

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.^{1,2} Its primary use was observed in chlorofluorocarbon (CFC) production.^{1,3} In the 20th century CCl₄ was widely used as a refrigerant and in lava lamps.⁴

CCl₄ is metabolized in the body primarily by the liver, but also in the kidney, lung and other tissues containing

Cytochrome P (CYP450).⁵ 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 CCl₄.⁶ In the liver the greatest accumulation of CCl₄ metabolites occurs in the centrilobular region, which has high CYP450 levels.⁷ Zangar et al in a study measured CCl₄ metabolic rate constants for human and animal hepatic microsomal preparations in vitro. Results suggested that the metabolic rate in humans is more similar to the rate in rats than in other rodent species.⁸

Nigella sativa Linn. (*N. sativa*) family Ranunculaceae is commonly known as black seed or black cumin. Original black cumin seed is *Carum bulbocastanum*. In South Asia it is known as Kalonji.⁹ It is found that *N. sativa* is an important medicinal herb; its oil is used as a natural remedy for a wide range of diseases including various allergies. It probably has an important role in the pharmacological effects. *N. sativa* possesses antioxidant property.¹⁰ The pharmacological actions include protection against nephrotoxicity and hepatotoxicity induced by either disease or chemicals. The oil and certain active ingredients showed beneficial immunomodulatory properties, augmenting the T cell and natural killer cell mediated immune responses. Most importantly, both the oil and its active ingredients expressed anti-microbial and anti-tumor properties toward different microbes and cancers.¹¹

Thus the present study is conducted to evaluate the protective role of *N. sativa* in CCl₄ induced hepatic changes in rabbits.

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*

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Received: May 6, 2016; Accepted: June 11, 2016

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, 3 of them showed sinusoidal congestion and periportal inflammation and 2 of them sinusoidal congestion and periportal inflammation and kupffer cell hyperplasia. 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 1).

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 2).

3.At 3rd 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 rabbits liver which were intoxicated with CCl₄, 1 of them showed steatosis, 1 of them showed piece meal necrosis, 2 of them showed bridging necrosis and 1 of them showed fibrosis. Histopathological findings of 5 rabbits liver which were given CCl₄ along with N. sativa, 2 of them showed no changes and 3 of them showed sinusoidal congestion and periportal inflammation (Table3).

Table 1: Histopathological Findings of Liver at 1st Week

Rabbit Distribution	No Findings	Sinusoidal congestion + periportal inflammation	Sinusoidal congestion + periportal inflammation + Kupffer cell Hyperplasia	Steatosis	Piece meal necrosis	Bridging necrosis	Fibrosis	Total	p-value
Control	5	0	0	0	0	0	0	5	0.07
CCl ₄	0	3	2	0	0	0	0	5	
CCl ₄ + N. sativa	3	2	0	0	0	0	0	5	
Total	8	5	2	0	0	0	0	15	

Table 2: Histopathological Findings of Liver at 2nd Week

Rabbit Distribution	No Findings	Sinusoidal congestion + periportal inflammation	Sinusoidal congestion + periportal inflammation + Kupffer cell Hyperplasia	Steatosis	Piece meal necrosis	Bridging necrosis	Fibrosis	Total	p-value
Control	5	0	0	0	0	0	0	5	0.029
CCl ₄	0	0	2	2	1	0	0	5	
CCl ₄ + N. sativa	3	2	0	0	0	0	0	5	

Table 3: Histopathological Findings of Liver at 3rd Week

Rabbit Distribution	No Findings	Sinusoidal congestion + periportal inflammation	Sinusoidal congestion + periportal inflammation + Kupffer cell Hyperplasia	Steatosis	Piecemeal necrosis	Bridging necrosis	Fibrosis	Total	p-value
Control	5	0	0	0	0	0	0	5	0.049
CCl ₄	0	0	0	1	1	2	1	5	
CCl ₄ + N. sativa	2	3	0	0	0	0	0	5	
Total	7	3	0	1	1	2	1	15	

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.⁷ 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.¹³ Another study by De-Groot and Noll reported CCl₄ produces necrosis and steatosis. This correlates with findings of the present study.¹⁴ 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 Tennekoon et al reported that there were no histological changes seen in animal model treated with N.sativa.¹⁵

Turkdogan et al have reported that N. sativa helps in the prevention of liver fibrosis in rabbits.¹⁶ 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.¹⁷ 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

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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.