Effect of Nigella Sativa Oil on Hepatotoxicity Induced by Antitubercular Drugs in Albino Rats

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Introduction

Drug induced liver damage is the most commonly encountered clinical entity of which anti-tubercular drugs constitute the major cause of hepatotoxicity in India. Isoniazid, Rifampicin, Pyrizinamide and Ethambutol are the most commonly used combination of which isoniazid, Rifampicin and Pyrizinamide combination is most hepatotoxic. The incidence of liver toxicity is 2-6 % with isoniazid and rifampicin. The antitubercular drug induced hepatotoxicity is found to be mediated through oxidative stress and free radical damage to hepatocytes.

The seed of Nigella sativa Linn, (Ranunculaceae), commonly known as black seed or black cumin, are used in folk medicine for treatment and prevention of diseases like asthma, diarrhoea and dyslipidemia. Much of the biological activity of the seeds has been shown to be due to thymoquinone present in Nigella sativa oil. It plays important role as antioxidant and acts as protective agent against chemical induced hepatic damage. Houghton PJ et al, 1995 showed that the fixed oil of Nigella sativa has both antioxidant and anti-eicosanoid effects.

Thus, this study was designed to evaluate the hepatoprotective activity of Nigella sativa oil (NS) against antitubercular drugs induced liver damage in rats.

Materials and Methods

Animals:
Adult albino rats of either sex of Wistar strain weighing 150-250 g were housed under standard conditions of temperature (22 ± 2°C), relative humidity (55 ± 5%) and light (12 h light/dark cycles). They were fed with standard pellet diet and water ad libitum. They were maintained in clean, sterile polypropylene cages. Protocol was approved by Institutional Animal Ethics committee and study was conducted according to guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals.

Drugs:
The doses of antitubercular drugs (isoniazid [I] - 27 mg/kg/day, rifampicin [R]-54mg/kg/day, pyrazinamide [Z]-135 mg/kg/day; Plethico Pharmaceuticals Ltd. Indore) were extrapolated from daily human dose using the conversion table based on body surface area. The Nigella sativa oil was obtained from Mahmodia Products, Karimnagar, A.P. and administered in the dose of 0.2 ml/kg intraperitoneally daily.

The animals were divided in 5 groups comprising of 6 animals each. The groups were treated as follows:

- Group I: Vehicle control i.e. 2% gum acacia suspension orally daily for 30 days
- Group II: (I+R+Z) suspension orally daily for 30 days
- Group III: (I+R+Z) suspension + NS oil 0.2 ml/kg body weight intraperitoneally daily for 30 days.
- Group IV: (I+R+Z) suspension orally from day 1 to day 30 + No treatment from day 31 to day 50.
Group V: (I+R+Z) suspension orally from day 1 to day 30 + NS oil 0.2 ml/kg body weight i.p. from day 31 to day 50.

Blood samples of animals from groups I, II and III were taken for liver function tests by cardiac puncture under ether anesthesia and their liver was removed for histopathological examination on 30th day. On 50th day animals of these two groups were sacrificed by ether anesthesia. Blood sample was taken by cardiac puncture for estimation of liver function tests and liver was removed for histopathological examination in each animal.

Assessment of liver damage:

Biochemical investigations:

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were estimated by Reitman and Frankel method. Serum protein, serum bilirubin and serum alkaline phosphatase (ALP) were estimated by Biuret method, Modified Jendrassik and Grofs method and King and King method respectively.

Histopathological examination of liver:

The liver tissue sections were fixed in 10% buffered neutral formalin for 48 hours and processed according to standard histological techniques and stained with hematoxylin and eosin and examined microscopically for histopathological changes.

The histopathological assessment of liver damage was done by scoring of structural changes described by National Health Services Meryland, USA.

The parameters were as followed:

a. Degeneration
   (0-No degeneration, 1-few vacuolated cells per lesion, 2-more than 10 vacuolated cells per lesion, 3-one to two rows of vacuolated cells around necrotic zone per lesion, 4-more than two rows of vacuolated cells around necrotic zone per lesion)

b. Necrosis
   (0-No necrosis, 1-Focal necrosis of one or two cells per lesion, 2-focal necrosis of more than two cells per lesion, 3-massive centrilobular necrosis, 4-massive centrilobular necrosis with necrotic tissue bridging the central vein)

c. Fibrosis
   (0-normal appearance of liver, 2-central necrosis, hydropic degeneration, no fibrosis, 2-fibrous tissue in periportal area only, 3-Fibrous tissues insinuating surrounding hepatic parenchyma, 4-formation of pseudolobules)

d. Regeneration
   (0-no Regeneration, 1-Mild, 2-Moderate, 3-Excellent)

Statistical Analysis

The data was analyzed using one way analysis of variance (ANOVA) with post hoc Dunnett’s test for biochemical parameters and Mann-Whitney U test for comparing histopathology scores. The values are represented as mean ± S.E.M. p<0.05 was considered statistically significant.

Results

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Total Protein (gm/dl)</th>
<th>Serum Bilirubin (mg/dl)</th>
<th>Sr. AST (units/ml)</th>
<th>Sr. ALT (units/ml)</th>
<th>Sr. ALP (KA units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>7.38±0.37</td>
<td>0.89±0.076</td>
<td>34.00±1.89</td>
<td>37.54±0.80</td>
<td>11.36±0.65</td>
</tr>
<tr>
<td>Group II</td>
<td>5.28±0.21##</td>
<td>2.35±0.18###</td>
<td>74.38±2.30###</td>
<td>172.26±5.46###</td>
<td>45.12±1.06###</td>
</tr>
<tr>
<td>Group III</td>
<td>6.68±0.40*</td>
<td>1.2±0.10**</td>
<td>38.24±1.98***</td>
<td>84.61±2.05***</td>
<td>14.83±0.91***</td>
</tr>
<tr>
<td>Group IV</td>
<td>6.13±0.13</td>
<td>1.23±0.19</td>
<td>68.35±1.83</td>
<td>75.32±1.30##</td>
<td>41.42±0.70</td>
</tr>
<tr>
<td>Group V</td>
<td>6.98±0.22***##@</td>
<td>0.97±0.15*##@@</td>
<td>32.25±1.20***@@</td>
<td>36.45±0.31###@@</td>
<td>11.25±0.36##@@</td>
</tr>
</tbody>
</table>

# = in comparison to group I, * = in comparison to group II, @ = in comparison to group IV, #,##,##,##,@@ p <0.05, #,##,##,##,@@ p <0.01, #######,##@ p <0.001
Effects of co-administration of Nigella sativa oil in preventing hepatotoxicity with anti-tubercular drugs

The animals which received anti-tubercular drugs for 30 days showed significant fall in total protein level along with rise in the levels of serum bilirubin, ALT, AST and ALP as compared to control group. Co-administration of Nigella sativa oil along with anti-tubercular drugs significantly prevented the rise of enzyme levels and fall in total serum proteins.

Effects of Nigella sativa oil in treating anti-tubercular drugs induced hepatotoxicity

Withdrawal of anti-tubercular drugs failed to produce significant reversal of any of the biochemical parameters within 20 days except ALT, which were decreased to a significant level on 50th day as compared to 30th day. The administration of Nigella sativa oil after withdrawal of anti-tubercular drugs lead to significant rise in sr. proteins and decrease in enzyme levels on 50th day as compared to their levels on 30th day. The change in liver function tests of this group was significant as compared to liver function tests for which Nigella sativa oil was not administered. On 50th day the levels were nearly normalized. The scores of degeneration, necrosis and fibrosis on 50th day for this group decreased significantly as compared to the scores on 30th day. There was a evidence of significant regeneration as compared to the 30th day. However the change in histopathological score of degeneration and fibrosis was not significant and histopathological score of necrosis and regeneration was significant for this group as

Fig. 1
Histopathology of normal liver having normal histological structures of hepatic lobules

Fig. 2
Histopathology of anti-tubercular drug treated liver showing congested vessels with degenerative changes (H & E, 10X)

Fig. 3
Histopathology of anti-tubercular drug treated liver showing hydropic vacuolation, cytoplasm of hepatocyte showing small and large vacuolations (H&E 40X)

Fig. 4
Histopathology of Nigella sativa oil treated liver showing areas of regeneration

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compared to the group that received no treatment after withdrawal of the 30th day anti-tubercular therapy.

**Discussion**

In the present study, administration of anti-tubercular drugs for 30 days significantly elevated the levels of serum bilirubin, serum Alanine aminotransferase, serum Aspartate aminotransferase and serum alkaline phosphatase, while the levels of serum total protein were significantly reduced showing hepatotoxicity. It also resulted in degeneration, necrosis and fibrosis.

Concurrent administration of Nigella sativa oil given along with antitubercular drugs significantly prevented the rise in the enzyme levels such as ALT, AST and ALP. It also reduced the serum bilirubin and the fall in serum total protein level as compared to group receiving antitubercular drug alone.

Administration of Nigella sativa oil for 20 days after receiving antitubercular drugs for 30 days showed significant reduction in levels of serum bilirubin as well as enzyme levels and rise in proteins as compared to mere withdrawal for 20 days. There was significant reduction in the scores at degeneration and necrosis. Thus Nigella sativa can be used to reduce hepatotoxicity caused by antitubercular drugs.

The varied chemical composition of Nigella sativa makes it difficult to assign its hepatoprotective property to one of its constituent chemicals. The hepatoprotective property of NS oil may also be because of its other properties like anti-inflammatory property which may prevent inflammatory hepatic damage, immunomodulatory property and anti-oxidant property thereby reducing the oxidative stress imposed by drugs; this antioxidant mechanism seems to be important as NS oil has been shown to reduce oxidative stress\(^2\) and oxidative stress has been found to be most important mechanism in hepatotoxicity of anti-tubercular drugs.\(^2\)

**Summary and Conclusion**

Thus it can be concluded that Nigella sativa oil can significantly prevented as well as reversed the hepatotoxicity produced by anti-tubercular drugs and the reversal of hepatotoxicity effected by the NS oil is superior to that effected by withdrawal of antitubercular drugs.

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**References**


