Lindane-induced biochemical perturbations in rat serum and attenuation by omega-3 and *Nigella sativa* seed oil

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Lindane (γ-hexachlorocyclohexane, γ-HCH), a highly persistent organochlorine insecticide is neurotoxic at acute doses and has been reported to induce oxidative stress in cells and tissues. In this study, we investigated the antioxidant property of *Nigella sativa* seed oil (N.O) and omega-3 polyunsaturated fatty acids (ω3) against γ-HCH-induced oxidative hepatic and renal damage in male rats serum. Rats were orally given sublethal dose of γ-HCH (12 mg/kg, 24 h prior to decapitation), while N.O (0.3 ml/kg) and ω3 (20 mg/kg) were given every 48 h for 20 days single or together, or also combined with γ-HCH. γ-HCH caused a significant increase in the levels of serum total lipids, cholesterol, and triglycerides by 49, 61 and 30% respectively, while HDL-cholesterol decreased by 45% compared to control group. Pretreatment with ω3 and N.O prior γ-HCH administration re-established the altered biochemical features and alleviated the harmful effects of γ-HCH on lipid profile. The concentration of serum total protein and albumin was significantly decreased by 35 and 45% respectively in rats treated with γ-HCH compared to control. γ-HCH also caused hepatic and renal damage, as observed from the elevated serum levels of urea, creatinine, total bilirubin and uric acid contents and aminotransferases (AST and ALT), phosphatases (ACP and ALP) and lactate dehydrogenase (LDH) activities. Co-administration of ω3 and N.O reversed the hazardous effects induced by γ-HCH on the liver and kidney and also protected acetylcholinesterase from the inhibitory action of γ-HCH as well as suppressed the lipid peroxidation. Thus, the results show that ω3 and N.O might prevent oxidative stress and attenuate the changes in the biochemical parameters induced by γ-HCH in male rats.

**Keywords:** Antioxidant enzymes, Lindane, Lipid profile, *Nigella sativa* oil; Omega-3, Oxidative stress, Hepato-specific enzymes

There has been mounting concern regarding the adverse health effects of environmental contaminants in general and organochlorine in particular. Lindane, the gamma isomer of hexachlorocyclohexane (γ-HCH) is an organochlorine pesticide widely used to control arthropod pests on food crops, timber, farm animals and humans. Its extensive use, chemical stability and bioaccumulation potential have resulted in its ubiquitous distribution in the ecosystem and thereby now considered to be a global pollutant. A vast array of literature exists showing neurotoxic and hepatotoxic effect of lindane.

Free radicals play an important role in toxicity of pesticides and environmental chemicals. Pesticide chemicals may induce oxidative stress, leading to generation of free radicals and alteration in antioxidants or oxygen free radicals (OFR) scavenging enzyme system. Lipid peroxidation has been suggested as one of the molecular mechanisms in pesticides-induced toxicity. OFR enzymatic scavengers like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPxs), glutathione reductase (GR), gamma-glutamyl transpeptidase (γ-GT), glutathione S-transferase (GST) etc, may protect the system from deleterious effects of free radicals. Further lymphocyte membrane contains cholinergic receptor as well as γ-GT which play an important role in the metabolism of pesticides.
The seeds of *Nigella sativa* L., also known as black seed, black cumin or habatul Barakah have long been used in the Middle-East as a traditional medicine for a variety of complaints, headache, cough, flatulence, as a choleric, antispasmodic and uricosuric. The seeds have been subjected to a range of pharmaceutical investigations. The data suggest that seeds and the major active constituent thymoquinone exhibit hepatoprotective effect against liver damage induced by carbon tetrachloride and tert-butyl hydroperoxide. Most of the hepatoprotective drugs belong to the group of free radical scavengers, and their mechanism of action involves membrane stabilization, neutralization of free radicals and immuno-modulation. The inhibitory effects of *N. sativa* crude fixed oil and pure thymoquinone on lipid peroxidation have been demonstrated.

Consumption of some ω3 polyunsaturated fatty acids, such as eicosapentaenoic (C20:5 n-3, EPA) and docosahexaenoic (C22:6 n-3, DHA) acids from fish oil has shown a preventive action against cardiovascular disease. Recently, the European Food Safety Authority (EFSA) has announced a qualified health claim for the use of EPA and DHA in conventional foods and dietary supplements. These fatty acids reduce the overall mortality related to infarction and sudden death in patients with coronary heart disease by inhibition of pro-inflammatory eicosanoid formation. Other mechanisms include decrease in plasma very low-density lipoproteins (VLDL) and triacylglycerol (TG), and production of both larger and less atherogenic low-density lipoproteins (LDL). In view of these findings, this study has been undertaken to determine hepatoprotective and antioxidant actions of *N. sativa* oil (N.O) and ω3 in lindane-intoxicated rats.

**Materials and Methods**

**Chemicals**

Lindane, the gamma isomer of hexachlorocyclohexane; γ-HCH (purity = 98%) was purchased from Aldrich Chem. Co., USA. Thiobarbituric acid and all other chemicals were purchased from Sigma Chemical Co. (St. Louis, USA). *Nigella sativa* seed oil (N.O) was purchased from Asala Co., Cairo, Egypt. Use of lindane was approved by the Animal Care Committee and met all guidelines for its use.

**Animals and experimental design**

Forty male rats with average body weight of 200 ± 20 g were obtained from National Research Institute, Cairo, Egypt and acclimatized for 2 weeks prior to the experiment. They were housed in universal galvanized wire cages at room temperature (22-25°C) and in a photoperiod of 14 h light/10 h dark per day. Animals received standard laboratory balanced commercial diet and water *ad libitum*. The animals were housed in groups of 10 rats each and divided randomly into 4 groups. The group I served as control and fed orally with corn oil, group II (γ-HCH) rats were treated with single dose of γ-HCH (12 mg/kg BW, orally) 24 h prior to decapitation, group III (ω3 + N.O) were fed orally with omega-3 (20 mg/kg BW) and *N. sativa* oil (0.3 ml/kg BW); treatments were carried out day by day for 20 days and group IV (γ-HCH + ω3 + N.O) treated with γ-HCH (12 mg/kg BW, 24 h prior to decapitation), followed by the administration of ω3 and N.O every 48 h for 20 days.

**Sample collection and biochemical parameters**

The animals were starved overnight for 12 h before blood was collected. Rats were anaesthetized with light ether and venous blood samples were collected by direct heart puncture in sterilized vials. Blood samples were centrifuged at 1,000 x g for 15 min at 4°C and serum was recovered.

Total lipids were measured using commercial kit (Bio ADWIC, Egypt), cholesterol, triglycerides, high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL) were measured using commercial kit (BioSystems Co., Spain). Uric acid was measured using commercial kit (Biocon® Diagnostik, Marienhagen, Germany), creatinine and urea were estimated using commercial kit (Diamond Co., Egypt), bilirubin level, total protein content was measured using commercial kit (Biodiagnostic Co., Egypt) and serum albumin was measured using commercial kit (Bio ADWIC, Egypt).

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated in blood serum using commercial kits (Bio M'erieux, France). Alkaline phosphatase (ALP) and acid phosphatase (ACP) activities were determined using commercial kit (Bio ADWIC, Egypt), lactate dehydrogenase (LDH) was also estimated. Serum gamma glutamyl transferase (γ−GT) was measured using commercial kit (BioSystems Co., Spain), Superoxide dismutase (SOD) and catalase (CAT) activities were measured using commercial kit (Biodiagnostic Co., Egypt). Serum acetylcholinesterase (AChE) activity was measured using...
commercial kit (Quimica Clinica Aplicada S.A., Spain).

Thiobarbituric acid reactive substances (TBARS) were measured according to the method described by Tapel and Zalkin. The color intensity of the TBARS reagents was measured at 532 nm and a molar extinction coefficient of 156,000 cm⁻¹ mol⁻¹ was used for calculation of the concentration.

Statistical analyses
Mean and standard error values were determined for all the parameters and the results were expressed as mean ± standard error for 10 rats in each group. The data were analyzed using a one-way analysis of variance (ANOVA). The student-Newman-keuls test was used to compare the treated and control groups and the significance is given as *p*<0.05, *p*<0.01 and *p*<0.001.

**Results**

**Lipid profile in serum**
Table 1 shows the overall means of serum TL, cholesterol, TG, and HDL and LDL-cholesterol. Results indicated that TL, cholesterol and TG were significantly (*p*<0.01) increased due to oral administration of γ-HCH, while HDL level was decreased. Pretreatment of rats with ω₃ and N.O prior to γ-HCH administration re-established these altered biochemical features, when compared with γ-HCH-treated group (group 2). Co-administration of ω₃ and N.O day by day for 20 days to rats significantly decreased (*p*<0.001) LDL-cholesterol, when compared with control group (Table 1).

**Liver and kidney function**
Oral administration of γ-HCH led to significant increase in urea, creatinine, uric acid and total bilirubin levels compared to control animals (Table 1). Treatment with ω₃ and N.O (group 3) had no significant effect on these parameters compared to control group, except lowering of creatinine level. Pretreatment of rats with ω₃ and N.O prior to oral administration of γ-HCH (group 4) counteracted the toxic effect of γ-HCH.

Administration of γ-HCH resulted in a significant decrease (*p*<0.01) in serum total protein (TP) and albumin compared to control group (Table 1). γ-HCH significantly (*p*<0.001) enhanced aminotransferases (AST and ALT), phosphatases (ACP and ALP), LDH and γ-GT levels compared to control group (Table 2). However, ω₃ and N.O pretreatment to γ-HCH-treated animals reversed the hazardous effect of γ-HCH on liver.

**Antioxidant enzymes in serum**
As shown in Table 2, SOD activity was increased (*p*<0.001) in γ-HCH treated groups as compared with control group, CAT activity was found to be insignificantly changed in γ-HCH at dose 12 mg/kg (Table 2). With ω₃+ N.O pretreatment in γ-HCH-treated rats, a dramatic restoration in SOD activity in serum was observed (Table 2).

**AChE activity and lipid peroxidation in serum**
Although the activity of AChE was decreased (*p*<0.05) in γ-HCH-treated rats, co-administration of γ-HCH + ω₃ + N.O was found to be significantly increased compared with control group (*p*<0.01). The color intensity of the TBARS reagents was measured at 532 nm and a molar extinction coefficient of 156,000 cm⁻¹ mol⁻¹ was used for calculation of the concentration.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>γ-HCH</th>
<th>ω₃ + N.O</th>
<th>γ-HCH + ω₃ + N.O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipids (g/dl)</td>
<td>3.29 ± 0.579</td>
<td>4.90 ± 0.398⁷</td>
<td>3.16 ± 0.070</td>
<td>4.01 ± 0.081⁴ᵇ</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>83.2 ± 3.76</td>
<td>134.2 ± 3.49⁹</td>
<td>83.8 ± 3.31</td>
<td>99.0 ± 10.00⁵ᶜ</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>99.8 ± 2.71</td>
<td>130 ± 9.86⁷</td>
<td>76.2 ± 8.33⁷</td>
<td>102.4 ± 1.36⁶ᵇ</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dl)</td>
<td>49.2 ± 0.93</td>
<td>27.2 ± 1.84⁴</td>
<td>42.6 ± 1.76⁷</td>
<td>39.4 ± 2.62⁷ᶜ</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dl)</td>
<td>65.6 ± 7.21</td>
<td>58.8 ± 1.21</td>
<td>37.9 ± 1.42⁴</td>
<td>57.8 ± 2.97</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>33.2 ± 3.76</td>
<td>44.6 ± 1.36⁷</td>
<td>30.2 ± 2.32</td>
<td>30.6 ± 4.00⁵ᶜ</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.608 ± 0.023</td>
<td>0.756 ± 0.052⁵</td>
<td>0.300 ± 0.093⁷</td>
<td>0.646 ± 0.026ᵇ</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>3.63 ± 0.229</td>
<td>4.97 ± 0.228⁴</td>
<td>3.89 ± 0.085</td>
<td>4.13 ± 0.21²ᵇ</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>1.49 ± 0.015</td>
<td>1.80 ± 0.115⁴</td>
<td>1.66 ± 0.303</td>
<td>1.45 ± 0.09⁷ᵃ</td>
</tr>
<tr>
<td>Total protein (mg/dl)</td>
<td>8.83 ± 0.478</td>
<td>5.78 ± 1.14⁷</td>
<td>7.78 ± 0.38²</td>
<td>5.93 ± 0.34⁵ᶠ</td>
</tr>
<tr>
<td>Albumin (mg/dl)</td>
<td>3.44 ± 0.750</td>
<td>1.88 ± 0.93⁷</td>
<td>2.12 ± 0.50⁴</td>
<td>4.06 ± 1.37⁵ᵈ</td>
</tr>
</tbody>
</table>

**Table 1**—Effect of omega-3 and *N. sativa* oil on lipid profile and liver and kidney functions in lindane-induced oxidative damage in rat serum

[Values are expressed as mean ± SEM; n= 10 for each treatment group]

γ-HCH, lindane; ω₃, omega-3; N.O, *N. sativa* oil; x, y, z, represent *p*<0.05, 0.01, 0.001 compared with control respectively; a, b, c, represent *p*< 0.05, 0.01, 0.001 in comparison between γ-HCH and γ-HCH + ω₃ + N.O, respectively.
Table 2—Effect of omega-3 and *N. sativa* oil on blood serum enzymes and TBARS in lindane-induced oxidative damage in rats (IU/L)

[Values are expressed as mean ± SEM; n = 10 for each treatment group]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>γ-HCH</th>
<th>ω3 + N.O</th>
<th>γ-HCH + ω3 + N.O</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>45.0 ± 1.79</td>
<td>77.2 ± 2.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.0 ± 3.29</td>
<td>56.6 ± 3.20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>AST</td>
<td>121.6 ± 9.67</td>
<td>166.6 ± 3.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>112.0 ± 4.47</td>
<td>143.4 ± 9.65&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALP</td>
<td>54.5 ± 3.15</td>
<td>85.6 ± 3.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.4 ± 3.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.7 ± 6.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ACP</td>
<td>37.2 ± 0.75</td>
<td>63.2 ± 4.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.6 ± 0.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.9 ± 3.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDH</td>
<td>674.6 ± 100.07</td>
<td>1144.2 ± 24.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>753.0 ± 96.14</td>
<td>887.4 ± 76.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>γ-GT</td>
<td>2.73 ± 0.462</td>
<td>10.55 ± 0.501&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.49 ± 0.251</td>
<td>4.98 ± 0.506&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SOD</td>
<td>475.0 ± 47.95</td>
<td>1200.0 ± 17.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>208.1 ± 36.38</td>
<td>515.4 ± 64.82&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT</td>
<td>720.0 ± 16.73</td>
<td>735.0 ± 36.05</td>
<td>615.0 ± 7.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>685.0 ± 18.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AChE</td>
<td>101.5 ± 24.52</td>
<td>70.0 ± 12.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>99.8 ± 9.08</td>
<td>121.1 ± 14.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TBARS</td>
<td>2.21 ± 0.235</td>
<td>4.62 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.56 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.49 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ω3 and N.O prior to γ-HCH treatment protected AChE from the inhibitory action by γ-HCH (Table 2). Significant alteration (*p*<0.001) in the levels of TBARS was found in γ-HCH-treated animals, when compared to control rats. ω3 and N.O pretreatment to γ-HCH-treated animals alleviated the toxic effect of γ-HCH (Table 2).

**Discussion**

Many studies have been carried out on the pharmacological effects of *N. sativa* seed oil<sup>31,32</sup>. The seed and its oil show no adverse effects on liver functions<sup>33,34</sup>. Potential deleterious effects of xenobiotics are manifested in tissues, due to peroxidation of membrane lipids, particularly the polyunsaturated fatty acids (PUFA)<sup>35</sup>. γ-HCH has been reported to cause lipid peroxidation in rats tissues<sup>36</sup>. The highly lipophilic nature of γ-HCH makes brain the most prone target<sup>37</sup>. Brain is considered highly vulnerable to oxidative stress than other organs of the body as it consumes high amounts of oxygen, contains high amounts of PUFA and low levels of antioxidant enzymes<sup>38</sup>. It is well-known that γ-HCH may provoke important metabolic derangements in the body. Feeding rats with γ-HCH-containing diets results in a significant increase of the serum TG, cholesterol and phospholipids levels<sup>39</sup>. Similar abnormalities have been found in albino mice that are on diet containing high dose of γ-HCH. Lower doses of γ-HCH do not produce substantial lipid abnormalities in serum<sup>40</sup>. Lipid abnormalities can be, at least in part, explained by lowering activities of liver lipogenic enzymes<sup>31</sup>.

In the present study, results indicated that TL, cholesterol and TG were significantly increased by γ-HCH treatment, while HDL level was decreased as compared to the control group, these results agreed with the previous studies on workers and rats<sup>42,43</sup>. Cartson and Kalmodin-Hedman<sup>44</sup> reported that the increase in the levels of serum cholesterol might be responsible for inducing atherosclerotic changes. They also reported that the accumulation of pesticides in liver is associated with the disturbance lipid metabolism and an elevation of serum cholesterol. Therefore, pesticides-induced increase in serum cholesterol can be attributed to the effect of pesticides on the permeability of liver cell membrane and/or liver dysfunction<sup>42</sup>. The present study confirmed the liver damage in γ-HCH-treated group by the increment of total bilirubin, AST and ALT in serum.

Results also showed that there was a significant increase in serum HDL of animals pretreated with ω3 and N.O prior γ-HCH administration compared to γ-HCH-treated group. HDL may hasten the removal of cholesterol from peripheral tissue to the liver for catabolism and excretion and high levels of HDL may compete with LDL receptor sites on arterial smooth muscle cells and thus partially inhibit uptake and
degradation of LDL. Also, HDL could protect LDL against oxidation in vivo because the lipids in HDL are preferentially oxidized before those in LDL. The reduction in lipidemia of animals supplemented with high EPA and DHA doses is well established. Some mechanisms involve increase of fatty acid oxidation in liver, inhibition of de novo fatty acid synthesis, higher binding affinity of LDL to membrane. ω3 polyunsaturated fatty acids are preferentially oxidized as compared to ω6 PUFA, resulting in decreased plasma TG levels. In human studies, only TG reduction has been observed after ω3 PUFA intake.

The increased blood urea and creatinine levels in rats treated with γ-HCH are considered as significant markers of renal dysfunction. Elevated blood urea is correlated with an increased protein catabolism in mammalian body or from more efficient conversion of ammonia to urea as a result of increased synthesis of enzyme involved in urea production. In the present study, pesticide induced increase in urea level and this might be due to the effect of pesticide on liver function, as urea is the end-product of protein catabolism and this was confirmed by the decrease in serum proteins and/or related to kidney dysfunction. The increase in serum total bilirubin concentrations in rats treated with γ-HCH was in accordance with the previous study in workers exposed to pesticides. The induction in serum bilirubin indicated malfunction in rat's liver. It is reported that elevation in bilirubin concentration could be due to the onset of periportal necrosis. Results also showed that antioxidant properties of ω3 and N.O counteracted the toxic effects of γ-HCH on serum bilirubin, urea and creatinine. The insignificant effect of ω3 and N.O on blood urea, uric acid and total bilirubin suggested that ω3 and N.O did not have any toxic effect on liver and kidney functions.

γ-HCH administration resulted in a significant decrease in serum TP and albumin. The protein depression might be due to loss of protein either by reduced protein synthesis or increased proteolytic activity or degradation. Also, the observed decrease in serum proteins could be attributed in part to the damaging effect of γ-HCH on liver cells, as confirmed by the increase in the activities of serum AST and ALT. Our study showed that serum levels of AST, ALT and ALP were significantly increased in γ-HCH-intoxicated rats in comparison with control. This finding was in agreement with results of other studies, indicating that γ-HCH may provoke hepatocytes injury. ALT is the most specific enzyme for liver among all enzymes. γ-HCH causes liver injury by inducing oxidative stress in hepatocytes.

Serum γ-GT activity was also increased after γ-HCH administration compared to control group. Rise in γ-GT activity has been reported in humans occupationally exposed to technical grade γ-HCH for 10 yrs in γ-HCH formulating plant. However, this rise is not detected in all exposed individuals, which suggesting that individual responses to γ-HCH may vary. These results indicate that serum γ-GT activity is less sensitive indicator to γ-HCH-induced liver injury. Elevated LDH level in γ-HCH-treated group might be attributed to cell membrane damage and/or to the well known ischemia-induced lactate accumulation.

Pesticides are capable of modulating immune responses in experimental animals and human poisoning cases. The result of the present study indicated the activities of γ-GT and AChE were altered following γ-HCH treatment. The decrease in serum AChE activity might be related to the neuroimmunoregulatory role of this enzyme in the poisoning cases. Since AChE and γ-GT are both membrane-bound enzymes, γ-GT could interact with the amino acid neurotransmitter acetyl choline which may be removed from the binding with AChE and may result in decreased activity of AChE. Co-administration of ω3 and N.O prior to γ-HCH treatment induced a significant increase in AChE activity, when compared to γ-HCH-treated rats.

In the present study, N. sativa oil had a marked protective action against γ-HCH-induced hepatic injury, an effect that was associated with suppression in the levels of lipid peroxidation and LDH. The anti-ulcerogenic effect of N.O could be attributed to the improvement of the antioxidant status of the animals, due to the presence of free radical scavenging substances, such as thymoquinone. Thymoquinone is the main active component in the volatile oil of N. sativa seeds and is able to inhibit lipid peroxidation. Moreover, its ability to preserve the cell membrane integrity could be proven by the restoration of LDH.

Under normal physiological conditions, a delicate balance exists between the rate of formation of H2O2 via dismutation of O₂⁻ by SOD activity and the rate of removal of H2O2 by CAT and glutathione peroxidase. Therefore, any impairment in this pathway will affect...
the activities of other enzymes in the cascade. The present study indicated that γ-HCH treatment resulted in increased SOD and insignificant increase in CAT activities which may be assumed to tackle the excessive free radical load. ω3 polyunsaturated fatty acids may stimulate α-tocopherol incorporation into membranes, increasing the level of CAT within both peroxisomes and cytoplasm, resulting in an enhanced defense against reactive oxygen species (ROS).

In conclusion, our results indicated that co-administration of N. sativa oil together with ω3 had renal and hepatoprotective action against γ-HCH intoxication, which could be attributed at least in part to their free radical scavenging property.

References
10 European Food Safety Authority (EFSA), Parma, Italy (2010) EFSA J 8, 1796
20 Jendrassik L & Gorf P (1938) Biochemistry 279, 7281-7297
37 Srivastava A & Shivanandappa T (2005) Toxicology 214, 123-130
54 Renner E L & Dalenbach A (1992) Ther Umsch 49, 281-286