Role of Nigella sativa in Carbon Tetrachloride induced Hepatotoxicity

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Abstract
Objective: To evaluate the protective role of Nigella sativa in carbon tetrachloride induced hepatic changes in rabbits.
Material and Methods: This Case control experimental study was conducted at Department of Pharmacology, ISRA University Hyderabad during July 2011 to November 2011. A total of 45 rabbits were divided into three groups consisting of 15 animals in each group A, B and C. Each group was further divided into three sub groups. Sub groups (A1, B1 and C1) received treatment for one week. Sub groups (A2, B2 and C2) received treatment for two weeks. Sub groups (A3, B3 and C3) received treatment for three weeks. The animals in group A (control) received normal saline. The animals in group B were treated with carbon tetrachloride. The animals in group C were treated with carbon tetrachloride and Nigella sativa.
Result: In present study sinusoidal congestion, periportal inflammation, kupffer cell hyperplasia, steatosis, necrosis and fibrosis were seen in carbon tetrachloride intoxicated rabbits. These findings were less marked in rabbits treated with Nigella sativa.
Conclusion: This study showed the hepatoprotective effects of Nigella sativa in carbon tetrachloride induced hepatotoxicity.
Key words: Carbon tetrachloride, Hepatoprotective, Hepatotoxicity, Nigella Sativa.

Introduction
Carbon tetrachloride (CCl4) has been used as a dry cleaning agent, fabric spotting fluid, solvent, reagent in chemical synthesis, fire extinguisher fluid and grain fumigant. Its primary use was observed in chlorofluorocarbon (CFC) production. In the 20th century CCl4 was widely used as a refrigerant and in lava lamps.
CCl4 is metabolized in the body primarily by the liver, but also in the kidney, lung and other tissues containing Cytochrome P (CYP450). As demonstrated in studies with CYP2E1 genetic knockout mice, this enzyme is required for the development of hepatotoxicity (as measured by elevated liver enzymes and liver histopathology) in mice exposed to CCl4. In the liver the greatest accumulation of CCl4 metabolites occurs in the centrilobular region, which has high CYP450 levels.

Material and Methods
This is a case control experimental study which was carried out in the Department of Pharmacology, ISRA University Hyderabad in collaboration of LUMHS Hyderabad. Sample size was 45 normal healthy rabbits of either sex with weight >1.5 Kg. The animals were divided in to 03 groups each comprising 15 animals.
- Group A: served as a control group
- Group B: received CCl4
- Group C: received CCl4 + N. sativa
Each group were sub divided into 03 sub groups (A1, A2 and A3), (B1, B2 and B3) and (C1, C2 and C3) according to the period of administration of CCl₄ alone and in combination. Each subgroup comprised of 05 animals. Five animals each of sub group (A1, B1 and C1) received treatment for one week and were sacrificed, five animals each of sub group (A2, B2 and C2) received treatment for two weeks and sacrificed while, five animals each of sub group (A3, B3 and C3) received treatment for three weeks.

**Group A:** (control group): All sub groups (A1, A2 and A3) were given 0.9% isotonic saline solution at a dose level 4 ml/kg on alternate day and were sacrificed at the end of their respective period of time and served as a control group.¹²

**Group B:** All sub groups (B1, B2 and B3) were given CCl₄ dissolved in olive oil (1:1 Ratio) at a dose level of 1.9 ml/kg orally on alternate day and were sacrificed at the end of their respective period of time.¹²

**Group C:** All sub groups (C1, C2 and C3) were given CCl₄ dissolved in olive oil at a dose level of 1.9 ml/kg along with suspension of N. sativa (1.25 g powder of N. sativa + 100 ml isotonic saline) at a dose level of 4 ml/kg on alternate day and were sacrificed at the end of their respective period of time.¹²

At the end of respective period of treatment, the animals were weighed and sacrificed. A mid line incision was given in the middle part of trunk and all the skin layers and fascia were opened and liver was identified and removed. The liver tissue was processed for histopathological examination followed by interpretation of results. The data was analyzed by SPSS version 16.0. The data was analyzed by applying the Chi-square to compare the histomorphological findings in animals of group A, B and C. p value of <0.05 was considered to be significant.

**Results**

Histopathological examination of the liver tissue for gross and microscopic findings was carried out. There were no gross changes or no any findings supporting the liver cirrhosis were observed.

1. **At 1st week:** 15 Rabbits were sacrificed, 5 rabbits from each sub group. Histopathological findings of 5 rabbit’s liver which were given isotonic saline showed no changes with normal liver architecture. Histopathological findings of 5 rabbit’s liver which were intoxicated with CCl₄, 2 of them showed sinusoidal congestion, periportal inflammation and kupffer cell hyperplasia, 2 of them showed steatosis and 1 of them showed piece meal necrosis. Histopathological findings of 5 rabbit’s liver which were given CCl₄ along with N. sativa, 3 of them showed no changes and 2 of them showed sinusoidal congestion and periportal inflammation (Table 2).

2. **At 2nd week:** 15 Rabbits were sacrificed, 5 rabbits from each sub group. Histopathological findings of 5 rabbit’s liver which were given isotonic saline showed no changes or normal liver architecture. Histopathological findings of 5 rabbit’s liver which were intoxicated with CCl₄, 2 of them showed sinusoidal congestion, periportal inflammation and kupffer cell hyperplasia, 2 of them showed steatosis and 1 of them showed piece meal necrosis. Histopathological findings of 5 rabbit’s liver which were given CCl₄ along with N. sativa, 3 of them showed no changes and 2 of them showed sinusoidal congestion and periportal inflammation (Table 3).

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**Table 1: Histopathological Findings of Liver at 1st Week**

<table>
<thead>
<tr>
<th>Rabbit Distribution</th>
<th>No Findings</th>
<th>Sinusoidal congestion + periportal inflammation</th>
<th>Sinusoidal congestion + Kupffer cell Hyperplasia</th>
<th>Steatosis</th>
<th>Piecemeal necrosis</th>
<th>Bridging necrosis</th>
<th>Fibrosis</th>
<th>Total</th>
<th>p-value</th>
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<tbody>
<tr>
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<td>5</td>
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<tr>
<td>CCl₄ + N. sativa</td>
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<td>0</td>
<td>5</td>
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</tr>
<tr>
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**Table 2: Histopathological Findings of Liver at 2nd Week**

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<th>Sinusoidal congestion + Kupffer cell Hyperplasia</th>
<th>Steatosis</th>
<th>Piecemeal necrosis</th>
<th>Bridging necrosis</th>
<th>Fibrosis</th>
<th>Total</th>
<th>p-value</th>
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</tr>
<tr>
<td>CCl₄ + N. sativa</td>
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Table 3: Histopathological Findings of Liver at 3rd Week

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<th>Rabbit Distribution</th>
<th>No Findings</th>
<th>Sinusoidal congestion + Periportal inflammation</th>
<th>Sinusoidal congestion + Periportal inflammation and Kupffer Cell Hyperplasia</th>
<th>Steatosis</th>
<th>Necrosis</th>
<th>Bridging Necrosis</th>
<th>Focal Necrosis</th>
<th>Total</th>
<th>p-value</th>
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Discussion

CCl4 is well-known for its hepatotoxic effects. It causes significant increases in absolute and relative liver weight, serum levels of ALT, AST and Alkaline phosphatase, and centrilobular hepatocellular vacuolar degeneration and necrosis. In the present study, sinusoidal congestion, periportal inflammation and kupffer cell hyperplasia were seen in CCl4 treated rabbits. These results are consistent with the findings of Tien et al. Another study by De-Groot and Noll reported CCl4 induces necrosis and steatosis. This correlates with findings of the present study. In current study, N. sativa proved to be hepatoprotective by reducing the toxic effects of CCl4. However, in contrast to the findings of the present study Tennekon et al reported that there were no histological changes seen in animal model treated with N. sativa. Turkdogan et al have reported that N. sativa helps in the prevention of liver fibrosis in rabbits. These findings are consistent with our present study, in which we also observed that N. sativa reduces steatosis, necrosis and fibrosis in liver. Al-Ghamdi has reported that N. sativa seeds appeared to be safe and possibly protective against CCl4 induced hepatotoxicity. This study supports the findings of the present study.

Conclusion

The present study has highlighted the important role of N. sativa which is directly related to the histological changes induced by CCl4 induced on liver morphology. This study showed the hepatoprotective effects of N. sativa in CCl4 induced hepatotoxicity.

Conflict of Interest

This study has no conflict of interest as declared by any author.

References


Authorship Contribution:

Author 1: Interpretation, analysis and discussion
Author 2: Concept, planning and Interpretation, analysis and discussion
Author 3: Concept and planning and active participation in research.