *N. sativa* on the prevention and treatment of liver injury induced by CCl₄ in rats; in addition, the levels of liver marker enzymes in serum, malondialdehyde (MDA) content, and the activities of antioxidants were measured in order to investigate its possible mechanisms.

2. Methods

2.1. Chemicals

CCl₄ and thiobarbituric acid were obtained from Merck Ltd. (Coimbatore, Tamilnadu, India). Antioxidant enzymes chemicals were procured from SD Fine Chemicals and Ranbaxy (Coimbatore, Tamilnadu, India).

2.2. Preparation of plant extract

The seeds of *N. sativa* were purchased from an ayurvedic shop located at Coimbatore, India. The seeds were cleaned, dried, and powdered well and then used to prepare 10% aqueous extract using the Soxhlet apparatus. The concentrated sample was dissolved in sterilized distilled water before oral administration by gavage to the experimental animals.

2.3. Preliminary phytochemical analysis

2.3.1. High performance thin layer chromatography analysis

2.3.1.1. Test solution preparation. The given extract was centrifuged at 3000 rpm for 2 minutes, after which the supernatant liquid was collected. Sample loading: 1.5 μL of the supernatant solution and 2 μL of standard solution were loaded as 5 mm band length in the 5 × 10 silica gel 60F254 TLC plate (Sigma, Bangalure, India) using a Hamilton syringe and Camag linomat 5 instrument (Sigma).

2.3.1.2. Spot development. The sample-loaded plate was kept in the TLC twin trough developing chamber (after saturation with solvent vapor) with the respective mobile phase (flavonoid, phenol, and saponin), and the plate was developed in the respective mobile phase up to 90 mm.

2.3.1.3. Photo-documentation. The developed plate was dried by hot air to evaporate the solvents from the plate. The plate was kept in a photo-documentation chamber (Camag reprostar 3; Sigma), and the images were captured at white light, UV 254 nm, and UV 366 nm.

2.3.1.4. Derivatization. The developed plate was sprayed with spray reagent and dried at 100°C in a hot air oven. The plate was photo-documented at daylight and UV 366 nm using photo-documentation (Camag reprostar 3) chamber.

2.3.1.5. Scanning. After derivatization, the plate was fixed in scanner stage (Camag TLC Scanner 3; Sigma), and scanning was done at UV 366 nm.

2.4. Experimental module

Toxicity studies were performed using male Wistar rats with an average weight of 120–150 g, which were purchased from Perundurai Medical College (Perundurai, Tamilnadu, India). The rats were housed in large spacious cages, which were environmentally controlled (25 ± 2°C, 12-h light–dark cycle), with free access to food and water ad libitum (ethical clearance number is 1282/ac/09/cpcsea). Rats were fed with standard laboratory chow diet during the study. Rats were divided into four groups of six animals each.

- Group I served as control.
- Group II received CCl₄ 1 mL/kg/bw (0.14 mL, IP, at 72-hour intervals for 14 days).
- Group III rats with CCl₄ (0.14 mL, IP, at 72-hour intervals for 14 days)-induced liver damage were additionally given 10% aqueous extract of *N. sativa* orally for 28 days.
- Group IV rats were pretreated with 10% aqueous extract of *N. sativa* for 28 days and then injected with CCl₄ (0.14 mL, IP, at 72-hour intervals for 14 days).

At the end of the experimental period, the rats were sacrificed via cervical decapitation. Blood was collected and then allowed to clot, and serum was separated at 2500 rpm for 15 minutes for further biochemical investigations. For each animal, the liver was dissected out and used for histopathological studies.

2.4.1. Serum biochemical assays

The following parameters were analyzed to evaluate the hepatotoxicity of *N. sativa* using the methods described below. Assays of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP), and lactate dehydrogenase (LDH) were determined by various methods, respectively. Determination of antioxidant activity included superoxide dismutase, catalase, peroxidase, glutathione reductase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, reduced glutathione, and total sulfydryl group. MDA in liver homogenate was determined by their action with thiobarbituric acid and used as an index of lipid peroxidation (LPO).
2.4.2. Histopathological examination

After the experimental period, animals were decapitated, and their livers were removed immediately, then sliced, and washed in saline. For histopathological analysis, liver specimens fixed in 10% formalin were embedded in paraffin, sliced at 5-μm thickness, and stained with hematoxylin and eosin (100×) for detection of hepatic damage. The pathological changes were assessed and photographed.

2.5. Statistical analysis

All experimental values were expressed as mean ± SD. Results were assessed by analysis of variance and Dunnett’s multiple comparison test. Differences were considered significant at \( p < 0.05 \).

3. Results

3.1. High performance thin layer chromatography fingerprint profile

Preliminary qualitative phytochemical screening showed the presence of phenols, flavonoids and saponins in *N. sativa* by the method high performance thin layer chromatography (HPTLC) fingerprint profile. \( R_f \) values and the relative percentage of the separated compounds were recorded. Analysis results of phenols, flavonoids, and saponins in *N. sativa* are recorded in Table 1. The fluorescent zone was detected in UV before and after derivatization in the chromatogram at UV 366 mode in the sample track and reference (rutin, quercetin, and saponin) track (Figs. 1–3), which confirmed the presence of phenols, flavonoids, and saponins as compared with rutin, quercetin, and saponin in the given plant extract. The \( R_f \) value of *N. sativa* was found to be 0.07, 0.36, 0.47, 0.55, 0.64, and 0.73 peaks of 2, 5–9 as confirmed phenols in the sample.

Analysis of the HPTLC result confirms that phenols, flavonoids (\( R_f \) value of 0.60, 0.74, and 0.82 and peaks of 9–11), and saponins (\( R_f \) value of 0.13, 0.17, and 0.32 and peaks of 1–3) were present in considerable amounts, which are gaining importance in therapeutics. Hence, it is confirmed that the plant may possess hepatoprotective and antioxidant activity.

<table>
<thead>
<tr>
<th>Track</th>
<th>Peak</th>
<th>( R_f )</th>
<th>Height</th>
<th>Area</th>
<th>Assigned substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin</td>
<td>1</td>
<td>0.45</td>
<td>59.3</td>
<td>1559.2</td>
<td>Rutin standard</td>
</tr>
<tr>
<td>Quercetin</td>
<td>1</td>
<td>0.78</td>
<td>380.9</td>
<td>9481.8</td>
<td>Quercetin standard</td>
</tr>
<tr>
<td>Saponin</td>
<td>1</td>
<td>0.17</td>
<td>201.6</td>
<td>6742.8</td>
<td>Saponin standard</td>
</tr>
<tr>
<td><em>N. sativa</em></td>
<td>5</td>
<td>0.36</td>
<td>162.2</td>
<td>11,394.6</td>
<td>Phenolic 1</td>
</tr>
<tr>
<td><em>N. sativa</em></td>
<td>6</td>
<td>0.47</td>
<td>257.3</td>
<td>13,542.9</td>
<td>Phenolic 3</td>
</tr>
<tr>
<td><em>N. sativa</em></td>
<td>7</td>
<td>0.55</td>
<td>116.1</td>
<td>5253.8</td>
<td>Phenolic 4</td>
</tr>
<tr>
<td><em>N. sativa</em></td>
<td>8</td>
<td>0.64</td>
<td>150.5</td>
<td>8809.7</td>
<td>Phenolic 5</td>
</tr>
<tr>
<td><em>N. sativa</em></td>
<td>9</td>
<td>0.73</td>
<td>459.7</td>
<td>19,858.2</td>
<td>Phenolic 6</td>
</tr>
<tr>
<td><em>N. sativa</em></td>
<td>9</td>
<td>0.60</td>
<td>299.2</td>
<td>13,761.5</td>
<td>Flavonoid 1</td>
</tr>
<tr>
<td><em>N. sativa</em></td>
<td>10</td>
<td>0.74</td>
<td>54.0</td>
<td>1701.2</td>
<td>Flavonoid 2</td>
</tr>
<tr>
<td><em>N. sativa</em></td>
<td>11</td>
<td>0.82</td>
<td>31.4</td>
<td>857.0</td>
<td>Flavonoid 3</td>
</tr>
<tr>
<td><em>N. sativa</em></td>
<td>1</td>
<td>0.13</td>
<td>80.8</td>
<td>1364.6</td>
<td>Saponin 1</td>
</tr>
<tr>
<td><em>N. sativa</em></td>
<td>2</td>
<td>0.17</td>
<td>48.0</td>
<td>724.5</td>
<td>Saponin 2</td>
</tr>
<tr>
<td><em>N. sativa</em></td>
<td>3</td>
<td>0.32</td>
<td>625.9</td>
<td>40,594.4</td>
<td>Saponin 3</td>
</tr>
</tbody>
</table>

Rutin, quercetin and saponin used as a standard for the following secondary components such as phenol, flavonoid, and saponin were identified by HPTLC.

3.2. Effect of *N. sativa* on serum liver marker enzymes

The effect of *N. sativa* on pre- and post-treatment of CCl\(_4\)-induced rats modification in serum ALT, AST, ALP, and ACP levels is shown in Table 2. CCl\(_4\) caused hepatotoxicity in rats as indicated by an increase in serum ALT, AST, ALP, and ACP activities before and after CCl\(_4\) administration, whereas animals pretreated with *N. sativa* exhibited a significant decrease in the activities of these marker enzymes, *N. sativa* administration also reversed the alteration of ALT, AST, ALP, and ACP levels when compared with the toxic group. Pre- and post-treatment with *N. sativa* decreased the elevated levels of serum LDH activity when compared to those in the toxic group. Decreased level of protein was observed in the CCl\(_4\)-treated group when compared to the control group, whereas

![Fig. 1. Densitogram display of rutin standard (phenol) and aqueous extract of Nigella sativa (scanned at 254 nm).](image-url)
N. sativa pre- and post-treated groups showed significantly increased levels compared to the control group (Fig. 4).

### 3.3. Effect of N. sativa on hepatic antioxidant defense system

The hepatic GSH level was reduced following CCl₄ injection. However, supplementation with 10% aqueous extract of *N. sativa* restored the decreased level of GSH caused by CCl₄ injection similar to the normal level, leading to the conclusion that *N. sativa* helped to maintain the same level of GSH despite CCl₄ injection. Beyond GSH depletion, CCl₄ also induced a substantial modification in hepatic antioxidative enzyme activities. Data showed that decreased levels of hepatic SOD and GPx activities are the result of CCl₄ injected as pre- and post-treatment in *N. sativa* groups. The pretreatment group showed an even higher GPx level than the normal group. In addition, although the observed differences did not reach statistical significance, the elevated GR and GPx activities in *N. sativa* groups indicated that *N. sativa* has antioxidative and beneficial effects for liver recovery from acute injury. Interestingly, CAT and PER activity of the *N. sativa* groups increased at a remarkable rate compared with the pretreated and treated groups than the control group.

### 3.4. Effect of N. sativa on hepatic oxidative damage

Table 3 shows increased hepatic LPO indices in the CCl₄-treated toxic group, which confirms that oxidative damage was induced. When CCl₄ was injected into rats that were pretreated with *N. sativa*, levels of LPO in liver were significantly reduced as compared with those in the toxic group. The observed pretreated and treated groups show suppression of oxidative damage in CCl₄-injured liver by *N. sativa* administration. This result suggests that *N. sativa* is antioxidative and hepatoprotective.

### 4. Discussion

The study of any herbal drug becomes more significant when it ameliorates some disease condition. In the present investigation, the hepatoprotective effect of *N. sativa* was studied based on CCl₄-induced liver damage in rats. Preliminary phytochemical analysis of *N. sativa* showed the presence...
of phenols, flavonoids, and saponins. The hepatoprotective effect of *N. sativa* against CCl₄-induced damage is demonstrated by the reduction in increased serum liver marker enzymes, and it also associated with a parallel significant inhibition of elevated hepatic lipid peroxide content and a significant increase in total sulfhydryl content in liver tissues. These findings may indicate that inhibition of LPO is the initial event in the mechanism by which *N. sativa* ameliorates CCl₄ toxicity and could be ameliorated by pretreatment with inhibitors of CCl₄ metabolism and antioxidants.²⁴

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II (CCl₄)</th>
<th>Group III (CCl₄ + N. sativa)</th>
<th>Group IV (N. sativa + CCl₄)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>113.82 ± 4.09</td>
<td>72.61 ± 5.63***</td>
<td>99.32 ± 2.009***</td>
<td>89.90 ± 2.09***</td>
</tr>
<tr>
<td>ALT</td>
<td>0.40 ± 0.05</td>
<td>1.64 ± 0.16***</td>
<td>0.49 ± 0.004***</td>
<td>0.64 ± 0.03**</td>
</tr>
<tr>
<td>AST</td>
<td>0.43 ± 0.09</td>
<td>1.06 ± 0.17***</td>
<td>0.44 ± 0.02***</td>
<td>0.46 ± 0.005***</td>
</tr>
<tr>
<td>ALP</td>
<td>3.24 ± 0.52</td>
<td>7.88 ± 0.55***</td>
<td>3.07 ± 0.103***</td>
<td>5.18 ± 0.25***</td>
</tr>
<tr>
<td>ACP</td>
<td>2.49 ± 0.25</td>
<td>8.30 ± 3.04***</td>
<td>3.86 ± 0.209***</td>
<td>4.19 ± 0.68**</td>
</tr>
<tr>
<td>LDH</td>
<td>0.20 ± 0.04</td>
<td>0.64 ± 0.08***</td>
<td>0.26 ± 0.033***</td>
<td>0.27 ± 0.01***</td>
</tr>
</tbody>
</table>

Values are mean ± SD for each group; units: protein mg/g tissue; AST, ALT, and LDH—μmol of pyruvate liberated/min/mg protein; ALP and ACP—μmol of phenol liberated/min/mg protein. ns = not significant; **p < 0.01; *p < 0.05.

a Group II is compared with Group I.
b Group III is compared with Group II.
c Group IV is compared with Group II.

Fig. 4. Histologic results of tissues stained with hematoxylin and eosin (100×) under light microscope in CCl₄-treated rats: (A) control rats; (B) CCl₄-induced rats; (C) CCl₄ + 10% aqueous extract of *N. sativa*; (D) 10% aqueous extract of *N. sativa* + CCl₄.

Increases in AST and ALT serum levels have been attributed to damage to the structural integrity of the liver.²⁵ They may be rapidly released from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage.²⁶ At a suitable dose, CCl₄ causes extensive necrosis in the liver centrilobular regions around the central veins.²⁷ It is generally accepted that CCl₄ hepatotoxicity results from activation of CCl₄ by the respective specific isozyme of the cytochrome P450 system in the endoplasmic reticulum of hepatocytes to form the reactive metabolite, trichloromethyl radical (CCl₃), which covalently binds to macromolecules,
protein, and lipid and also interacts with O₂ to yield highly reactive trichloromethylperoxy radical (CCl₃O₂). This, in turn, initiates peroxidative degeneration of the membrane lipids of the ER-rich polyunsaturated fatty acids, thereby disrupting the structure and function of cell membrane. AST and ALT, especially the former, are highly localized in hepatocyte cytosols. The crude ethanolic extracts of N. sativa probably acted to preserve the structural integrity of the plasma cellular membrane of the hepatocytes to protect it against breakage by the reactive metabolites produced from exposure to CCl₄. This prevented further damage to more hepatocytes and hence reduced further leakage of AST and ALT due to cell destruction. This may explain the lower levels of these transaminases observed in rats treated with the N. sativa extracts before and after exposure to the toxin.

LPO is one of the principal causes of CCl₄-induced liver injury and is mediated by the free radical derivatives of CCl₄. As expected, MDA, a product of LPO, was increased in rat liver by CCl₄ induction. However, the results showed that N. sativa significantly reduced MDA formation. In other words, the mechanism of the inhibitory effects, by which the N. sativa protects against LPO, may involve radical scavenging capability. This observation implies that these plant extracts may provide phytochemicals that inhibit LPO in the rat liver. In the present study, the development of liver cell injury by the administration of CCl₄ was associated with a significant increase in hepatic lipid peroxide content and a significant decrease in hepatic total sulfhydryl content, a potent factor in the control of LPO.

In addition, the antioxidant activity and/or the inhibition of free radical generation are important in terms of protecting the liver from CCl₄-induced damage. Thus, antioxidant enzymes also play an important role in the detoxification of xenobiotics, catalyzing their conjugation with reduced GSH. The antioxidative and free radical scavenging activities of many substances have been assessed, and many substances that possess antihepatotoxic activity also show strong antioxidative activity.

In this study, exposure to CCl₄ caused GSH depletion and decreased activities of SOD, CAT, GPx, and GR, as well as an increased level of LPO in the liver, implying the down-regulation of numerous antioxidative reactions in the liver. The GSH antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. This system consists of GSH and an array of functionally related enzymes, of which GR is responsible for the regeneration of GSH, whereas GPx works with GSH in the decomposition of hydrogen peroxide and other organic hydroperoxides. GPx and GR activities were significantly reduced in the toxic group when compared with controls. It is conceivable that N. sativa may be initially ascribed to a reduction and inhibition of the GSH-related enzyme activities and then restoration of the GSH content in CCl₄-induced liver damage. Gpx plays a pivotal role in H₂O₂ catabolism and the detoxification of endogenous metabolic peroxides and hydroperoxides which catalyze GSH. Antioxidant activity and the inhibition of free radical generation are important in terms of protecting the liver after treatment. A sufficient amount of GSH thus increased the detoxification of active metabolites through the involvement of GPx.

Animals pretreated and treated with N. sativa showed a significant reduction in the levels of hepatic peroxidative markers with concomitant improvement in the hepatic antioxidative defense system. Phytochemicals have also been shown to stimulate the synthesis of antioxidant enzymes and detoxification systems at the transcriptional level through antioxidant response elements, and to increase γ-glutamyl cysteine synthesis. This suggests that N. sativa may be protecting against the oxidation on hepatic cellular membrane damage via free-radical scavenging property.

The hepatoprotective activity of the aqueous extract of N. sativa was studied on CCl₄-induced liver toxicity in rats. Hence, the hepatoprotective effect observed in the present study may be mainly due to the presence of phenolic compounds in N. sativa, which exhibited prominent antioxidant and hepatoprotective potential against CCl₄-induced damage, in accordance with the description on the pharmacological properties of phenolic compounds in the aforementioned reports.
Phytochemical analysis based on HPTLC indicated the presence of various phenolic compounds, which may contribute to the pharmacological activities of *N. sativa*. Our findings support the reported therapeutic use of this seed as a hepatoprotective agent in the Indian system of medicine. Further studies are needed to explore the possible mechanism.

Acknowledgments

The first author is grateful for the financial support provided by the UGC-Rajiv Gandhi National Fellowship, which made this investigation possible. The authors also thank Periyar University, Salem 11, for providing facilities during the course of this study.

References