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# The Protective Effects of Nigella Sativa Seed Oil against $CCl_4$-Induced Hepatic Injury in Wistar Rats

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**Abstract:** Carbon tetrachloride ($CCl_4$) is a well-established hepatotoxic agent used to induce oxidative liver injury in animal models. Nigella sativa oil (NSO) possesses antioxidant, anti-inflammatory, and hepatoprotective properties attributed primarily to thymoquinone. To evaluate the protective effects of Nigella sativa oil on biochemical and physiological parameters in Wistar rats exposed to chronic $CCl_4$-induced hepatotoxicity. Twenty-four Wistar rats (n=6 per group) were allocated into four groups: Normal, $CCl_4$-treated, $CCl_4$+N. sativa oil (NSO), and NSO-only. $CCl_4$ (0.8mL/kg, s.c.) was administered twice per week for eight weeks in $CCl_4$-treated and $CCl_4$+N. sativa oil (NSO) treated groups. NSO (1 mL/kg/day, oral gavage) was administered for eight weeks in $CCl_4$+N. sativa oil (NSO), and NSO-only groups. During the study period, body weights of all groups were recorded on weekly basis and after $8^{th}$ week rats were slaughtered to obtain blood samples for biochemical estimations. Serum was separated to evaluate AST, ALT, ALP and bilirubin levels. Statistical analysis was done by calculating mean± SD. Results showed that $CCl_4$ administration in rats caused marked elevation in serum AST, ALT, ALP, and bilirubin levels which is indicative of liver toxicity. The serum biomarkers were reduced in $CCl_4$+NSO group suggesting it ameliorative role against $CCl_4$ induced hepatic damage. Control and NSO treated groups showed almost similar levels of the serum parameters. It is concluded that the toxic effects of $CCl_4$ are opposed by NSO suggesting its hepatoprotective potential thus can be used as a natural remedy for cure and prevention.

**Keywords:** NSO, liver, toxicity, rat.

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## Introduction

Liver is a natural detoxification center in our body that aims to transform and facilitates removal of metabolic wastes. But in turn it may become harmed by the toxic substances produced during metabolic transformations. $CCl_4$ is an important chemical used to create oxidative stress and eventual hepatic tissue damage in experimental animals [1]. Chronic $CCl_4$ exposure disrupts liver enzyme homeostasis and alters metabolic markers, which serve as clinical indicators of hepatic dysfunction [2].

Nigella sativa has a long history of usage as a medicinal plant and belongs to family Ranunculaceae. Nigella sativa oil (NSO) is thought to provide some protection against liver injury (hepatoprotection) from chemical agents (toxins) such as carbon tetrachloride (CCl4). The mechanisms through which NSO provides hepatoprotection are thought to be through the suppression of the production of free radicals, which are harmful molecules that can cause cell death (apoptosis) and cellular damage (lipid peroxidation), as well as through the inhibition of pro-inflammatory cytokines, which are molecules that promote inflammation [3,4].

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Several in vitro studies have suggested that NSO has the ability to inhibit oxidative stress and inflammation produced by CCl4. However, there is limited data available regarding the use of NSO as a potential therapeutic for the prevention of CCl4-induced liver damage in vivo [5].
Therefore, the primary purpose of this study was to investigate whether or not NSO provides any form of protection against liver damage caused by CCl4 in vivo. Specifically, the study examined if administration of NSO would prevent changes in serum biochemical markers associated with liver damage (such as increased liver enzymes), changes in body weight, and histopathological changes seen in the livers of rats that received CCl4 compared to control rats that did not receive CCl4.

## Method

### Experimental Animals

Age and body weight matched male Wistar rats were selected and purchased for this study. All procedures were in accordance with the international guidelines for animal care and rights. The rats were acclimatized for 7 days prior to any treatment and had free access to standard diet and water. Twenty-four Wistar rats were randomly assigned to one of four different treatment groups (n=6): a control group, a CCl4 treatment group, a CCl4 treatment group plus NSO treatment group, and an NSO-only treatment group. Each group had six rats.

### Study Duration and Treatment

All rats were housed in the same animal room and fed the same diet. All treatments began when the rats were 24 weeks old. The rats in the control and CCl4 treatment groups were weighed once a week for the first 8 weeks. The rats in the CCl4 treatment group received 0.8 mL/kg of CCl4 subcutaneously (under the skin) two times a week. The rats in the CCl4 treatment group plus NSO treatment group received 0.8 mL/kg of CCl4 subcutaneously two times a week and 1 mL/kg of NSO orally once a day for 8 weeks. The rats in the NSO-only treatment group received 1 mL/kg of NSO orally once a day for 8 weeks.

### Blood Collection and Biochemical Measurements

One week after the last injection of CCl4 (or one week after the last administration of NSO), all rats were euthanized and blood samples were collected and placed in tubes containing EDTA to measure serum biochemical markers of liver damage, specifically AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), and bilirubin [6,7].

### Statistical Analysis

All biochemical measurements were expressed as Mean ± SD.

## Results and Discussion

The rats receiving CCl4 lost approximately 30% of their initial body weight at 8 weeks. The rats receiving both CCl4 and NSO gained back some of the weight they lost and regained almost all of their lost weight by the end of the 8-week period. During the whole study period the body weights of rats in control and NSO treated groups did not show major variations in their body weights which is indicative of no toxicity created by NSO (Table 1).
The rats treated in control and NSO groups did not show any signs of toxicity as indicated by similar levels of serum indicators of liver injury i.e. AST, ALT, ALP & bilirubin. On the other hand rats in CCl4 treated groups exhibited enhanced levels of these liver damage indicators whereas rats in CCL4+NSO treated groups showed reversal of liver toxicity as their levels of AST, ALT, ALP & bilirubin were declined as compared with CCl4 treated groups (Table 2).

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**Table 1: Body Weight Changes in Control, $CCl_4$ treated, $CCl_4$+NSO treated and NSO treated rats.**

| GROUP | WEEK | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Control | 135±4.4 | 170.8± 20.7 | 153.8± 9.5 | 178 ±7.7 | 159.2 ±5.9 | 149.4 ±6.07 | 136±6.5 | 134.2 ±8.7 |
| CCL4 treated | 147.33 ±7.76 | 138.33± 6.76 | 143.33 ±9.95 | 178 ±16.85 | 132.33 ±10.1 | 132.66 ±9.7 | 88 ±8.7 | 129.33 ±9.76 |
| CCL4+NSO | 141.33±6.7 | 140± 4.5 | 139± 5.3 | 139.5±6.8 | 140.3±3.2 | 140.6±8.5 | 141±7.8 | 142±4.3 |
| NSO ONLY | 145±6.5 | 145± 6.66 | 144± 7.5 | 1444.34±5.6 | 144.1±6.4 | 144±4.3 | 144.2±8.7 | 144.3±7.5 |

Data is presented as Mean ± SD

**Table 2: Serum Enzymes and Bilirubin Level in Control, $CCl_4$ treated, $CCl_4$+NSO treated and NSO treated rats.**

| SERUM MARKERS | Normal | CCL4 treated | CCL4+NSO | NSO |
|---|---|---|---|---|
| AST (IU/L) | 8.56 ±0.11 | 10.5 ±0.55 | 17.85 ±2.192 | 14.6± 2.497 |
| ALT (IU/L) | 25.152 ±1.72 | 23.89 ±1.11 | 6.88±2.941 | 13.12±4.16 |
| ALP (IU/L) | 461.118 ±167.85 | 1391.61 ±1155.18 | 139.38± 25.370 | 248.7 ± 21.582 |
| BILIRUBIN (mg/dl) | 0.41 ±0.0432 | 0.56 ±0.024 | 0.504±0.2545 | 0.5328± 0.3792 |

Data is presented as Mean ± SD

The findings of this study shows that Nigella sativa seed oil has anti-inflammatory properties probably because of presence of antioxidants and thymoquinone and has potential to revert oxidative stress induced hepatic damage in rats and could be adopted as natural remedy to prevent and cure hepatic ailments [8]. Our results are in accordance with other studies [9, 10].

## Conclusion

Liver being detoxification center is susceptible to damage produced by chemicals during their metabolic transformation. Nigella Sativa seed oil consumption resulted in reversal of liver toxicity created by CCl4 in rats therefore could be a choice of treatment for hepatotoxicity caused by xenobiotics.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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None

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