Hepatoprotective Effect of Nigella Sativa Linn (Kalajira) On Paracetamol-induced Liver Damage

D R Pal¹, S Nahar², K R Rimi³, S A Talukder⁴, M M Hossain⁵, P C Paul⁶, E O Eva⁷

Abstract
Paracetamol is the widely used non-steroidal anti-inflammatory drugs for the treatment of mild to moderate pain. It causes hepatotoxicity in therapeutic dose for prolonged time. It can induce centrilobular hepatic necrosis in large over doses. Nigella sativa (kalajira) is a medicinal plant has a protective role against hepatotoxicity. Therefore, the present study was designed to observe the protective role of Nigella sativa on paracetamol induced liver damage biochemically in Long Evans rats. The experiment was carried out in the Department of Anatomy, Dhaka Medical college, in the period of July 2003 to June 2004. Sixty matured Long Evans rats of either sex, age of 10-12 weeks and weighing between 150-200 gm were used in this study. They were divided into four equal groups. Group A was vehicle (distilled water) control group.

Key words: Paracetamol, Nigella sativa

Introduction
Many environmental factors, chemicals, drugs and other contaminated food affect the liver physiology up to a certain extent, which may lead to other secondary physiological changes. Inappropriate use of drugs, excessive consumption of alcohol and certain toxin lead to a various kind of liver disorders. In spite of tremendous research in modern medicine, there are hardly any drugs that stimulate liver functions, offer protection to the liver from damage or help regeneration of hepatic cells. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used of all therapeutic agents. They are frequently prescribed for rheumatic musculoskeletal complaints and are often taken without prescription for minor aches and pain. Paracetamol is one of the most important and also popular drugs among NSAIDs. It can causes hepatotoxicity in a low dose and therapeutic dose for prolong time. It can induce centrilobular hepatic necrosis in large overdoses. Use of herbal drugs in the treatment of liver diseases has a long tradition, especially in eastern medicine. It is a widely held belief that herbal preparations are natural and are therefore intrinsically harmless. Nigella sativa (N. sativa) is an herbaceous plant commonly known as black seed, belongs to botanical family of Ranunculaceae. It is commonly used as a natural food additive. Seeds of N. sativa are frequently used in folk medicine in the Middle East and some Asian countries for the promotion of good health & treatment of many diseases. In recent years, it has been suggested that oil of N. sativa has protective role against carbon tetrachloride and D-galactosamine-induced hepatic damage in rats. In rabbits, prior administration of N. sativa has prevented experimental liver cirrhosis and fibrosis induced by carbon tetrachloride. It has been reported that N. sativa oil protects liver against Schistosoma mansoni induced liver damage. Thymoquinone is the main constituent of N. sativa essential oil. The possible hepatoprotective action of thymoquinone is related to preservation of intracellular glutathione. It has been reported that thymoquinone protects organs against oxidative damage induced by free radical generating agents and conditions like CCl₄-hepatotoxicity, cisplatin nephropathy, Diabetes mellitus. Therefore, in the present study, hepatoprotective effects of N. sativa were examined bio-chemically.

Materials and Methods
The experiment was carried out on 60 adult healthy Long Evans rats of both sexes. They were 10-12 weeks and weighing between 150-200gm and was kept in metallic cages in the animal house of the department of Pharmacology, Dhaka Medical College. They were allowed to live at room temperature with standard rat feed and water. Nigella sativa linn: The oil of Nigella sativa was used. Extraction of N. sativa oil from N. sativa seeds: N. sativa seeds were cleaned, washed in water and dried. The seeds were crushed in a blender. Dried and powdered seeds (1000) were extracted with petroleum ether (40-60°C).

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(4×3L) at room temperature for 72 hours in the BCSIR Laboratory. The solvent was removed from the extract under reduced pressure (40°C). Evaporation of petroleum ether left brownish residue (23.1%).

**Experimental procedure**

The animals were divided into four groups consisting of 15 rats each. Grouping of animal, their dietary and drug allotment is presented in table-I.

On 30th day after being fasted overnight except water, 2cc blood was withdrawn from the heart by a 5cc disposable syringe. The blood was collected in plain test tube for estimation of serum bilirubin, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) and serum alkaline phosphatase (ALP). The collected blood samples were kept vertically in a test tube rack for separation of serum. Serum was prepared from the collected blood samples by centrifuging the blood at 300 rpm for 8 minutes.

**Statistical evaluation**

Students unpaired ‘t’ test was performed to evaluate the degree of significance.

**Results**

Serum bilirubin, ALT, AST and ALP level in different groups of rat is presented in Table-II. As evident from study, the mean serum bilirubin, ALT, AST and ALP level was normal in Group-A (0.80±0.18, 18.26±5.24, 25.26±5.09 and 8.90±1.85 accordingly) and in Group-B (0.88±0.12, 17.86±4.92, 24.80±5.83 and 8.74±1.72 accordingly). In Group-C, serum bilirubin, ALT, AST and ALP level (3.28±0.61, 71.33±5.71, 68.40±7.67, 31.40±4.07 accordingly) was significantly elevated after paracetamol administration. These elevated serum level was significantly antagonized by prior administration of N. sativa oil (800mg/kg body weight) in Group-D animals. Serum bilirubin, ALT, AST and ALP level (0.84±0.13, 37.20±5.69, 37.93±6.61, 15.61±3.15 accordingly) was decrease in Group-D as compared to Group-C.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose</th>
<th>Route of administration</th>
<th>Duration of treatment</th>
<th>Day of sacrifice</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Distilled water</td>
<td>3 cc</td>
<td>Single dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Nigella sativa oil</td>
<td>800mg/kg</td>
<td>Daily dose for 28 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Paracetamol</td>
<td>3gm/kg</td>
<td>oral</td>
<td>Single dose on 29 day</td>
<td>30th day of experiment</td>
</tr>
<tr>
<td>D</td>
<td>Nigella sativa oil</td>
<td>800mg/kg</td>
<td>bw</td>
<td>Daily dose for 28 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paracetamol</td>
<td>3gm/kg</td>
<td>bw</td>
<td>Single dose on 29 day</td>
<td>6th day of experiment</td>
</tr>
</tbody>
</table>

Table I: Grouping of the animals, doses of drug/vehicle and Nigella sativa oil, duration of experiment and sacrificing schedule.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug Dose</th>
<th>Route of administration</th>
<th>Duration of treatment</th>
<th>Day of sacrifice</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Distilled water 3 cc</td>
<td>Single dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Nigella sativa oil 800mg/kg</td>
<td>Daily dose for 28 days</td>
<td></td>
<td></td>
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<tr>
<td>C</td>
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</tr>
</tbody>
</table>

Table-II: Serum bilirubin, ALT, AST, ALP level in different groups of rats

<table>
<thead>
<tr>
<th>Group</th>
<th>No of rats</th>
<th>Serum bilirubin</th>
<th>Serum ALT</th>
<th>Serum AST</th>
<th>Serum ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/dl</td>
<td>U/L</td>
<td>U/L</td>
<td>U/L</td>
</tr>
<tr>
<td>A</td>
<td>15</td>
<td>0.80±0.18</td>
<td>18.26±5.24</td>
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</tbody>
</table>

**Discussion**

The present study, hepatotoxicity was induced by single oral administration of paracetamol (3gm/kg body weight). The dose was selected according to Sharma et al.16 N. sativa oil was used at a dose of 800mg/kg body weight/day for 28 days. The dose, route of administration and duration was selected according to El-Dakhakhny et al.8 The result of bio-chemical studies suggested that paracetamol 3gm/kg body weight, in a single dose produced hepatotoxicity which was assessed by significant (P<0.001) increase in serum bilirubin, ALT, AST and ALP in Group-C animals. Same enzyme level elevation was found by Davidson and Eastham in human.17 Boyer and Rouf.18 Johnson and Tolman3, Gerber et al.19 In the present study, N. sativa oil at the given dose has no significant biochemical changes in Group-B as compared to Group-A. This result agrees with that reported by Tenekoon et al.20 In this study, effects of N. sativa oil on paracetamol-induced hepatotoxicity were evaluated bio-chemically. 28 days treatment with N. sativa oil in Group-D, the mean serum bilirubin, ALT, AST and ALP level was significantly (P<0.001) lower as compared to Group-C. Data of the present study were closely associated with the result done by El-Dakhakhny et al.9 Al-Gharably et al.13 also observed the similar findings measured by ALT level and malondialdehyde (MDA) level. They suggested that thymoquinone (the active constituent of N. sativa volatile oil) is an efficient cytoprotective agent against chemically induced hepatic damage. Meral et al.15 also suggested that N. sativa treatment increase antioxidant defense system.

**Conclusion**

N. sativa oil did not produce any significant changes in serum enzymes (ALT, AST and ALP) and serum bilirubin level in normal rats. Paracetamol in a toxic dose produces hepatic damage to the liver which can be prevented by N. sativa oil. However, more studies are recommended for establishing as potent, safe and useful antihepatotoxic herbal plant as well as its mechanism of action regarding hepatoprotective properties.
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