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# Hepatoprotective Effect of Black Seed *(Nigella sativa)* oil on Carbon Tetrachloride ($CCl_4$) Induced Liver Toxicity in Adult Wistar Rats.

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***Abstract:*** *The aim of this study was to investigate the effect of N. sativa oil on carbon tetrachloride (CCL4)-induced liver damage using 35 adult Wistar rats. The experimented animals weighed between 130-180g and were randomly divided in to seven groups. Each group comprised of 5 rats. Group 1 rats were administered normal Saline (volume per body weight) orally for 2 weeks. Group 2 rats were administered olive oil 4m1/kg body weight orally for 2 weeks. Group 3 rats were administered 2ml/kg body weight of N. sativa oil orally for 2 weeks. Group 4 rats were administered 4ml/kg body weight of N. sativa oil orally for 2 weeks. Group 5 rats were administered 4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil) orally for 2 weeks. Group 6 rats were administered 2ml/kg body weight of N. sativa oil plus4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil) orally for 2 weeks. Group 7 rats were administered 4ml/kg body weight of N. sativa oil plus 4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil) orally for 2 weeks. Histopathological or biochemical changes were not evident following administration of N. sativa alone. Serum levels of aspartic transaminase (AST) was significantly increased in animals treated with CCL4 when compared to the control group while L-alanine aminotransferase (ALT) and alkaline phosphatase (ALP) was significantly decreased in animals treated with CCL4 when compared to the control group. This increase and decrease in liver enzymes was almost restored to normal in animals treated with N. sativa and CCl4 has that of the control. Antioxidant status in the blood serum was estimated by determining the activities of superoxide dismustase (SOD), catalase (CAT), thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) levels. For histopathological evaluation, liver of all rats were excised and processed for light microscopy. CCl4 caused elevated level of TBARS and marked depletion of liver endogenous antioxidant enzymes. N. sativa treatment positively protects the alterations in these biochemical variables in the CCl4 + N. sativa-treated rats. N. sativa markedly reduced elevated TBARS and significantly increased the levels of antioxidant enzymes. The histological examination of the liver tissues in group 1, 2, 3 and 4 showed normal liver architecture. The histology of the liver in group 5 showed wide spread inflammation, oedema, vascular congestion, dilated sinusoidal spaces, N .sativa oil showed hepatoprotection on histological section of the liver in groups 6 and 7. In conclusion, our results demonstrated that N. sativa through its antioxidant activity effectively protects CCl4- induced livertoxicity.*

***Key Words****: $CCl_4$, N. sativa, DNA, Liver, Biochemical, Wistar Rats*

## I. Introduction

Carbon tetrachloride ($CCl_4$) is one of the oldest and most widely used toxins for experimental induction of liver fibrosis in laboratory animals (Tsukamoto *et al.,* 1990). This model has been used in various studies on examined the deposition of extracellular matrix in the fibrotic and cirrhotic liver (Hernandez-Munoz *et al.,* 1994; Muriel *et al.,* 1998). $CCl_4$ is a selective hepatotoxic chemical agent. $CCl_4$- induced reactive free radicals initiate cell damage through two different mechanisms of covalent binding to the membrane proteins and cause lipid peroxidation. A number of investigators have utilized this chemical to produce liver cirrhosis in experimental animals (Parola *et al.,* 1992). Production of reactive oxygen species and lipid peroxidation induced by iron overload (Bacon *et al.,* 1990), cholestatic injury (Parola *et al.,* 1996) and intoxication by ethanol (Kamimura *et al.,* 1992) and CCl4 (Parola *et al.,* 1992) is associated with liver fibrosis and cirrhosis. These effects are partially prevented by antioxidant compounds including α-tocopherol (Parola *et al.,* 1992; Halim *et al.,* 1997), silymarin (Mourelle *et al.,* 1989) and salvianolic acid (Hu *et al.,* 1997). The seed of *Nigella sativa* L (NS), an annual *Ranunculaceae* herbaceous plant, has been used traditionally for centuries in the Middle East, Northern Africa, Far East and Asia for the treatment of asthma. NS contains more bthan 30 of a fixed oil and 0.40-0.45 w/w of a volatile oil. The volatile oil has been shown to contain 18.4-24% thymoquinone and 46% many monoterpenes such as p-cymene, and α-pinene (El Tahir *et al.,* 1993). Recently conducted clinical and experimental researches have shown many therapeutic effects of NS extracts such as immunomodulator, antiinflammatory and anti-tumour, antibacteria agents (Rogozhin *et al.,* 2011; Alam *et al.,* 2010; Houghton *et al.,* 1995; Mehmet *et al.,* 2005; El Daly *et al.,* 1998; El-Kadi et al., 1987).

## II. Materials And Methods

**Experimental Animals**

A total number of 35 young adult Wistar rats were purchased from the Department of Pharmacology, Ahmadu Bello University Zaria. The animals were housed in the animal house of the Department of Human Anatomy, Ahmadu Bello University Zaria. The animals were between the ages of six and seven weeks and weighed between 130-180g. The animals were kept and maintained on standard laboratory condition of room temperature, humidity and under twelve hours dark – light cycle. The animals were fed with standard pellet diet and water. The animals were allowed to acclimatize to their new condition for two weeks before the commencement of the experiment.

**Experimental Protocol**

The 35 animals were randomly divided in to seven groups. Each group comprised of 5 rats and each rat in every group was marked for identification. The experimental animals weighed between 130-180 g and were randomly divided in to seven groups. Each group comprised of 5 rats. Group 1 rats were administered normal Saline (volume per body weight) orally for 2 weeks. Group 2 rats were administered olive oil 4m1/kg body weight orally for 2 weeks. Group 3 rats were administered 2ml/kg body weight of *N. sativa* oil orally for 2 weeks. Group 4 rats were administered 4ml/kg body weight of *N. sativa* oil orally for 2 weeks. Group 5 rats were administered 4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil) orally for 2 weeks. Group 6 rats were administered 2ml/kg body weight of *N. sativa* oil plus4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil) orally for 2 weeks. Group 7 rats were administered4ml/kg body weight of *N. sativa* oil plus 4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil) orally for 2 weeks.

**SACRIFICE OF ANIMALS**

On day 15, all animals were humanely sacrificed. Blood was collected from the jugular vein of the animals under deep anaesthesia with chloroform for biochemical analysis. The liver tissues were harvested for histological examination.

**BIOCHEMICAL STUDY**

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels were determined using autoanalyzer (Leica, Japan).

## III. Estimation Of Oxidative Parameters

**Determination of Catalase Activity**

Catalase activity was determined spectrophotometer at 570 nm using the method described by Sinha (1972). 5 % Potassium heptaoxochromate (VI), $K_2Cr_2O_7$: 5 g of $K_2Cr_2O_7$ was dissolves in little quantity of distilled water and made up to 100 ml. 0.2 M $H_20_2$: 0.6 ml of $H_20_2$ will be in dissolved in little quantity of water distilled and made up to 100 ml. it is stores at 4°C. 0.0lM phosphate buffer: 1.2g of $NaH_2P0_4$ and 1.4lg of $Na_2HPO_4$ was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 10.2. Diebroinate/Acetic acid solution 5 % Potassium heptaoxochromate (VI) $K_2Cr_2O_7$ was mixed with gacial acetic acid in the ratio 1:3, and was stored in brown bottle at room temperature.0.9 ml or distilled water was added to 0.1 ml of microsome and mixed thoroughly. 2.5 ml of phosphate buffer was put in a small conical flask; 0.5 ml of microsome was added; and 2.0 ml of $H_20_2$ added, starting the stop watch. The reaction mixture will be thoroughly mixed and the reaction will be stopped after every 60 seconds for 3 minutes with Dichroniate/Acetic acid solution. It was heated in a water bath for 10 minutes at 80°C. Absorbance was read at 570 nm.

**Determination of superoxide dismutase activity**

Superoxide Dismutase (SOD) activity was determined spectrophotometer by a method described by Fridovich (1989). The ability of superoxide dismutase (SOD) to inhibit auto oxidation of adrenaline at pH 10.2 formed the basses of this assay. 0.05 M phosphate buffer: 6.97 g of diphosphate $K_2HPO_4$ and 1.36 g of $KH_2PO_4$ was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 7.8. 0.05 Carbonate buffer: 14.3g of $Na_2CO_3$) and 4.2g Of $NaHC0_3$) was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 10.2. 0.3 mM Adrenaline: 0.01 g of adrenaline was dissolved in 17 ml of distilled water. The solution was prepared fresh. 0.1 ml of microsome will be diluted in 0.9 ml of distilled water to make 1:10 dilution of microsome. An aliquant mixture of 0.20 ml of the diluted mierosome was added to 2.5 ml of 0.05 M Carbonate buffer. The reaction started with the addition of 0.3m1 of 0.3 mM adrenaline. The reference mixture contains 2.5 ml of 0.05 M Carbonate buffer. 0.3rnl of 0.3 mM adrenaline and 0.20 ml of distilled water. Absorbance was measured ever 30 up to 150 s at 48Onm. 1 unit

of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adenochrome in 1 minute.

**Assessment of Lipid Peroxidation**

Lipid peroxidation as evidenced by the formation of TBARS was measured by the modified method of Niehaus and Samuelson (1968) and described by Akartji et al. (2009). To 150µl of serum, (O.25M sucrose solution) were treated with 2m1 of (1:1:1 ratio) TBA-TCA-HCL reagent (thiobarbituric acid 0.37%, 0.25N HCL and 15% TBA) and place in water bath for l hour at 90°C. The mixture was cooled and centrifuged at 3000rpm for 5mm at 4°C. The absorbance of the pink supernatant 2.0ml was measured against a reference blank using spectrophotometer at 535nm.

**Assay of Reduced Glutathione Concentration**

Reduced glutathione (GSH) concentration measurements were done according to ElIman (1959) as described by Rajagopalan et al. (2004). 0.2M phosphate buffer: 8.40g of $NaH_2PO_4$ and 9.94 of $Na_2HPO_4$ was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 8. To 150µ1 of serum or tissue homogenate (in phosphate -saline PH 7.4), 1.5m1 of 10% TCA was added and centrifuged at 1 500g for 5mm. 1 ml of supernatant was treated with 0.5 ml of ElIman's reagent (19.8 mg of *5,* 5'-clithiobis (nitro benzoic acid) (DNTB) in 100 ml of 0.1% sodium nitrate) and 3 ml of phosphate buffer (0.2 M, p1-I 8). The absorbance was read at 412 nm.

## HISTOLOGICAL ANALYSIS

Small liver specimens were extracted, placed in formalin solution, and processed routinely by embedding in paraffin. Tissue sections (4-5 µm) were stained with haematoxylin-eosin for general features and examined under light microscope (Celestron).

## STATISTICAL ANALYSIS

Data are presented as mean ± standard deviation. For establishing significant differences between groups, data were analyzed by the One-way ANOVA of variance followed by the Tukey *post hoc* test. Values were considered statistically significant if P value is less than 0.05 ($p < 0.05$), using sigmastat 2.0 for Window (soft stat, jan Raff, CA).

## IV. Result

The effects of *N. sativa* on $CCL_4$ induced hepatotoxicity in rats were evaluated by recording changes in serum AST, ALT and ALP levels as well as histopathological changes. The increased serum AST level in the CCL4 (240.0 ± 14.14 IU/L)-treated animals compared to that of the control group (159.0 ± 5.66 IU/L). This effect was also reversed in the animal groups that were given *N. sativa* and CCL4 (i.e. 2ml/kg body weight of *N. sativa* oil plus4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil and 4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil) orally). Serum AST levels were significantly elevated when compared to the CCL4 group. However, AST serum level of animals treated with *N. sativa* alone demonstrated no significant changes when compared to the control animals (Table 1).

The data in Table 1 demonstrate a trend of decrease levels of serum ALT in the CCL4 (330.0 ± 14.14 IU/L) treated animals compared to the control (574.0 ± 5.66 IU/L). ALT of animals treated with *N. sativa* alone remained within normal levels as that of the control group. This effect was reversed in the animal groups that were given *N. sativa* and $CCL_4$ (i.e. 2ml/kg body weight of *N. sativa* oil plus4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil and 4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil) orally). ALT serum levels were significantly elevated when compared to both the control and CCL4 groups.

There is decrease levels of serum ALP in the CCL4 (303.5 ± 106.8 IU/L) treated animals compared to the control (495.0 ± 7.07 IU/L). ALP of animals treated with *N. sativa* alone remained within normal levels as that of the control group. This effect was reversed in the animal groups that were given *N. sativa* and $CCL_4$ (i.e. 2ml/kg body weight of *N. sativa* oil plus4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil and 4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil) orally). ALP serum levels were significantly elevated when compared to both the control and CCL4 groups. On the other hand, animals treated with *N. sativa* alone remained within normal levels as the control (Table 1).

**Table 1: Showing the effect of *N.sativa* and $CCl_4$ on the Liver Enzymes.**

| Groups | AST (IU/L) | ALT (IU/L) | ALP (IU/L) |
|---|---|---|---|
| **1. Control** | 159.0±5.66* | 574.0±5.66* | 494.0±7.07* |
| **2. Olive Oil 4m1/kg body** | 167.0±4.24* | 515.0±35.36* | 499.5±6.71* |
| **3. Extract 2ml/kg body weight** | 166.5±12.02* | 519.5±71.42* | 484.5±50.21* |
| **4. Extract 4ml/kg body weight** | 162.5±3.54* | 570.0±14.14* | 517.5±53.03* |
| **5. 4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil)** | 240.0±14.14** | 330.0±14.14** | 303.5±106.8** |
| **6. CCL4 + Extract 2ml/kg body weight** | 178.0±2.83 | 395.0±7.07* | 439.0±41.01 |
| **7. CCL4 + Extract 4ml/kg body weight** | 170.0±42.43 | 480.0±84.85 | 486.5±19.09 |

***P<0.05, **P<0.01**

Antioxidant status in the blood serum was estimated by determining the activities of superoxide dismustase (SOD), catalase (CAT), thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) levels. Oxidative stress was characterized by increased lipid peroxidation and/or altered non-enzymatic and enzymatic antioxidant systems. The liver antioxidant activity of SOD, CAT and GSH significantly decreased, while liver TBARS significantly increased in the CCl4- treated, group of rats. The control group of rats maintained optimal value activity of the antioxidants studied. Administration of *Nigella sativa* significantly decreased the elevated TBARS, and also significantly increased the reduced antioxidant enzyme activities. Furthermore, *Nigella sativa* proved significantly better in restoring the altered activity of antioxidant enzymes like SOD, CAT, GSH and TBARS towards their normal values in the kidney homogenates. The animals treated with *Nigella sativa* alone showed no significant change in the levels of SOD, CAT, TBARS and GSH (Figs. 1, 2, 3 and 4).

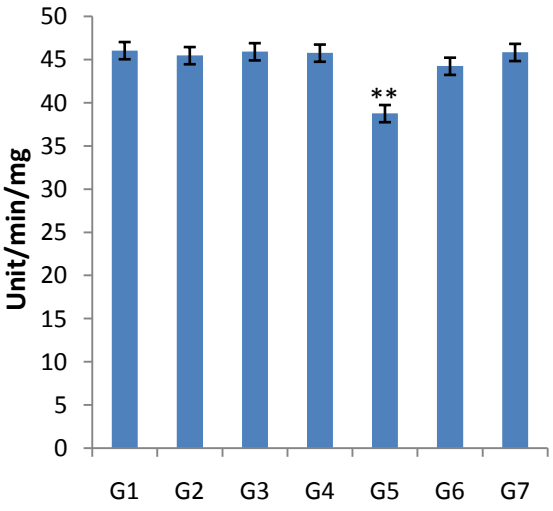


**Fig 1 effect of *N.sativa* and $CCl_4$ on the Liver SOD.**
**= p<0.01

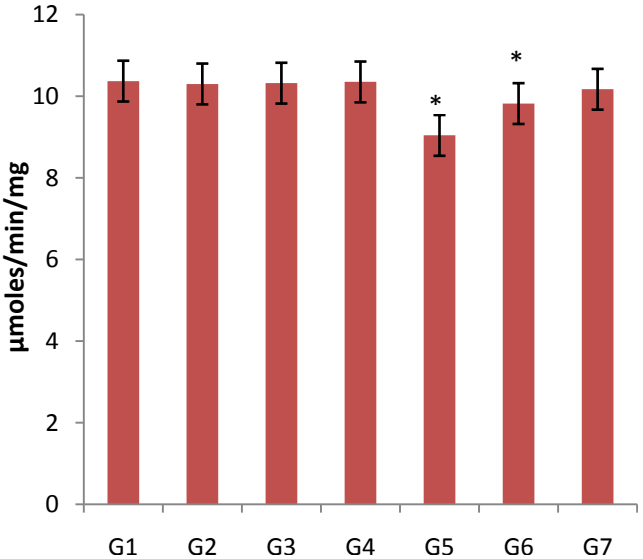


**Fig.2 effect of *N.sativa* and $CCl_4$ on the Liver CAT.**
*=p<0.05

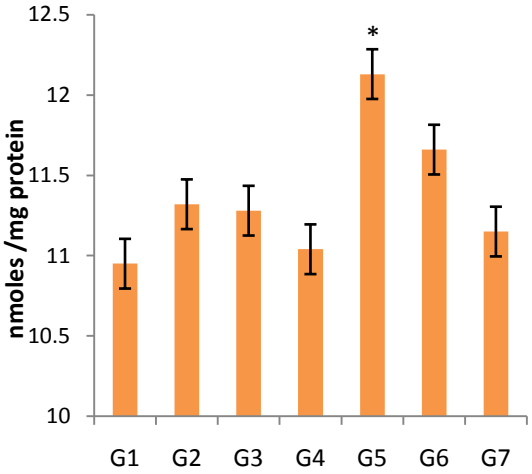


**Fig3 effect of *N.sativa* and $CCl_4$ on the Liver LPO.**
*=p<0.5

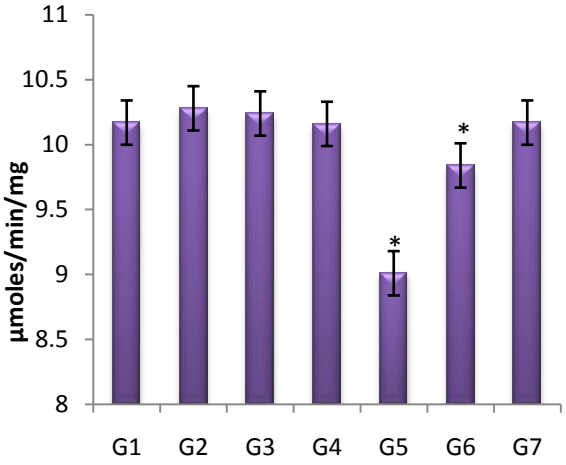


**Fig4 effect of *N.sativa* and $CCl_4$ on the Liver GSH**
*=p<0.05

The histological examination of the liver tissues in groups 1, 2, 3 and 4 (Plate 1, 2, 3 and 4) showed normal liver architecture. The histology of the liver in group 5 (Plate 5) showed wide spread inflammation, oedema, vascular congestion, dilated sinusoidal spaces. *N .sativa* oil showed hepatoprotection on histological section of the liver in groups 6 and 7 (Plate 6 and 7).

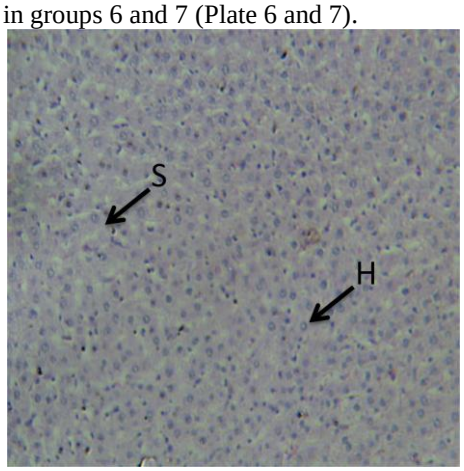


**Plate 1: Microscopic section of the liver of group 1 (control) rats showing normal histology of the liver; sinusoid (S) and hepatocytes (H) (H and E x 100).**

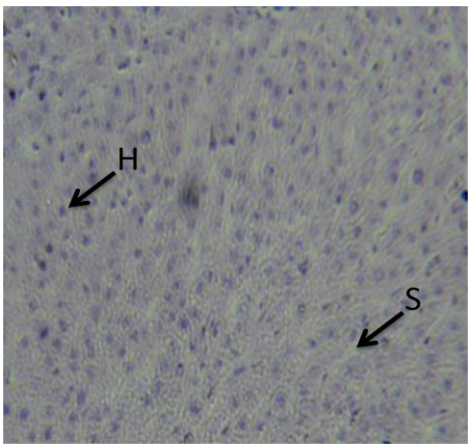


**Plate 2: Microscopic section of the liver of group 2 (2ml/kg olive oil) rats; sinusoid (S) and hepatocytes (H), (H and E x 100).**

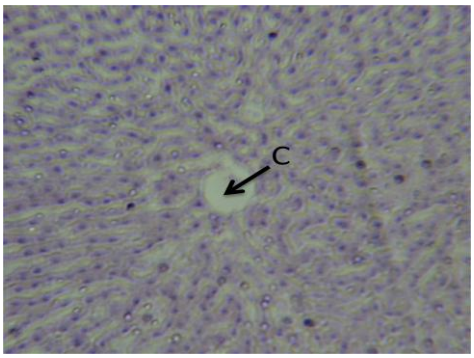


**Plate 3: Microscopic section of the liver of group 3 (2ml/kg *N. sativa*) rats; showing normal histology of the liver: central vein (C) sinusoid (S) and hepatocytes (H) (H and E x 100).**

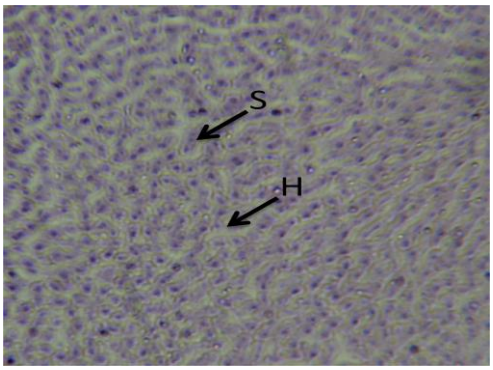


**Plate 4: Microscopic section of the liver of group 4 (2ml/kg *N. sativa*) rats; showing normal histology of the liver: sinusoid (S) and hepatocytes (H) (H and E x 100).**

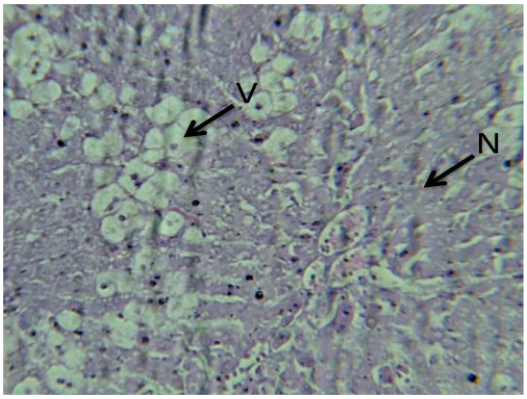


**Plate 5: Microscopic section of the liver of group 5 (4ml/kg $CCl_4$) rats; vacuolation (V) and necrosis (N), (H and E x 100).**

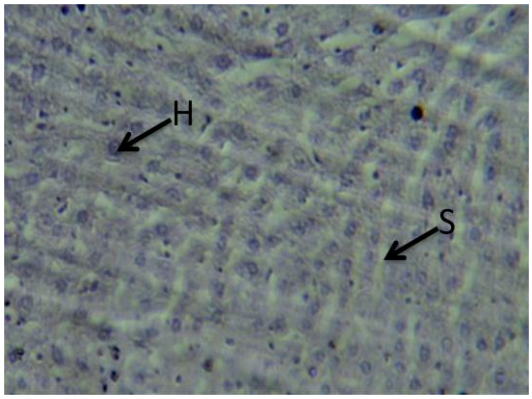


**Plate 6: Microscopic section of the liver from group 6 (2ml/kg *N. sativa* + 4ml/kg $CCl_4$) rats; hepatocytes (H) and sinusoid (S), (H and E x 100)**

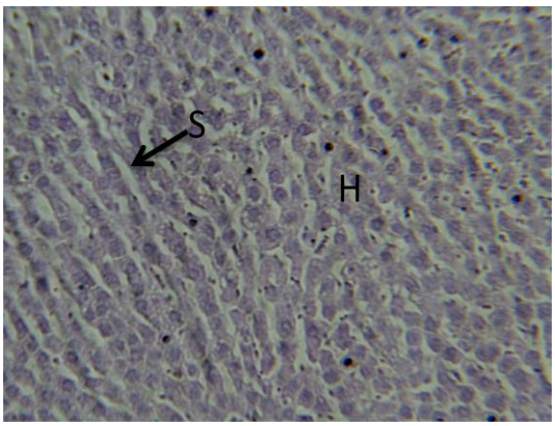


**Plate 7: Microscopic section of the liver from group 7 (4ml/kg *N. sativa* + 4ml/kg $CCl_4$) rats; sinusoid (S) and hepatocytes (H) (H and E x 100).**

## V. Discussion

Treatment of animals with $CCl_4$ is known to cause severe hepatic injury (Terblanche *et al.,* 1995). In this study, the repeated $CCl_4$ treatment for 14 days increased the lipid peroxidation and liver enzymes, and also decreased the antioxidant enzyme levels. It has been suggested that the lipid peroxidation may be a link between tissue injury and liver fibrosis by modulating collagen gene expression (Parola *et al.,* 1993). It was reported that CCl4 is suitable to induce lipid peroxidation in experimental animals within a few minutes after administration and its long-term use results in liver fibrosis and cirrhosis by lipid peroxidation pathway (Sherlock, 1970). It is generally thought that $CCl_4$ toxicity is due to reactive free radical ($CCl_3$), which is generated by its reductive metabolism by hepatic cytochrome P450. The reactive intermediate is believed to cause lipid peroxidation and breakdown of cellular membranes (De Groot *et al.,* 1989). It was found that the fixed oil of *N. sativa* has both antioxidant and anti-eicosanoid effects greater than thymoquinone which is its active constituent (Houghton *et al.*, 1995). *N. sativa* has antioxidant activity by suppressing the chemiluminescence in phagocytes (Haq *et al.,* 1995). Recently, Turkdogan *et al.*, 2003 observed that *N. sativa* has a significant hepatoprotective effect in $CCl_4$-administrated rabbits. However, Turkdogan *et al.*, 2001 found that *N. sativa* can prevent liver fibrosis and cirrhosis, suggesting that *N. sativa* protects liver against fibrosis possibly through immunomodulator and antioxidant activities. In our study, we found that *N. sativa* treatment alone showed normal LPO, antioxidant enzyme and liver enzyme levels as that of the control and also increased the reduced antioxidant enzyme levels in $CCl_4$-treated rats. Previously performed clinical and experimental investigations have shown that *N. sativa* has a protective effect against oxidative damage in isolated rat hepatocytes (Daba and Abdel-Rahman, 1998). Our biochemical results demonstrated that *N. sativa* treatment prevented $CCl_4$-induced hepatotoxicity in rats by decreasing the lipid peroxidation and increasing the antioxidant defense system activity. However, Kanter *et al.,*

2003 also showed the *N. sativa* increased the antioxidant defense system activity in experimentally CCl4-treated rats.

## VI. Conclusion

In conclusion, *N. sativa* decreased lipid peroxidation and liver enzymes, and increased antioxidant defense system activity in the $CCl_4$-treated rats.

## ACKNOWLEDGMENT

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