Hepatic Regeneration and Reno-Protection by Fish oil, *Nigella sativa* Oil and Combined Fish Oil/*Nigella sativa* Volatiles in CCl₄ Treated Rats

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Abstract: The aim of the present research was to investigate the effect of fish oil, crude *Nigella sativa* oil and combined fish oil/*Nigella sativa* volatile oil as hepato-regenerative and renal protective supplements. The oils were administered as emulsions to rat model with liver injury induced by CCl₄. Plasma activities of transaminases (AST and ALT) were evaluated as liver function indicators, while plasma creatinine and urea and creatinine clearance were determined as markers of kidney function. Plasma malondialdehyde (MDA), nitrite (NO) and tumor necrosis factor-α (TNF-α) were estimated to assess the exposure to oxidative stress and subsequent inflammation. Liver fat was extracted and their fatty acids’ methyl esters were determined using gas chromatography. Results showed that plasma activities of AST and ALT were significantly higher in CCl₄ control group compared to control healthy group. Plasma levels of creatinine and urea increased significantly in CCl₄ control, while creatinine clearance was reduced significantly in the same group. All rat treated groups given the three oil emulsions showed improvement in liver function pointing to the initiation of liver regeneration. The combination of fish oil/*Nigella sativa* volatiles showed the most promising regenerative activity. Oxidative stress and inflammation which were increased significantly in CCl₄ control showed improvement on administration of the three different oil emulsions. Fatty acids methyl ester of liver fat revealed that rats treated with fish oil/*Nigella sativa* volatile oil presented the highest content of unsaturated fatty acids (45.52% ± 0.81) while fish oil showed the highest saturated fatty acids (53.28% ± 1.68). Conclusion; Oral administration of oil emulsions of native fish oil, *Nigella sativa* crude oil and combined fish oil/*Nigella sativa* volatile oil reduced liver and kidney injury in rat model of CCl₄, through exerting anti-inflammatory and antioxidant activity. Fish oil/*Nigella sativa* volatile oil emulsion was the most promising hepato-regenerative and reno-protective formula among the different groups.

Key words: liver regeneration, carbon tetrachloride, fish oil, *Nigella sativa* crude oil, *Nigella sativa* volatile oil, renal dysfunction

1 Introduction

The liver plays an important role in different vital processes including metabolism, detoxification and immunity. Liver damage could occur through exposure to chemicals, extreme fat deposition and/or viral infection which all lead ultimately to additional functional renal failure. The advancement of both liver and kidney dysfunction might be related to both high oxidative stress and inflammatory conditions⁴. Fortunately, the liver has unmatched capacity for regeneration after exposure to hepatic damage factors²⁻³. Regeneration is also a part of remodeling of the liver that occurs in different other cases like cirrhosis, the replenishment of lost cells after hepatotoxic insults, and the transient hepatomegaly during pregnancy in response to the increased metabolic demand by the fetus⁴. The cells associated with liver regeneration are closely related to the degree of liver damage. Multiple genes and cytokines as well as the liver microenvironment play crucial roles in the dynamic regulation of regeneration via complex mechanisms that are not fully understood at present⁴.

Beside the inherent capacity of liver for self-regeneration, some bioactive plant constituents like silymarin and...
silibinin can stimulate the regeneration process\textsuperscript{6}. In addition dietary components like curcumin, vitamin K2 and vitamin E can also play a crucial role in enhancing liver regeneration\textsuperscript{7-9}. Omega-3-polyunsaturated fatty acids (ω-3 PUFA) have been shown to promote liver regeneration and functional recovery in living liver donors after resections for liver transplantation\textsuperscript{10}. They also showed effectiveness for treatment of patients after hepatectomy when administered in the form of lipid emulsion\textsuperscript{11}.

Based on the advantages of ω-3 PUFA in the enhancement of liver regeneration, the authors of the current work planned to examine the possibility of further enhancement of liver regeneration ability of ω-3 PUFA by combination with a small amount of the volatile oil fraction of black cumin (\textit{Nigella sativa}) seeds. This plant-derived volatile oil is co-extracted simultaneously with the crude oil of \textit{N. sativa} during seeds’ pressing. It was chosen due to its liver-related biological activity that includes protection against injury\textsuperscript{12}, reduction of formation of malondialdehyde, and enhancement of antioxidant enzyme activities which all lead to potential treatment of fibrosis\textsuperscript{13}. The volatile oil of \textit{N. sativa} was also found to be responsible for the protecting activity of its parent bearing crude oil against fatty liver in rat model\textsuperscript{14}. The main components of the volatile oil fraction of \textit{N. sativa} namely thymoquinone and p-cymene were deemed to be responsible for the anti-inflammatory, hepato and reno-protective effect of the whole crude oil of \textit{N. sativa}\textsuperscript{14,15}.

For these reasons it is hypothesized that combination of \textit{N. sativa} volatile oil with fish oil (as a rich source of ω-3 PUFA) could potentially elicit a recognized liver regeneration together with prevention of renal failure. Therefore, the current work was designed to evaluate the enhancement of liver regeneration and subsequent prevention of renal failure in rat model deliberately injured by carbon tetrachloride. The liver regenerating agents (oils) planned to be studied in the current work are the whole crude oil of \textit{N. sativa}, fish oil and fish oil combined with the volatile oil fraction of \textit{N. sativa} (at a percentage similar to its natural abundance in the whole crude oil of \textit{N. sativa}).

2 Experimental

2.1 Chemicals, fish oil and plant material

\textit{CCl}_4 was purchased from (Sigma-Aldrich St. Louis, MO USA). Fish oil was purchased from Natrol Inc. (Chatsworth, CA 91311 USA). Mature dried seeds of black cumin (\textit{Nigella sativa} L, family Ranunculaceae) were purchased from local herbal retail store specialized in medicinal and aromatic plants trading (Cairo, Egypt).

2.2 Animals

Male Sprague Dawley rats weighing 143–179 g (age: 6-8 weeks) were used in the present study. Animals were obtained from Animal house of National Research Centre, Cairo, Egypt. Animals were kept individually in metabolic stainless steel cages; water and food were given \textit{ad-libitum}.

2.3 Preparation of carbon tetrachloride for dosing

A certain volume of carbon tetrachloride was added to equal volume of corn oil and the whole mixture was stirred till homogeneity.

2.4 Diet

A balanced diet composed of 12.5% casein, 10% corn oil, 23.3% sucrose, 46.7% maize starch, 3% fiber, 3.5% salt mixture and 1% vitamin mixture was prepared for feeding rats all over the experimental period according to the literature\textsuperscript{16}.

2.5 Extraction of \textit{N. sativa} whole crude oil

The crude oil of \textit{N. sativa} was extracted from the seeds according to the previous study\textsuperscript{17}. In detail, the seeds were exhaustively pressed for two successive cycles by using a commercial scale expeller screw pressing machine located at the same herbal retail store from which the seeds were purchased. The extracted crude oil of \textit{N. sativa} was filtered and received in dark brown glass bottles equipped with screw caps and stored immediately under refrigeration conditions at $-4\degree C$ until used.

2.6 Isolation of \textit{N. sativa} volatile oil from its parent crude oil

Part of the whole crude oil of \textit{N. sativa} which was obtained from the previous extraction step was subjected to a hydro-distillation process as described in the same previous study\textsuperscript{18} to isolate its volatile oil fraction. In detail, 100 g of the crude oil (which is corresponding to \textasciitilde 500 g seeds) was mixed with distilled water (1:7 w/v, respectively) in a 5 liter round-bottom flask and distilled for three hours using the Clevenger-type apparatus. The cup that receives the pure volatile oil fraction in the side arm of the apparatus was wrapped with aluminum foil to protect the volatile oil from light. At the end of the distillation process the volatile oil was collected, dried over anhydrous sodium sulfate and its yield percent relative to the weight of the parent crude oil was assessed from three different extractions \pm S.D. Finally the collected samples of the volatile oils were stored at $-4\degree C$ until used.

2.7 Preparation of different sets of oil emulsions for rat dozing

Three different sets of oil-in-water emulsions were prepared having the same quantitative composition but differ only in the type of the oil phases. These oils included: 1- the whole crude oil \textit{N. sativa}, 2- pure fish oil, 3- pure fish oil.
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Oil combined with the isolated volatile oil fraction of *N. sativa* which was spiked at 2.6% of the fish oil’s weight. That chosen percentage is typically similar to the natural abundance of that volatile oil fraction in the whole crude oil of *N. sativa* (as would be seen from the results section).

The three emulsions were formulated with 6% total oil phase, 1.2% surfactant (Tween 80) and 92.8% distilled water. First, the surfactant was dispersed in each oil phase separately using mechanical stirring for 20 min. Then, each oil-surfactant mixture was added drop wise to the aqueous phase (distilled water) to form spontaneously a coarse milky white emulsion. The three different oil emulsions were further homogenized to reduce the particle size of the dispersed oil droplets by using high speed rotor-stator homogenizer (WiseTis HG-15D, Wise Laboratory instruments, Korea) at 3000 rpm for 3 min. The final emulsions were kept in refrigerator at 4°C and administered to rats at the next day of formulation. All oil emulsions were stored for only 10 days during the feeding experiment, after which the emulsions were discarded even if they did not manifest any signs of separation. Consequently fresh new batches of the three oil emulsions were re-formulated regularly every 10 days till the end of the experimental period. Beside the three oil emulsions, a vehicle composed of 1.2 wt% surfactant and 98.8 wt% distilled water was also formulated without any kind of oil phase, for comparison.

2.8 Design of animal experiment

Thirty male rats were divided into five groups, each comprised of 6 rats. Group one served as normal control. Group two was that of control rats with induced liver injury. The other three groups (3-5) were the test groups. Hepatic injury was induced in rats of groups 2-5 according to literature, with some modifications, by intra-peritoneal injection of CCl₄/corn oil (at a dose of 2 ml CCl₄/kg rat body weight thrice during ten days). After induction of hepatic injury rats of groups 3-5 were treated by daily oral dose of the oil emulsions separately that provide 300 mg oil/kg rat body weight, for 20 days. Rats of the two control groups were given daily oral dose of the vehicle. After elapse of experimental period (a month); blood samples were withdrawn from eye vein orbital of anaesthetized fasted rats and received in heparinized tubes for separation of plasma. Twenty-four hours urine samples were collected during the last 24 h of the experiment for determination of creatinine and for calculation of creatinine clearance. Rats were dissected and livers were immediately separated, weighed and stored at −20°C till being analyzed. Plasma activities of alanine transaminases and aspartate transaminase (ALT and AST) were estimated as indicator of liver function. Plasma creatinine and urea were also determined, respectively to assess kidney function. Plasma tumor necrosis factor-α (TNF-α) was estimated as inflammatory marker. Plasma nitrite (NO) was assessed as both oxidative stress and inflammatory biomarker. Plasma malondialdehyde (MDA) as indicator of lipid peroxidation was determined.

Animal procedure was performed in accordance with the Ethics Committee of the National Research Centre, Cairo, Egypt, and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.9 Extraction, preparation and gas chromatographic analysis of hepatic lipids

Total hepatic lipids were extracted according to published procedure, which was modified by other authors. Fatty acids methyl esters of hepatic lipids were prepared for GC analysis according to AOAC (2000). Assessment of the methyl ester was carried out by injecting 2 µL into a Hewlett Packard HP-system 6890 gas chromatograph equipped with FID. HP-5 capillary column (30 m x 0.32 mm i.d.; 0.25 µm film thickness) was used to separate the different methyl esters. The chromatographic analysis conditions were: initial temperature 70°C with a hold for 1 min, then raised to 120°C at a rate of 40°C /min with 2 min hold then the temperature was finally raised to 220°C at a rate of 4°C /min with another 20 min hold. The injector and detector temperatures were 250°C and 280°C respectively. Identification of the fatty acid methyl esters were carried out by direct comparison of retention times of each of the separated compounds with standards of the fatty acid methyl esters analyzed under the same conditions. Quantization was based on peak area integration.

2.10 Statistical analysis

The results of biological experiment were expressed as the mean ± SE and analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan’s test. In all cases *p* < 0.05 was used as the criterion of statistical significance.

3 Results

A whole crude oil was obtained from the seeds of *N. sativa* reaching ~21% of the seeds’ weight after being subjected to a screw pressing process. This crude oil was found to contain a natural volatile oil fraction accounting for 2.6% of its weight, as revealed from the hydro-distillation process of that crude oil.

Table 1 showed that plasma AST and ALT activities were significantly higher in CCl₄ control group than normal control group, indicating liver dysfunction. Plasma levels of creatinine and urea as biomarkers of kidney function increased significantly together with significant reduction of creatinine clearance in CCl₄ control group compared to normal control. Oral administration of the three different
Fish oil/N. sativa volatile oil was the most effective treatment for improving AST and ALT. In addition, plasma nitrite level was significantly elevated in CCl4 control group compared with normal control. Plasma levels of MDA and TNF-α were significantly high in CCl4 control group than control normal rats. Stearic was significantly higher in CCl4 control group concerning saturated fatty acids; palmitic, lignoceric and behenic and the unsaturated linoleic acid. On the other hand, oleic acid was significantly higher in normal control group only on administration of N. sativa crude oil. Creatinine clearance showed insignificant change from control normal rat only on treatment with fish oil/volatile oil combination.

Fatty acids content of liver fat of different experimental groups as percentage of total fatty acids.

Table 1 Biochemical parameters of different experimental groups (mean ± SE).

|                          | Normal control | CCl4 control | N. sativa crude oil emulsion | Fish oil emulsion | Fish oil / volatile oil emulsion |
|--------------------------|----------------|--------------|------------------------------|------------------|---------------------------------|
| Plasma ALT (U/L)         | 9.83±0.75      | 21.00±1.46   | 8.67±0.71                    | 10.66±1.63       | 8.33±0.42                       |
| Plasma AST (U/L)         | 33.33±1.38     | 83.67±2.93   | 50.16±1.25                   | 48.17±2.95       | 48.00±1.91                      |
| Plasma urea (mg/dL)      | 25.74±1.10     | 34.56±0.92   | 25.40±1.10                   | 29.07±0.57       | 28.55±0.87                      |
| Plasma creatinine (mg/dL)| 0.69±0.02      | 1.32±0.03    | 1.00±0.07                    | 0.96±0.08        | 0.81±0.06                       |
| Plasma TNF-α (pg/mL)     | 18.17±1.25     | 49.33±2.27   | 34.00±1.81                   | 38.83±2.09       | 32.00±1.81                      |
| Plasma NO (µmol/L)       | 20.65±0.71     | 35.13±1.71   | 19.25±0.90                   | 22.09±1.44       | 21.04±1.22                      |
| Plasma MDA (nmol/mL)     | 8.26±0.58      | 17.28±0.44   | 8.72±0.84                    | 8.89±0.87        | 9.22±0.62                       |
| Creatinine clearance     | 1.00±0.05      | 0.53±0.02    | 0.72±0.05                    | 0.77±0.09        | 0.90±0.08                       |

In each column same letters mean non-significant difference; different letters mean significant difference at 0.05 probabilities.

Table 2 Fatty acids content of liver fat of different experimental groups as percentage of total fatty acids (mean ± SE).

|                          | Normal control | CCl4 control | N. sativa crude oil emulsion | Fish oil emulsion | Fish oil / volatile oil emulsion |
|--------------------------|----------------|--------------|------------------------------|------------------|---------------------------------|
| Palmitic (C16:0)         | 22.93±0.58     | 22.44±0.26   | 21.09±0.30                   | 23.41±0.00       | 23.93±2.55                      |
| Stearic (C18:0)          | 14.64±0.84     | 11.67±0.86   | 12.79±0.42                   | 10.05±0.03       | 7.68±0.65                       |
| Oleic (C18:1)            | 15.34±0.92     | 19.16±0.29   | 18.35±0.55                   | 23.80±0.27       | 24.07±0.71                      |
| Linoleic (C18:2)         | 20.28±0.11     | 19.44±0.10   | 21.16±0.25                   | 18.57±0.10       | 21.45±0.11                      |
| Behenic (C22:0)          | 21.07±0.49     | 18.23±0.21   | 11.92±5.12                   | 16.38±0.99       | 16.63±4.12                      |
| Lignoceric (C24:0)       | 1.18±0.32      | 1.62±0.01    | –                            | 3.45±0.71        | 0.68±0.32                       |
| Total saturated fatty acids | 58.42±1.05    | 53.95±0.92   | 45.79±5.84                   | 53.28±1.68       | 48.92±0.61                      |
| Total unsaturated fatty acids | 35.58±0.81 | 38.60±0.18   | 39.51±0.80                   | 42.37±0.38       | 45.52±0.81                      |

In each column same letters mean non-significant difference; different letters mean significant difference at 0.05 probabilities.

sets of oil emulsions used in the present study showed significant improvement in liver and kidney function with different degrees, pointing to liver regeneration. It was also inferred from Table 1 that the deliberate combination of fish oil/N. sativa volatile oil was the most effective treatment for improving AST and ALT. In addition, that oil combination was the most promising in improving creatinine and creatinine clearance.

Plasma levels of MDA and TNF-α were significantly high in CCl4 control group compared with normal control referring to elevated oxidative stress and inflammation. Also plasma nitrite level was significantly elevated in CCl4 control compared to normal control. Plasma levels of TNF-α as inflammatory biomarker reduced significantly in all oil emulsions treated groups with the lowest level (32.0±1.81 pg/mL) observed for rats given the combination of fish oil/volatile oil. On the other hand, oral administration of N. sativa whole crude oil emulsion (which is naturally containing 2.6 wt% of the volatile oil) was the most promising in improving MDA and NO levels.

All treatments were able to normalize plasma ALT activity, NO and MDA. Plasma urea matched the control level only on administration of N. sativa crude oil. Creatinine clearance showed insignificant change from control normal rat only on treatment with fish oil/volatile oil combination. Fatty acids’ content of liver fat (Table 2) revealed no distinct variation between control normal rats and CCl4 control group concerning saturated fatty acids; palmitic, lignoceric and behenic and the unsaturated linoleic acid. On the other hand, oleic acid was significantly higher in CCl4 control group than control normal rats. Stearic was significantly high in control normal rats compared to CCl4 control group. Total unsaturated fatty acids were significantly high in CCl4 control group compared to normal group. Upon administration of the three oil emulsions, rats given oral administration of N. sativa crude oil emulsion showed the lowest content of liver saturated fatty acids (45.79%) followed by rats given oral administration of fish oil/volatile oil combination (48.92%), however no significant change was present among test groups and CCl4 control. Regarding the effect of treatments on unsaturated fatty acids it was found that rats treated with fish oil/volatile oil combination presented the highest content of unsaturated fatty acids (45.52%) followed by rats adminis-
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Table 3  Nutritional parameters and liver/body weight % of different experimental groups (mean ± SE).

|                          | Normal control | CCl4 control | N. sativa crude oil emulsion | Fish oil emulsion | Fish oil / volatile oil emulsion |
|--------------------------|----------------|--------------|------------------------------|-------------------|---------------------------------|
| Initial body weight (g)  | 159.00± 5.23   | 158.83± 3.75 | 158.50± 3.05                 | 158.67± 3.72      | 158.67± 4.62                   |
| Final body weight (g)    | 220.50± 5.14   | 228.33± 4.45 | 219.17± 4.08                 | 218.00± 4.19      | 220.50± 4.37                   |
| Body weight gain (g)     | 61.50± 2.14    | 69.50± 1.87  | 60.67± 1.99                  | 59.33± 2.23       | 61.83± 1.33                    |
| Total food intake (g)    | 529.83± 5.29   | 492.00± 8.08 | 512.17± 4.93                 | 505.00± 13.35     | 523.00± 4.16                   |
| Feed efficiency ratio    | 0.116± 0.003   | 0.141± 0.003 | 0.118± 0.003                 | 0.117± 0.002      | 0.118± 0.002                   |
| Liver weight (g)         | 7.31± 0.21     | 7.99± 0.16   | 7.34± 0.10                   | 7.67± 0.34        | 7.63± 0.13                     |
| Liver/body weight %      | 3.33± 0.14     | 3.51± 0.12   | 3.35± 0.05                   | 3.54± 0.22        | 3.47± 0.08                     |

In each row same letters mean non-significant difference; different letters mean significant difference at 0.05 probabilities.

4 Discussion

Hepatic diseases are the most common of all the pathologies worldwide and constitute up to 83% of all the cases. Among the causative factors of hepatic toxicity are food additives, alcohol, toxic industrial chemicals and air and water pollutant. The liver is a unique organ with ability to regenerate and recover its original function after extensive resection or injury. This occurs due to the hyperplasia of the residual lobes and mitosis of the hepatocytes which are quiescent under normal conditions. Some studies have speculated that slight genesis and/or formation of low level of free radicals are important for stimulating liver regeneration and necessary for their natural course. These speculations seem to be only coincided on low concentration of reactive oxygen species because logically elevated oxidative stress could contribute to liver damage. It is mandatory that the continual hepatic exposure to oxidative stress and inflammation could hinder the intrinsic liver regeneration and require external intervention with antioxidant and anti-inflammatory agent. In order to fulfill that requirement, the current study evaluated the liver regeneration potentials of some natural oils like fish oil, N. sativa whole crude seeds oil and fish oil / Nigella volatile oil combination.

Based on the above mentioned, the current study was initiated by inducing hepatic injury in rats using CCl4 which is one of the most potent inducers of acute liver injury. It is also used in experimental model to investigate the hepatoprotective role of natural products and drugs. Liver injury was observed in the present study through significant elevation of ALT and AST as previously reported. CCl4 induced toxicity depends on the dose and duration of exposure. At low CCl4 doses, transient effects occur, including the loss of Ca2+ sequestration, impaired lipid homeostasis and release of several cytokines. Longer CCl4 exposure alters fatty acid metabolism, and induces fibrosis, cirrhosis and cancer. One distinctive feature of CCl4 toxicity is rapid triglycerides accumulation in the liver, similar to observation in liver steatosis.

In CCl4 treated control rats plasma levels of MDA, NO and TNF-α were significantly elevated pointing to the incidence of lipid peroxidation, oxidative stress and inflammation. All these changes are expected consequences of treatment with CCl4 which induces elevation of reactive oxygen species (ROS) and MDA that lead to destruction and disruption of lipids of the membranous system, cellular proteins, intracellular organelles and causes DNA damage inducing several pathological changes. CCl4 is transformed in the liver to trichloromethyl free radical that disrupts Ca2+ homeostasis and induces lipid peroxidation that leads to hepatocellular injury.

In the present study significantly elevated plasma levels of creatinine and urea together with reduction of creatinine clearance indicated kidney dysfunction, in CCl4 control. The results are in agreement with other studies which reported that CCl4 induced nephrotoxicity in rats through elevation in creatinine and blood urea nitrogen. This nephrotoxicity might be due to direct action of CCl4 or could be...
attributed to either elevated ROS and TNF-α or a consequence of severe liver injury.

Administration of different oil emulsions in the present study produced reduction in oxidative stress and inflammation reflected in inhibiting MDA, NO and TNF-α level in CCl4 treated rats. This effect leads to improvement in both liver and kidney functions as noticed from the reduction in plasma ALT and AST activities, creatinine and urea with the simultaneous increase in creatinine clearance.

The hepato-renal improvement in rats treated with the whole crude oil of N. sativa emulsion might originates from its natural content of the volatile oil fraction, which reached 2.6% of its weight in the current study. This volatile oil contains a major component namely thymoquinone (TQ) in addition to p-cymene which are considered to be responsible for the protective effect of that volatile oil fraction and its parent crude oil that bears that volatile fraction. The content of TQ and p-cymene in the current investigation reached 68.13% and 20.11%, respectively of the total volatile oil composition, as determined by full GC analysis that was illustrated in our recent study. That previous study was conducted concomitantly with the current study by using the very same seeds of N. sativa and its whole crude oil under the same extraction and storage conditions. Therefore it can be comfortably indicated that the chemical composition of the volatile oil and its content of TQ and p-cymene in the current study is exactly the same as that illustrated before. One should also bear in mind that the other non-volatile components of the whole crude oil of N. sativa like the vitamin E, phytosterols and linolenic acid can also participate beside the volatile oil fraction in the hepato-renal improvement effect of that crude oil due to their anti-inflammatory and/or anti-oxidant activities. It is also worth indicating that the whole crude oil of N. sativa previously showed hepato and reno-protective effect in rat model treated with galactosamine hydrochloride and fed on high fructose diet.

The hepato-renal ameliorating effect of fish oil seen in the present study can be justified based on its content of the anti-inflammatory ω-3 fatty acids like eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). Previous reports indicated that EPA has protective effect towards glomerulonephritis and can improve liver MDA in CCl4 induced liver dysfunction. Despite the high unsaturation of fish oil fatty acids however improvement in MDA and NO was noticed in the present study which agreed with other studies. In addition, ω-3 fatty acids were demonstrated to have remedial effect in experimental lupus nephritis. The anti-inflammatory activity of ω-3 fatty acids is related to amelioration of cytokine. These fatty acids were also reported to reduce oxidative damage in various tissue which agreed with the current study. However other authors demonstrated elevated thiobarbituric acid reactive substance in liver of mice treated by fish oil. It is worth noting that fatty acids composition of fish oil was not determined in the present study. However in our previous work, the fatty acids of this oil was assessed by GC-FID and GC-MS and the main fatty acids were EPA (20.15%), DHA (13.77%), palmitic (13.44%), oleic (10.85%) and other minor fatty acids including linoleic (1.57%).

The evaluation of the liver regeneration ability of fish oil/ N. sativa volatile oil emulsion combination is considered to be the main target of the current study and the first report in this field. The data in the present study indicated that this oil combination was almost the best among the other sets of oils in its liver ameliorative effect which is an indication of regeneration. The mechanism by which this oils’ combination exert its action is axiomatically inferred from the additive regenerative activity of fish oil as a source of ω-3 fatty acids along with N. sativa volatile oil as a rich source of thymoquinone. Although there were insignificant difference between the inflammation, oxidative stress, liver and kidney function biomarkers of rats treated by the whole crude oil of N. sativa, fish oil and fish oil/N. sativa volatile oil combination but the values of the latter indicates almost the highest regenerative effect.

The liver is a major metabolic organ involved in fatty acid metabolism. Lipid homeostasis and fatty acids metabolism are strongly affected by exposure to CCl4. A decrease in saturated fatty acids and an increase in unsaturated fatty acids were noticed after treatment with CCl4 which agreed with the data of the current study. In the present work; CCl4 treatment induced significant increase in oleic without affecting linoleic with significant reduction in stearic acid. CCl4 treatment provoked fatty metamorphosis in liver. It was also reported that oleic acid and linoleic acid were increased in liver tissue after partial hepaotropic. These authors suggested that some significant signals are transmitted during the regeneration process owing to alterations in the membrane structure by the high levels of fatty acid after partial hepatectomy.

In the present study; the three different oils treatment produced significant increase in both oleic and linoleic acid (except for linoleic acid in case of fish oil) compared to normal rats reflecting the possible increase in regeneration process as previously reported. The fatty acid profile in liver was rarely studied during intake of ω-3 or ω-6 rich oils or even after treatment with CCl4; the scarce studies found could not explain the mechanism by which alteration in hepatic fatty acids profile occur. Fish oil was reported to produce hepatic increase in genes expression related to beta-oxidation of fatty acids and reduce expression of those induce lipogenesis. The body is dependent on the exogenous supply of omega-6 (present in Nigella sativa crude oil) and omega-3 (present in fish oil). These essential fatty acids are key players in regulating metabolic signaling. Omega-3 fatty acids exert anti-inflammatory properties in addition to its beneficial effect on hepatic steatosis, re-
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It was revealed that intake of omega-3 fatty acids by normal mice produced enrichment of $\omega-3$ in liver and plasma phosphatidylcholine, lysophosphatidylcholine, cholesterol ester and free fatty acids which are important bioactive lipids. In the present results no $\omega-3$ fatty acid was seen among the analyzed hepatic fatty acids during treatment with $\omega-3$ fatty acids represented by either fish oil or fish oil/N. sativa volatile. This could be due to the complex metabolic effect of fish oil including the increased fatty acids beta-oxidation and the reduction in hepatic lipogenesis and liver fats along with increased export of lipids from liver that could alter the expected hepatic fatty acids profile. Fatty acids composition could be influenced by the rate of lipid peroxidation and the activity of delta-6 desaturase enzyme. PUFAs up regulate proximal proliferator-activated receptors that lead to increased genes transcription that are responsible for fatty acids degradation. These fatty acids also down regulate steroid-responsive element binding protein that increases transcription of genes responsible for fatty acids synthesis. Linoleic acid ($\omega-6$) is the major fatty acid in Nigella sativa seed oil accounting for 54.0-70.0%. This fatty acid showed significant increase in the liver of rats treated by N. sativa oil compared to normal control group, CCl$_4$ group and the rats treated with fish oil which could be a reflection of its intake. However the non significant difference of liver linoleic acid between the rats given N. sativa oil and those treated by fish oil/volatile is hardly to be explained. Full identification of the fatty acid composition of the crude oil of N. sativa was determined in our previous article where the major fatty acids were linoleic (60.4%), oleic (21.9%), palmitic (9.5%), stearic (3.3%) and other minor fatty acids.

In the present study; the body weight gain of CCl$_4$ control group increased significantly compared with different groups also final body weight increased non-significantly. This result agreed with that of previous study. The apparent increase in body weight gain is actually due to ascitis that results from drainage of fluid in the abdominal region that was noticed during rats’ dissection in addition to the slight hepatomegaly but not to an increase in the lean or fat compartment of the body. The significant reduction of total food intake of CCl$_4$ control group supports this explanation. Oral administration of different oil emulsions attenuated elevation in body weight to reach normal level which reflects an improvement in the damaged liver and subsequent reduction of ascitis. Also, $\omega-3$ fatty acids were reported previously to reduce body weight through decrease hepatic and total body fats. Crude oil of N. sativa produced significant reduction in rat body weight in a previous study which supports the present results.

Conclusion

Oral administration of N. sativa crude oil, fish oil and/or fish oil supplemented with N. sativa volatile oil in emulsion form could reduce liver injury and enhance liver regeneration with concomitant reno-protection in rat model of CCl$_4$. These activities were accomplished through the anti-inflammatory and antioxidant effects which were excreted by these natural oils. Fish oil/N. sativa volatile oil combination was superior to the other oils. This work can potentially be a new achievement in the dietary supplements filled that can help in protection and/or amelioration of liver dysfunction.

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