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Genetic and histopathological alterations induced by cypermethrin in rat kidney
and liver: protection by sesame oil

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Running title: Protective role of sesame oil on biohazards of cypermethrin
Abstract

Pesticides are widespread synthesized substances used for public health protection and agricultural programs. However, they cause environmental pollution and health hazards. This study aimed to examine the protective effects of sesame oil (SO) on the genetic alterations induced by cypermethrin (CYP) in the liver and kidney of Wistar rats. Male rats were divided into four groups, each containing 10 rats; the control group received vehicle; SO group (5 mL/kg b.w); CYP group (12 mg/kg b.w), and protective group received SO (5 mL/kg b.w) plus CYP (12 mg/kg b.w). Biochemical analysis showed an increase in albumin, urea, creatinine, GPT, GOT and lipid profiles in CYP group. Co-administration of SO with CYP normalized such biochemical changes. CYP administration decreased both the activity and mRNA expression of the examined antioxidants. SO co-administration recovered CYP down regulate the expression of glutathione-S-transferase (GST), catalase and superoxide dismutase. Additionally, SO co-administration with CYP counteracted the CYP-altered the expression of renal interleukins (IL-1 and IL-6), tumor necrosis factor alpha (TNF-α), heme oxygenase-1 (HO-1), anigotensinogen (AGT), AGT receptors (AT1) and genes of hepatic glucose and fatty acids metabolism. CYP induced degenerative changes in the kidney and liver histology which are ameliorated by SO. In conclusion, SO has a protective effect against alterations and pathological changes induced by CYP in the liver and kidney at genetic and histological levels.

Keywords: Cypermethrin, Genetic regulation, Histopathology, Sesame oil protection.

Introduction
Insecticide toxicity is a global problem with occurring in the developing nations (1). A public concern over the amounts of insecticides being applied to the land and their possible adverse effects on human and animal health; as well as, the impact on the environment has risen sharply. With the advancement of agriculture there is also an upsurge of unexplained diseases.

Cypermethrin (CYP) is widely used and has become the dominant insecticide for agricultural and public health purposes by farmers in developing countries and all over the world (2). Pesticide residues have been found in dairy and meat products, food products, soft drinks and in water. CYP is primarily absorbed from gastrointestinal tract and may also be absorbed by inhalation of spray mist and only simply through the intact skin. Due to its lipophilic nature, cypermethrin has been found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries and brain. CYP is the most effective means of pest eradication, but their use has arrived at frightening rate due to a number of undesirable effects on non-target organisms including human beings (3). Exposure to CYP induces various deleterious effects such as anemia, defective blood coagulation, brain and nerve damage, paralysis, jaundice, hepatic fibrosis, kidney problems, cancer, genetic disorders, birth defects, impotence, and infertility or sterility (4, 5). CYP effects cause abnormal generation of reactive oxygen species (ROS) and significant damage to cell structure, lipids, proteins, carbohydrates, and nucleic acids (6). ROS is the cause for toxicity induced by various pesticides such as CYP (6). In mammals, CYP accumulates in fat cells, skin, liver, kidneys, adrenal glands, ovaries, lung, blood, and the heart causing organs dysfunction (7). Administration of CYP to rats caused significant increase in the levels of urea and creatinine together with renal pathological affections (8). Rats exposed to CYP induced significant increases in the serum levels of free amino
acids, total proteins, urea, urea nitrogen, uric acid and creatinine (9). Currently, all reported findings focused on blood and histological changes, very little data is available regarding genetic alterations.

Sesame oil (SO) is the extract of plant Sesamum indicum, family: Pedaliaceae. Sesamin and sesaminol are the major phenolic constituents of SO with broad spectrum pharmacological effects, including antimutagenic, antioxidant, antihypertensive, anti-inflammatory and antithrombotic (10). SO abolishes oxidative stress and multiple organ failure that is triggered by endotoxin in rats. SO decreases LPO by inhibiting the generation of reactive oxygen free radicals (11). SO is easily available in markets and its molecular protective effects are still unclear. The increasing usage of CYP in agriculture makes it as an interesting subject to investigate its possible adverse effects on the kidney and liver, which are the main target organs for different xenobiotics. Therefore, this study was designed to examine the protective effect of SO on oxidative stress, genetic alterations of antioxidants, cytokines, glucose and fatty acids metabolism after CYP exposure. In addition, histopathological changes occurring in the kidney and liver were examined.

Materials and Methods

Chemicals and kits

Cypermethrin, Ethidium bromide and agarose were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Wistar albino rats were purchased from King Fahd center for Scientific Research, King Abdel-Aziz University, Jeddah, Saudi Arabia.
Serologic kits for glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), superoxide dismutase (SOD), malondialdehyde (MDA), albumin, total proteins, creatinine and urea were purchased from Bio-diagnostic Co., Dokki, Giza, Egypt. The deoxyribonucleic acid (DNA) ladder was purchased from MBI, Fermentas, Thermo Fisher Scientific, USA. Qiazol for RNA extraction and oligo dT primer were purchased from QIAGEN (Valencia, CA, USA).

Animals and Experimental design

Forty male Wistar rats 8 weeks old, weighting 170–200 g were selected randomly and given free access to food and water. Rats maintained at 12h/12h day and light all experimental periods. After 2 weeks of acclimatization, rats were assigned for experimental procedures. Rats were subdivided into 4 subgroups. Control (normal rats n=10) and gained free access to food and water. Sesame oil group (n =10) was given a normal diet together with sesame oil (5mL/kg/day) for 28 days. Cypermethrin group was given normal diet and administered cypermethrin orally (12 mg/kg b.w) for 28 days. Protective group (sesame oil plus cypermethrin), was administered cypermethrin (12 mg/kg b.w) with sesame oil (5mL/kg/day) for 28 days. The dose of sesame oil and CYP was determined based on studies of Abdou et al., (8) and Hussien et al., (12). At the end of experimental procedures, all rats were anesthetized using diethyl ether after overnight fasting and blood was collected for serum extraction. Liver and kidney tissues were taken on formalin for histopathology and on TriZol for RNA extraction and gene expression (RT-PCR).
Serum extraction and chemistry analysis

Blood was collected from the eye using heparinized capillary tubes inserted into retro-orbital venous plexuses. Blood was left to clot at room temperature then in the refrigerator for 15 minutes and centrifuged for 10 minutes at 4 °C and 5000 rpm, supernatant serum was taken and stored at -20 °C till assays. Fasting blood glucose levels were determined using spectrophotometric assay. Serum creatinine, urea, albumin, GPT, GOT, total triglycerides (TG), total cholesterol and HDL were measured using commercial available kits that are based on spectrophotometric analysis and were purchased from Al-Asaafra Laboratories, Alexandria. Egypt.

Determination of liver antioxidant activity

For SOD and MDA activity measurements, one gram of liver or kidney slices was homogenized in 5ml of cold buffer (50mM potassium phosphate buffer; PBS, pH 7.4) for SOD and MDA. Cold buffer of SOD activity contains 1mM EDTA and 1mL/l Triton X-100. After centrifugation at 4000 x g for 15 minutes at 4ºC, the supernatant was removed and stored frozen at -80 ºC until the time of analysis of SOD (U/mg protein) and MDA (nmol/g protein). The protein content of the liver and kidney extract was determined by the method of Lowry using bovine serum albumin as a standard. The activities of SOD and MDA were determined by ELISA reader (Absorbance Microplate Reader ELx 800TM BioTek®, Seattle, WA, USA). Results of SOD and MDA activities were calculated according to the manufacturer’s instructions.

RNA Extraction, cDNA Synthesis and Semi-quantitative RT-PCR Analysis
Total RNA was extracted from liver and kidney tissues preserved in Qiazol reagent (50 - 100 mg per sample). RNA was extracted using chloroform-isopropanol extraction assay. After extraction, RNA pellets were washed with 70% ethanol, briefly dry up, and then dissolved in Diethylpyrocarbonate (DEPC) water.

For cDNA synthesis, a mixture of 3 µg total RNA and 0.5 ng oligo dT primer in a total volume of 11 µl sterilized DEPC water was incubated in the PeX 0.5 thermal Cycler (Thermo Electronic Corporation, Milford, Ma) at 65°C for 10 min for denaturation. Then, 4 µl of 5X RT-buffer, 2 µl of 10 mM dNTPs and 100 U Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase (SibEnzyme Ltd. Ak, Novosibirsk, Russia) were added and the total volume was completed up to 20 µl by DEPC water. The mixture was then re-incubated in the thermal Cycler at 37°C for one hour, then at 90°C for 10 min to inactivate the enzyme.

For semi-quantitative RT-PCR analysis, specific primers for examined genes (table 1) were designed using Oligo-4 computer program and synthesized by Macrogen (Macrogen Company, GAsa-dong, Geumcheon-gu. Korea). PCR was conducted in a final volume of 25 µl consisting of 1 µl cDNA, 1 µl of 10 pM of each primer (forward and reverse), and 12.5 µl PCR master mix (Promega Corporation, Madison, WI). The volume was brought up to 25 using sterilized, deionized water. PCR was carried out using Bio-Rad thermal Cycle with the cycle sequence at 94 °C for 5 minutes one cycle, followed by variable cycles ranged from 30 to 35 cycles for examined genes and 25 cycles for glyceraldehyde-3-phosphate dehydrogenase (G3PDH). Each PCR cycle consists of denaturation at 94 °C for one minute, annealing at the specific temperature corresponding to each primer (table 1) and extension at 72 °C for one minute with additional final extension at 72 °C for 7 minutes. As a reference, expression of G3PDH
mRNA was examined (table 1). PCR products were electrophorized on 1.5% agarose (Bio Basic INC. Konrad Cres, Markham Ontario) gel stained with ethidium bromide in TBE (Tris-Borate-EDTA) buffer. PCR products were visualized under UV light and photographed using gel documentation system. The intensities of the examined bands were quantified densitometrically using ImageJ software (http://imagej.en.softonic.com).

Liver and Kidney Histopathology

Liver and kidney of all experimental animals were incised. Tissues were then removed from the rats and fixed overnight in a 10% buffered neutral formalin solution. Fixed tissues were processed routinely including washing, dehydration, clearing, paraffin embedding, casting, sectioning to 5 μm sections for using in hematoxylin and eosin staining.

Data analysis

Results are expressed as means ± S.E. for 5 independent rats per each group. The statistical significance of the differences between groups was assessed using analysis of variance (ANOVA), and post hoc descriptive tests by SPSS software version 11.5 for Windows (SPSS, IBM, Chicago, IL, USA). Values at p<0.05 were considered significant.
Results

Protective Effect of SO on CYP induced changes in serum biochemical profiles, renal and hepatic function tests in Wistar rats

The results in table 2, show that oral administration of CYP for 28 days increased the serum levels of fasting blood sugar (FBS), GPT, GOT, triglycerides (TG), cholesterol and low density lipoproteins (LDL) in serum of CYP administered rats compared to control and SO administered rats. Co-administration of SO together with CYP normalized such alterations. Moreover, CYP administered rats showed an increase in serum levels of urea, creatinine and decrease in total proteins, high density lipoproteins (HDL) and albumin levels. SO co-administration with CYP restored such alterations to normal levels. Administration of CYP induced a significant increase in serum levels of MDA that are decreased to control values when CYP co-administered with SO (table 2).

Protective effect of SO on CYP induced changes in MDA and SOD activity of liver and kidney in Wistar rats

Destruction of lipids and proteins reported in CYP group (table 2) lead to oxidative stress and an increase in ROS. Administration of CYP for consecutive 28 days induced significant increases in MDA activity and a decrease in SOD activity of both liver and kidney. Such oxidative damage was normalized when SO co-administered with CYP (table 3).

Protective effect of SO on CYP induced changes in renal IL-1β, IL-6, TNF-α and IL-10 expression in Wistar rats
Administration of CYP for 28 days increased mRNA expression of proinflammatory cytokines IL-1β, IL-6 and TNF-α (Fig. 1a, b and c). SO alone decreased the expression of IL-1 and IL-6. Co-administration of SO with CYP normalized such increase in cytokines expression reported in cypermethrin group. Unlike IL-1β, IL-6 and TNF-α, the expression of IL-10 is down regulated during CYP toxicity and upregulated in SO group. Co-administration of SO with CYP increased IL-10 expression that was decreased in CYP group (Fig. 1d).

**Protective effect of SO on CYP induced changes in hepatic catalase, GST and SOD expression in Wistar rats**

SO administration increased the mRNA expression of catalase, GST and SOD (Fig. 2a, b and c). In contrast, CYP administration for consecutive 28 days decreased the antioxidants mRNA expression. SO co-administration with CYP inhibited changes in the antioxidants expression reported in CYP administered rats (Fig. 2a, b and c).

**Protective effect of SO on CYP induced changes in hepatic fatty acids and glucose metabolism in Wistar rats**

The expression of genes such as FAS and PPAR-α that are related to lipid metabolism (Fig. 3a and b) and enolase and GLUT-2 that are involved in glucose metabolism (Fig. 3c and d) were shown in figure 3. FAS expression was decreased in CYP group and normalized when SO co-administered with CYP for 28 days (Fig. 3a). PPAR-α has been upregulated in the CYP group due to the increase in lipolysis and fatty acid oxidation (Fig. 3b). SO co-administration induced partial inhibition in PPAR-
α expression. Regarding genes of glucose metabolism, both α-enolase and GLUT-2 expression were increased in CYP group to support body cells with energy to compensate the metabolic alterations induced after CYP administration. Of interest, enolase, not GLUT-2, showed more additive stimulatory effect on mRNA expression in the protective group (Fig. 3 c & d).

Protective effect of SO on CYP induced changes in renal heme oxygenase-1 (HO-1) and angiotensinogen (AGT) expression in Wistar rats

Due to the degenerative changes induced by CYP reported in table1, 2 and figure 1, the expression of HO-1 was increased in CYP administered rats as seen in figure 4a. SO alone increased HO-1 expression compared to control. Coadministration of SO with CYP inhibited the overload in HO-1 expression. AGT and AT1 expression were increased in CYP administered rats and SO co-administration with CYP inhibited upregulation in AGT and angiotensinogen receptor-1(AT1) expression to maintain renal oxidative stress and hypertension within normal range (Fig. 4 c and d).

Protective effect of SO on CYP induced hepatic and renal histopathological changes in Wistar rats

Liver of healthy control rats (Fig. 5 a), showed normal hepatic architecture represented by hepatic lobule with a thin walled central vein and normal hepatic cords radiating towards the periphery alternating with hepatic sinusoids. In SO administered rats (Fig. 5 b), liver of rats showed normal cell architecture. In CYP group, the liver showed congestion of the central vein, lymphocytic infiltration and hydropic degeneration in the hepatocytes (Fig. 5c). Liver of the protective group (SO plus CYP) showed
disappearance of the degenerative changes in hepatocytes except edema in the central vein and few hepatic cells showed hydropic degeneration (Fig. 5 d).

Renal changes in control and SO administered rats showed a normal renal architecture represented by renal tubules and renal corpuscles (Fig. 5 e and f). In CYP administered group, renal histology showed congestion of the renal blood vessels, vacuolation of the renal tubules and degeneration in the cells of the proximal and distal convoluted tubules (Fig. 5g). In the protective group, SO induced recovery and regeneration in renal structure from biohazards of CYP (Fig. 5h).

Discussion

This study shows that CYP induce alterations in serum proteins, lipid profiles, kidney and liver function parameters. Co-administration of SO together with CYP ameliorated and normalized such alterations (table 2 and 3). Antioxidant expression was decreased after CYP administration and up-regulated post SO co-administration (Figure 2). The increase in MDA, GPT and GOT indicate liver toxicity, oxidative stress and LPO induced by CYP administration. As known, oxidative stress occurs when there is an imbalance in the biological oxidant-to-antioxidant ratio (13). CYP is metabolized through the cytochrome P450 microsomal system resulting in oxidative stress (14). Studies involving serum proteins have noted a decrease in the level of total proteins in serum of young rabbits because of CYP toxicity (15). The changes in enzyme activity reported may be due to the inhibition of transcriptional rate, enhanced clearance rate, pH change and inhibition/induction of mono-oxygenase enzyme system (14, 16). Such enzymatic changes explain and support the degree of protection induced by SO to
overcome chronic toxicity induced by CYP. The increase in GPT and GOT levels in CYP administered rats may be due to more cell damage and leakage of inner cellular enzymes. CYP is a lipophilic molecule that can easily pass through the cell lipid bilayer and damages its integrity (17). LPO disturbs the integrity of cellular membranes and the leakage of cytoplasmic enzymes (12), therefore, LPO plays an important role in the pathogenesis of numerous diseases. Here, CYP induced oxidative stress and lipid peroxidation and affected several pathways in the liver and kidney.

SO possesses the ability to protect the body from alterations in LPO and normalized changes in antioxidants expression and activity. PPAR-α is the main regulator of fatty acids β-oxidation, and mediation of oxidative stress (18). Therefore, the disturbance of fatty acid oxidation and impairment of mitochondrial function may play a very important role in CYP induced oxidative stress. ROS scavenging moiety of sesame lignans can protect body cells from the free radical injury (19). Sesame oil increases the hepatic detoxification of chemicals and protects against oxidative stress and hepatic gene expression (20). Sesame oil could protect against blood pressure and lipid peroxidation and increases enzymatic and nonenzymatic antioxidants. Sesame oil has been regarded as a daily nutritional supplement that increases cell resistance to LPO because of its antioxidant activity.

SO possesses the ability to protect the body from alterations in LPO and normalized changes in antioxidants expression and activity. PPAR-α is the main regulator of fatty acids β-oxidation and mediation of oxidative stress (18). Therefore, the disturbance of fatty acid oxidation and impairment of mitochondrial function may play a very important role in CYP induced oxidative stress. PPAR-α expression and SO
coadministration ameliorated CYP alterations at the molecular levels. ROS scavenging moiety of sesame lignans can protect body cells from the free radical injury (19). SO increases the hepatic detoxification of chemicals and protects against oxidative stress and hepatic gene expression (20, 21). SO could protect against blood pressure and lipid peroxidation and increases enzymatic and non-enzymatic antioxidants (10). SO has been regarded as a daily nutritional supplement that increases cell resistance to LPO. Moreover, SO could be considered as a potent antioxidant, which appears superior to corn oil or mineral oil in attenuating oxidative stress caused by a variety of chemicals in rats (22).

Cytokines (IL-1β, IL-6, TNF-α and IL-10) are secreted in response to infection and/or toxicity induced by environmental contaminants such as CYP (23). Little is known about the role of IL-10 during CYP challenge. IL-10 down regulates the expression of Th1 cytokines and acts as an anti-inflammatory and immunoregulator (24). IL-10 inhibits IL-1 and IL-6 production from macrophages (25). Therefore, the increase in IL-10 expression is to control inflammation induced by CYP and to down regulate mRNA expression of cytokines increased during CYP challenge.

The increase in serum glucose levels may reflects the increase in glucose mobilization through breakdown of dietary or reserve complex carbohydrates. This postulation was strengthened by the mRNA expression of genes related to glucose metabolism such as GLUT-2 and enolases. GLUT-2 is the principal transporter of glucose between the liver and blood and for renal glucose reabsorption (26). It is likely that the increase in fatty acids oxidation reported is the cause for down regulation in FAS expression. Enolases are a family of cytoplasmic proteins involved in glycolytic metabolism and energy regulation (27). Alpha enolase is the glycolytic enzyme that
catalyzes the production of phosphoenolpyruvate from 2-phosphoglycerate (28). α-enolase participates in the maintenance of intracellular ATP levels in cardiomyocytes exposed to ischemic hypoxia (29). In the current study, administration of CYP with SO up-regulated hepatic α-enolase mRNA expression, which indicates its ability to stimulate glycolysis.

Chronic CYP administration ameliorated HO-1 and angiotensinogen expression. HO-1 catalyzes the oxidation of heme to generate several biologically active molecules such as carbon monoxide, biliverdin, and ferrous ion. These active molecules serve as a second messenger affecting several cellular functions (30). Upregulation of HO-1 causes either suppression of immune effector functions or adaptive response to several injuries in the body (31). The up-regulation in the expression of IL-1β, IL-6 and TNF-α is the cause for the increase HO-1 expression. SO co-administration with CYP down regulated HO-1 expression compared to CYP administered rats. Therefore, SO worked in a way to control the integrity and reduce the severity of toxicity induced by CYP. It has been shown that augmentation of intra-renal AGT synthesis, secretion, and excretion is associated with the development of hypertension, renal oxidative stress, and tissue injury (32). Therefore, the changes occurred in the expression of AGT and its receptor AT-1 in CYP administered group are due to alteration in oxidative stress and tissue injury reported in renal histology and controlled by SO co-administration.

Renal and hepatic histopathological findings reported in this study are in agreement with study of Abdou et al, (8). They observed degenerative changes in the hepatocytes such as congestion, lymphocytic infiltration, pyknosis, necrosis and vacuolation. Acute CYP administration caused significant degeneration in the histological structure of liver tissues (hyperplasia, disintegration of hepatic mass and
focal coagulative necrosis (33). SO co-administration repaired the degenerative changes reported in the kidney and liver. All together, these data confirm the protective role of SO on CYP induced genetic and histopathological changes in the kidney and liver.

**Conclusion**

This study confirms the ability of sesame oil to protect rats against biohazards induced by chronic administration of cypermethrin. The protective effects of sesame oil occurred at the biochemical, molecular and histopathological levels. Further *in vitro* studies are needed to clarify the exact signaling pathways stimulated by sesame oil to overcome cypermethrin side effects.

**Acknowledgments**

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**Declaration of interest**

The authors report no conflicts of interest.

**References**


Table 1. PCR conditions for examined genes in kidney and liver of Wistar rats after cypermethrin administration for 28 days.

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Forward primer (5’-3’)</th>
<th>Reverse primer (5’-3’)</th>
<th>Annealing Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β (218 bp)</td>
<td>ATGGCAACCGTACCTGAA CCCA</td>
<td>GCTCGAAATGTCCCAAGG AA</td>
<td>61</td>
</tr>
<tr>
<td>IL-6 (450bp)</td>
<td>AGTTGAGCTTTTGGGACTG ATGT</td>
<td>TGGCTCTGAAATGACTTGAGC TTTG</td>
<td>56</td>
</tr>
<tr>
<td>TNF-α (256 bp)</td>
<td>CCACCACGCTCTCTGCTTCT AC</td>
<td>ACCACAGTTGCTGTCCTTG TG</td>
<td>58</td>
</tr>
<tr>
<td>IL-10 (320bp)</td>
<td>GGAGTGAAGACCAAAAGG AC</td>
<td>TCTCCGAGGAATCAATG G</td>
<td>57</td>
</tr>
<tr>
<td>Catalase (652 bp)</td>
<td>GCGAATGGAGAGGCAGTG TAC</td>
<td>GAGTGAGCGTTGCTCTTCAATT AGCCTG</td>
<td>55.5</td>
</tr>
<tr>
<td>GST (575 bp)</td>
<td>GCTGGAGTGGATTTGGAAG GAA</td>
<td>GTCCCTGACCAGCTCAACAT AG</td>
<td>55</td>
</tr>
<tr>
<td>SOD (410 bp)</td>
<td>AGGATTAACCTGAAGGCAGCAT</td>
<td>TCTACAGTATGACCCAGGGCAG CAG</td>
<td>55</td>
</tr>
<tr>
<td>FAS (345 bp)</td>
<td>CCAGAGCAGCAGACGAGA AG</td>
<td>GACGCCAGTGTTCTGTTCC</td>
<td>61</td>
</tr>
<tr>
<td>Gene</td>
<td>Primer Sequences</td>
<td>Length (bp)</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------------------------</td>
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<td></td>
</tr>
<tr>
<td>GLUT-2 (330 bp)</td>
<td>AAGGATCAAGCCATGTTGG</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Enolase (278 bp)</td>
<td>ATCCTACTGCCAGAACCTCAC</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>HO-1 (250 bp)</td>
<td>CTTGCAGAGAAAAGCTCATGA</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>AGT (263 bp)</td>
<td>TTGTGAGAGCTTGGTGAGCCATCA</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>AT1 (440 bp)</td>
<td>GCACAAATCGCCATAATATTCC</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>PPAR-α (680 bp)</td>
<td>GAGGTCCGATTTTCCACTG</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>GAPDH (309 bp)</td>
<td>AGATCCCAACGGGATACCTT</td>
<td>35</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Serum biochemical changes after cypermethrin (CYP), sesame oil (SO) and SO plus CYP administration for consecutive 28 days in Wistar rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SO</th>
<th>CYP</th>
<th>SO + CYP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Albumin (mg/dL)</strong></td>
<td>3.7 ± 0.07</td>
<td>3.9 ± 0.2</td>
<td>2.1 ± 0.11*</td>
<td>3.1 ± 0.07#</td>
</tr>
<tr>
<td><strong>Urea (mg/dL)</strong></td>
<td>31 ± 0.5</td>
<td>33.6 ± 2.8</td>
<td>41 ± 3.6*</td>
<td>34 ± 1.2#</td>
</tr>
<tr>
<td><strong>Creatinine (mg/dl)</strong></td>
<td>0.7 ± 0.03</td>
<td>0.6 ± 0.04</td>
<td>1.3 ± 0.1*</td>
<td>0.56 ± 0.07#</td>
</tr>
<tr>
<td><strong>Total proteins (mg/dl)</strong></td>
<td>6.6 ± .05</td>
<td>6.8 ± 0.2</td>
<td>4.5 ± 0.34*</td>
<td>6.4 ± 0.03#</td>
</tr>
<tr>
<td><strong>GOT (U/L)</strong></td>
<td>59 ± 1.5</td>
<td>69.6 ±1.4</td>
<td>187 ±2.8*</td>
<td>85 ± 2.9#</td>
</tr>
<tr>
<td><strong>GPT (U/L)</strong></td>
<td>63 ± 3.7</td>
<td>65.7 ± 2.6</td>
<td>159 ± 3.3*</td>
<td>73 ±2.02#</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/dL)</strong></td>
<td>52 ± 4.5</td>
<td>69.3 ±3.8</td>
<td>67 ± 2.3*</td>
<td>50 ± 1.2#</td>
</tr>
<tr>
<td><strong>Cholesterol (mg/dL)</strong></td>
<td>91.3 ± 3.1</td>
<td>88.3 ± 6.4</td>
<td>118. 7 ± 3.8*</td>
<td>55.3 ± 2.02#</td>
</tr>
<tr>
<td><strong>LDL (mg/dL)</strong></td>
<td><strong>33.9 ± 2.3</strong></td>
<td><strong>43 ± 1.3</strong></td>
<td><strong>40.9 ± 1.7</strong></td>
<td><strong>31.7 ± 0.9</strong></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>SO</td>
<td>CYP</td>
<td>SO + CYP</td>
</tr>
<tr>
<td>--------------------</td>
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</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA (nmol/g protein)</td>
<td>11.78 ± 1.9</td>
<td>12.7 ± 1.0</td>
<td>20.2 ± 0.5*</td>
<td>14.6 ± 0.4*</td>
</tr>
<tr>
<td>SOD (U/g protein)</td>
<td>29 ± 4.1</td>
<td>34 ± 1.7*</td>
<td>19 ± 1.1*</td>
<td>30.9 ± 1.3*</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA (nmol/g protein)</td>
<td>8.78 ± 1.9</td>
<td>9.7 ± 1.0</td>
<td>18.2 ± 0.5*</td>
<td>10.6 ± 0.4*</td>
</tr>
<tr>
<td>SOD (U/g protein)</td>
<td>21 ± 2.1</td>
<td>26 ± 0.7*</td>
<td>12 ± 1.9*</td>
<td>23.9 ± 3.3*</td>
</tr>
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</table>

*Values are means ± standard error (SE); n=10 for each treatment group; Values are statistically significant at *p<0.05 Vs. control and #p<0.05 Vs. cypermethrin group.

GOT = Glutamate oxalate transaminase, GPT = Glutamate pyruvate transaminase, LDL = low density lipoprotein, HDL = High density lipoprotein, FBS = Fasting blood sugar, MDA = malondialdehyde.

Table 3. Protective effect of sesame oil (SO) on cypermethrin (CYP) induced changes in hepatic and renal MDA and SOD activity in Wistar rats.
Values are means ± standard error (SE); n=10 for each treatment group; Values are statistically significant at *p<0.05 Vs. control and #p<0.05 Vs. cypermethrin group. 

MDA = malondialdehyde (nmol/g protein), SOD= superoxide dismutase (U/g protein).
Figure 1. Protective molecular effects of sesame oil (SO) on cypermethrin (CYP) induce changes in the expression of IL-1β, IL-6, TNF-α and IL-10 expression in kidney tissue of Wistar rats using semi-quantitative RT-PCR analysis. RNA was extracted and reverse transcribed (1 μg) and RT-PCR analysis was carried out for of IL-1β, (A) IL-6 (B), TNF-α (C) and IL-10 (D) expression as described in materials and methods. Densitometric analysis was carried for 3 different experiments. Data are means ± SEM.
for 3 independent experiments. Values are statistically significant at *$p<0.05$ Vs. control, #$p<0.05$ Vs. cypermethrin and $p<0.05$ Vs. control.

Figure 2. Protective molecular effects of sesame oil (SO) on cypermethrin (CYP) induce changes in the expression of catalase, GST and SOD expression in liver tissue of Wistar rats using semi-quantitative RT-PCR analysis. RNA was extracted and reverse transcribed (1 $\mu$g) and RT-PCR analysis was carried out for catalase (A), GST (B) and SOD (C) expression as described in materials and methods. Densitometric analysis was carried for 3 different experiments. Data are means ± SEM for 3 independent experiments. Values are statistically significant at *$p<0.05$ Vs. control, #$p<0.05$ Vs. cypermethrin and $p<0.05$ Vs. control.
Figure 3. Protective molecular effects of sesame oil (SO) on cypermethrin (CYP) induced changes in the expression of FAS, PPAR-α, enolase and GLUT-2 expression in liver tissue of Wistar rats using semi-quantitative RT-PCR analysis. RNA was extracted and reverse transcribed (1 μg) and RT-PCR analysis was carried out for FAS (A), PPAR-α (B), enolase (C) and GLUT-2 (D) as described in materials and methods. Densitometric analysis was carried for 3 different experiments. Data are means ± SEM for 3 independent experiments. Values are statistically significant at *p<0.05 Vs. control and #p<0.05 Vs. cypermethrin group and $p<0.05 Vs. sesame oil.
**Figure 4.** Protective molecular effects of sesame oil (SO) on cypermethrin (CYP) induce changes in the expression of HO-1, angiotensin (AGT) and angiotensin receptor-1 (AT1) expression in liver tissue of Wistar rats using semi-quantitative RT-PCR analysis. RNA was extracted and reverse transcribed (1 µg) and RT-PCR analysis was carried out of HO-1 (A), AGT (B) and AT1(C) as described in materials and methods. Densitometric analysis was carried for 3 different experiments. Data are means ± SEM for 3 independent experiments. Values are statistically significant at *p<0.05 Vs. control and #p<0.05 Vs. cypermethrin group and $. p<0.05 Vs. sesame oil.

**Fig. 5. a,** photomicrograph of the liver of healthy control rats shows a normal hepatic architecture represented by hepatic lobule with a thin walled central vein (CV) and hepatic cords (H) radiating towards the periphery alternating with hepatic sinusoids.
(H&E x 100). b, photomicrograph of the liver of sesame oil rats group shows normal architecture as that of the control group (CV) and hepatic cords (H) (H&E x 100). c, photomicrograph of the liver of cypermethrin rats shows congestion of the central vein (C), lymphocytic infiltration (L) and hydropic degeneration in the hepatocytes (HD), restoration of normal hepatic architecture with disappearance of fat droplets from hepatocytes cytoplasm (arrows) and regeneration of hepatic parenchyma (H&E x100). d, photomicrograph of the liver of the treated group shows disappearance most of the degenerative changes except edema in the central vein and few hepatic cells showed hydropic degeneration (HD) (H&E x 100). In e and f, photomicrograph of the kidney of healthy control and sesame oil administered rats shows normal renal architecture represented by renal tubules and renal corpuscles (G) (H&E x 100). In g, photomicrograph of the kidney of cypermethrin administered rats shows congestion of the renal blood vessels (c), vacuolation of the renal tubules (V) and degeneration of the cells of the proximal and distal convoluted tubules (d) (H&E x 100). In h, photomicrograph of the kidney of treated group shows recovery of the renal tissue to normal structure (G) (H&E x 100).