GENETIC AND HISTOPATHOLOGICAL ALTERATIONS INDUCED BY CYPERMETHRIN IN RAT KIDNEY AND LIVER: PROTECTION BY SESAME OIL

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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 regulating the expression of glutathione-S-transferase (GST), catalase and superoxide dismutase. Additionally, SO co-administration with CYP counteracted the CYP- altering the expression of renal interleukins (IL-1 and IL-6), tumor necrosis factor alpha (TNF-a), 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.

Insecticide toxicity is a global problem occurring in developing nations (1). Public concern exists concerning the amounts of insecticides being applied to the land and their possible adverse effects on human and animal health; furthermore, 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 all over the world, and in particular in developing countries (2).

Kew words: cypermethrin, genetic regulation, histopathology, sesame oil protection

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Pesticide residues have been found in dairy and meat products, food products, soft drinks and in water. CYP is primarily absorbed by the gastrointestinal tract as well as by inhalation of spray mist or only simply through skin contact. 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 its use has arrived at frightening rate and causes 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 result of 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 organ dysfunction (7). Administration of CYP to rats caused significant increase in the levels of urea and creatinine together with renal pathological affections (8). In rats exposed to CYP, significant increases were induced 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 the 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 commerce and its molecular protective effects are still unclear. The increasing usage of CYP in agriculture makes 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 acid 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 the King Fahd center for Scientific Research, King Abdel-Aziz University, Jeddah, Saudi Arabia. Serologic kits for glutamate pyruvate transaminase (GPT), glutamate oxalacetate 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. Ratswere maintained at 12h/12h day/night cycle during all experimental periods. After 2 weeks of acclimatization, the rats were assigned for experimental procedures. The rats were subdivided into 4 subgroups. Controls (normal rats n=10) gained free access to food and water. The sesame oil group (n =10) was given a normal diet together with sesame oil (5mL/kg/day) for 28 days. The cypermethrin group was given normal diet and administered cypermethrin orally (12 mg/kg b.w) for 28 days. The 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 the 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 min and centrifuged for 10 min 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 commercially available kits 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 5 ml of cold buffer (50 mM potassium phosphate buffer; PBS, pH 7.4). Cold buffer of SOD activity contains 1 mM EDTA and 1mL/l Triton X-100. After centrifugation at 4000 x *g* for 15 min at 4ºC, the supernatant was removed and stored frozen at -80ºC 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 Lowry method, 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 dried, and then dissolved in Diethylpyrocarbonated (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 a PeX 0.5 thermal Cycler (Thermo Electronic Corporation, Milford, Ma, USA) 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 I) 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, USA). The volume was brought up to 25 µl using sterilized, deionized water. PCR was carried out using Bio-Rad thermal Cycle with the cycle sequence at 94°C for 5 min one cycle, followed by variable cycles ranging 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 I) and extension at 72°C for one minute with additional final extension at 72°C for 7 min. As a reference, expression of G3PDH mRNA was examined (Table I). 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 II 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

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

mRNA	Forward primer (5'-3')	Reverse primer (5'-3')	Annealing Temp (°C)
IL-1β (218 bp)	ATGGCAACCGTACCTGAACCCA	GCTCGAAAATGTCCCAGGAA	61
IL-6 (450bp)	AGTTGCCTTCTTGGGACTGATGT	TGCTCTGAATGACTCTGGCTTTG	56
TNF-α (256 bp)	CCACCACGCTCTTCTGTCTAC	ACCACCAGTTGGTTGTCTTTG	58
IL-10 (320bp)	GGAGTGAAGACCAAAGG	TCTCCCAGGGAATTCAAATG	57
Catalase (652 bp)	GCGAATGGAGAGGCAGTGTAC	GAGTGACGTTGTCTTCATTAGCACTG	55.5
GST (575 bp)	GCTGGAGTGGAGTTTGAAGAA	GTCCTGACCACGTCAACATAG	55
SOD (410 bp)	AGGATTAACTGAAGGCGAGCAT	TCTACAGTTAGCAGGCCAGCAG	55
FAS (345 bp)	CCAGAGCCCAGACAGAGAAG	GACGCCAGTGTTCGTTCC	61
GLUT-2 (330 bp)	AAGGATCAAAGCCATGTTGG	GGAGACCTTCTGCTCAGTGG	55
Enolase (278 bp)	ATCCTACTGCCAGAACTTCAC	CCACAACATTCAGTTTCTTGCT	55
HO-1 (250 bp)	CTTGCAGAGAGAAGGCTACATGA	AGAGTCCCTCACAGACAGAGTTT	54
AGT (263bp)	TTGTTGAGAGCTTGGGTCCCTTCA	CAGACACTGAGGTGCTGTTGTCCA	57
AT1 (440 bp)	GCACAATCGCCATAATTATCC	CACCTATGTAAGATCGCTTC	54
PPAR-α (680 bp	GAGGTCCGATTTTCCACTG	ATCCCTGCTCTCCTGTATGG	58
GAPDH (309bp)	AGATCCACAACGGATACATT	TCCCTCAAGATTGTCAGCAA	52

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

	Control	SO	CYP	SO + CYP
Albumin (mg/dL)	3.7 ± 0.07	3.9 ± 0.2	2.1 ± 0.11*	3.1 ± 0.07#
Urea (mg/dL)	31 ± 0.5	33.6 ± 2.8	41 ± 3.6*	34 ± 1.2#
Creatinine (mg/dl)	0.7 ± 0.03	0.6 ± 0.04	1.3 ± 0.1*	0.56 ± 0.07#
Total proteins (mg/dl)	6.6 ± .05	6.8 ± 0.2	4.5 ± 0.34*	6.4 ± 0.03#
GOT (U/L)	59 ± 1.5	69.6 ±1.4	187 ±2.8*	85 ± 2.9#
GPT (U/L)	63 ± 3.7	65.7 ± 2.6	159 ± 3.3*	73 ±2.02#
Triglycerides (mg/dL)	52 ± 4.5	69.3 ±3.8	67 ± 2.3*	50 ± 1.2#
Cholesterol (mg/dL)	91.3 ± 3.1	88.3 ± 6.4	118. 7 ± 3.8*	55.3 ± 2.02#
LDL (mg/dL)	33.9 ± 2.3	43 ± 1.3	40.9 ± 1.7*	31.7 ± 0.9#
HDL (mg/dL)	23.6 ± 1.7	23 ± 0.6	17.6 ± 0.3*	24 ± 0.9#
FBS (mg/dL)	72 ± 4	83.3 ± 3.5	97.7 ± 2*	80.3 ±2.6#
MDA (nmol/L)	5.4 ± 0.4	4.5± 0.5	18.7 ± 2.5*	8.3 ±2.6#

*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 III. *Protective effect of sesame oil (SO) on cypermethrin (CYP) induced changes in hepatic and renal MDA and SOD activity in Wistar rats.*

Experimental groups				
Parameters	***Control***	***SO***	***CYP***	***SO + CYP***
Liver				
MDA (nmol/g protein)	11.78 ± 1.9	12.7 ± 1.0	20.2 ± 0.5*	14.6 ± 0.4#
SOD (U/g protein)	29 ± 4.1	34 ± 1.7*	19 ± 1.1*	30.9 ± 1.3#
Kidney				
MDA (nmol/g protein)	8.78 ± 1.9	9.7±1.0	18.2 ± 0.5*	10.6 ± 0.4#
SOD (U/g protein)	21 ± 2.1	26 ± 0.7*	12 ± 1.9*	23.9 ± 3.3#

*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).*

normalized such alterations. Moreover, CYP-administered rats showed an increase in serum levels of urea, creatinine and a 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 were decreased to control values when CYP was co-administered with SO (Table II).

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 the CYP group (Table II) led to oxidative stress and an increase in ROS. Administration of CYP for 28 consecutive 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 was co-administered with CYP (Table III).

Protective effect of SO on CYP induced changes in renal IL-1b, IL-6, TNF-a and IL-10 expression in Wistar rats

Administration of CYP for 28 days increased mRNA expression of proinflammatory cytokines IL-1b, IL-6 and TNF-a (Fig. 1 a, b and c). SO alone decreased the expression of IL-1 and IL-6. Co-administration of SO with CYP normalized such increase in cytokine expression reported in cypermethrin group. Unlike IL-1b, IL-6 and TNF-a, the expression of IL-10 was down regulated during CYP toxicity and upregulated in the 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. 2 a, b and

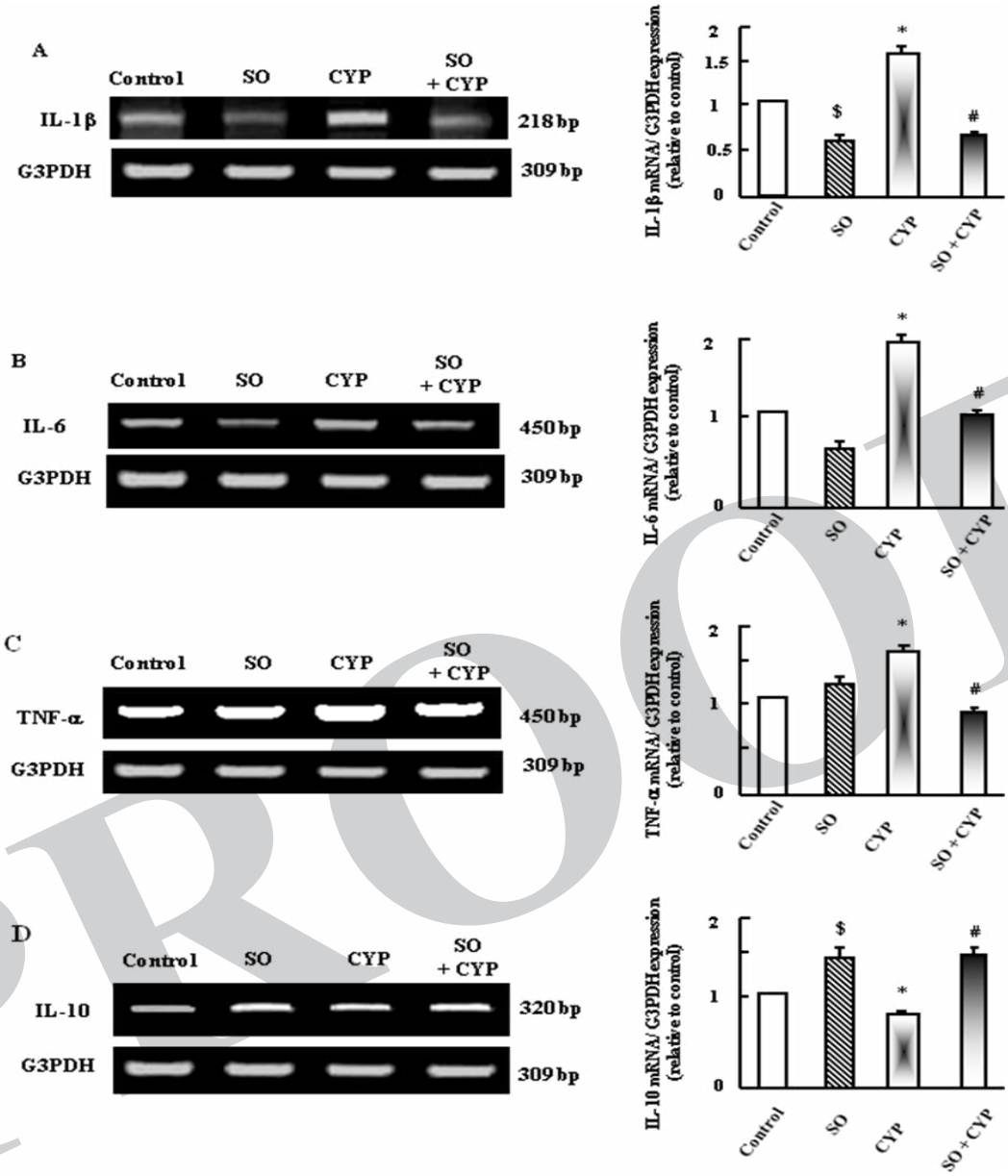

Fig. 1. *Protective molecular effects of sesame oil (SO) on cypermethrin (CYP) induced changes in the expression of IL-1b, IL-6, TNF-a and IL-10 expression in kidney tissue of Wistar rats using semi-quantitative RT-PCR analysis. RNA was extracted and reverse transcribed (1 mg) and RT-PCR analysis was carried out for IL-1b, (**A**) IL-6 (**B**), TNF-a (**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.*

c). In contrast, CYP administration for 28 consecutive days decreased the antioxidant mRNA expression. SO co-administration with CYP inhibited changes in the antioxidants expression reported in the CYP-administered rats (Fig. 2 a, 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-a that are related to lipid metabolism (Fig. 3 a and b)

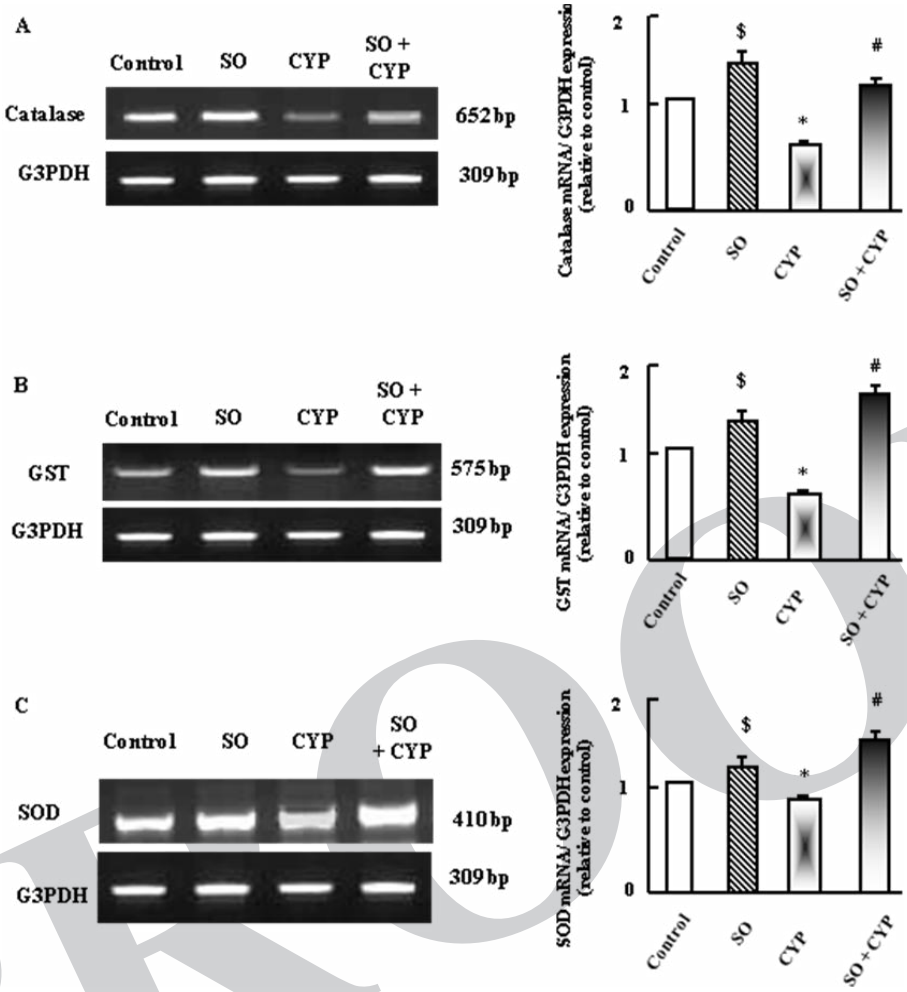

Fig. 2. *Protective molecular effects of sesame oil (SO) on cypermethrin (CYP) induced changes in the expression of catalase, GST and SOD expression in liver tissue of Wistar rats using semi-quantitative RT-PCR analysis. RNA was extracted and reverse transcribed (1 mg) and RT-PCR analysis was carried 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.*

and enolase and GLUT-2 that are involved in glucose metabolism (Fig. 3 c and d) are shown in Fig. 3. FAS expression was decreased in the CYP group and normalized when SO was co-administered with CYP for 28 days (Fig. 3a). PPAR-a was 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-a expression. Regarding genes of glucose metabolism, both a-enolase and GLUT-2 expression were increased in the 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 and 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 Tables I and II and Fig. 1, the expression of HO-1 was increased in CYP administered rats as

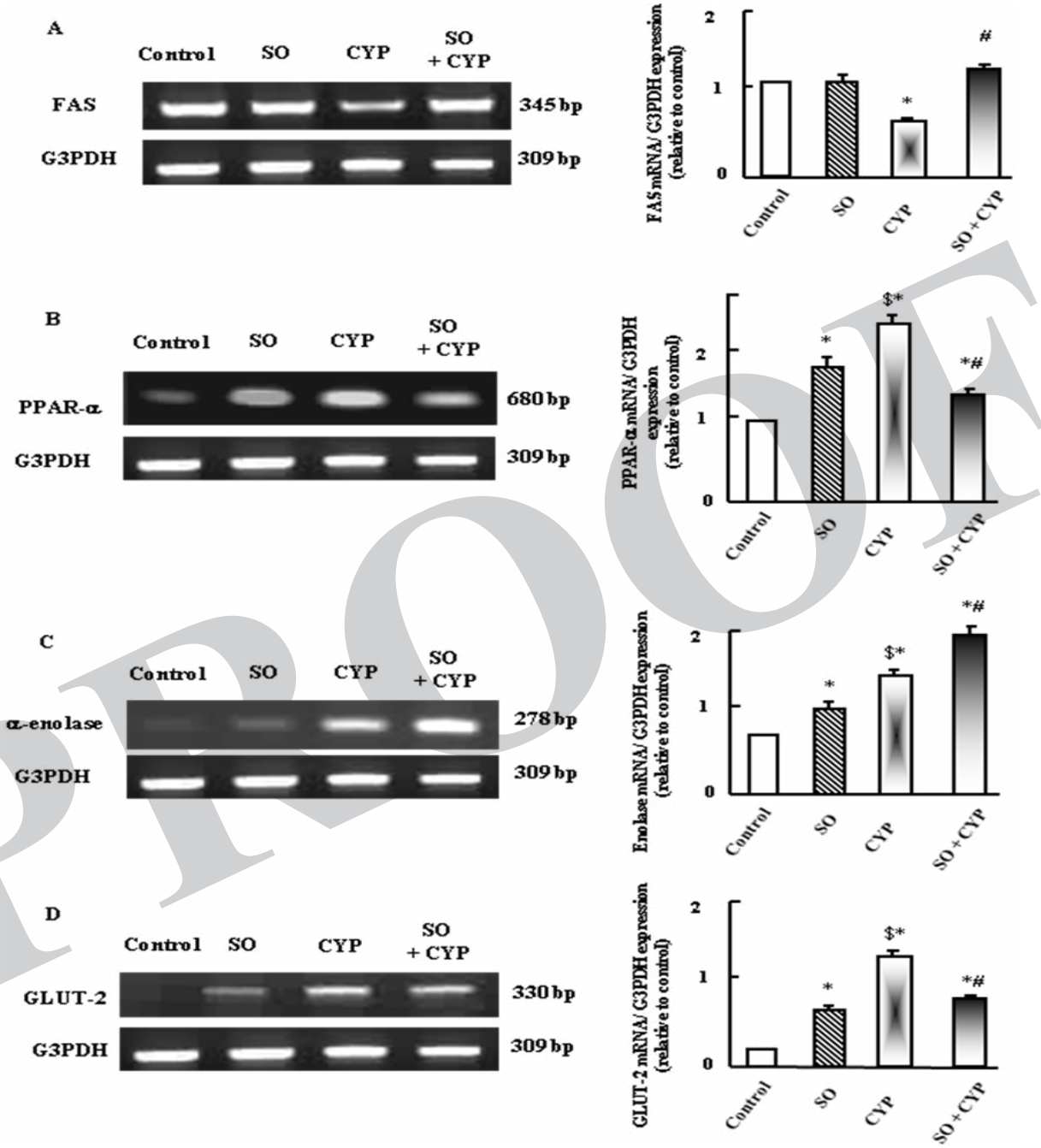

Fig. 3. *Protective molecular effects of sesame oil (SO) on cypermethrin (CYP) induced changes in the expression of FAS, PPAR-a, enolase and GLUT-2 expression in liver tissue of Wistar rats using semi-quantitative RT-PCR analysis. RNA was extracted and reverse transcribed (1 mg) and RT-PCR analysis was carried out for FAS (**A**), PPAR-a (**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.*

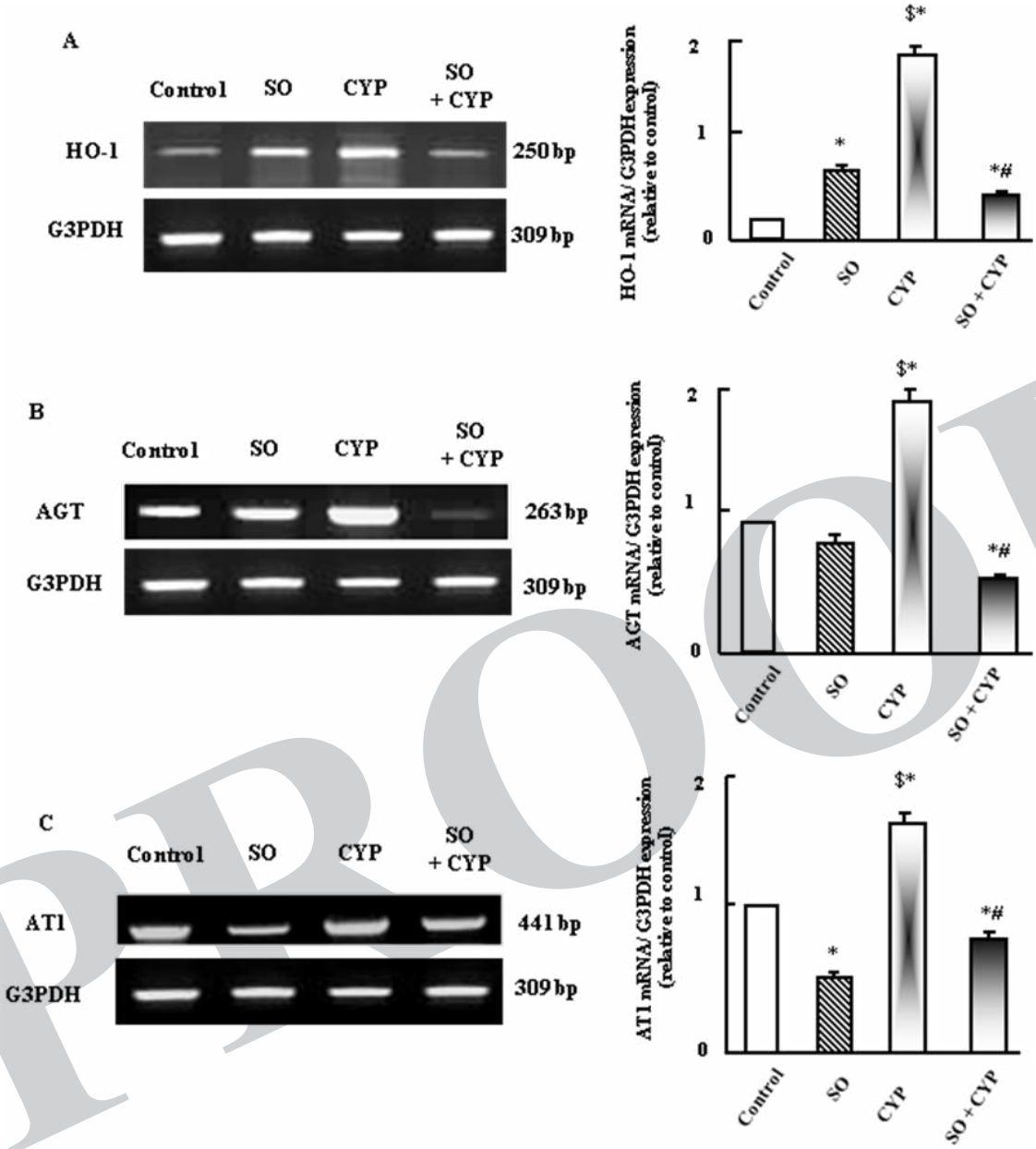

Fig. 4. *Protective molecular effects of sesame oil (SO) on cypermethrin (CYP) induce changes in the expression of HO-1, angiotensin (AGT) and angiotensin receptor-1 (AT1) expression in liver tissue of Wistar rats using semi-quantitative RT-PCR analysis. RNA was extracted and reverse transcribed (1 mg) 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.*

seen in Fig. 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

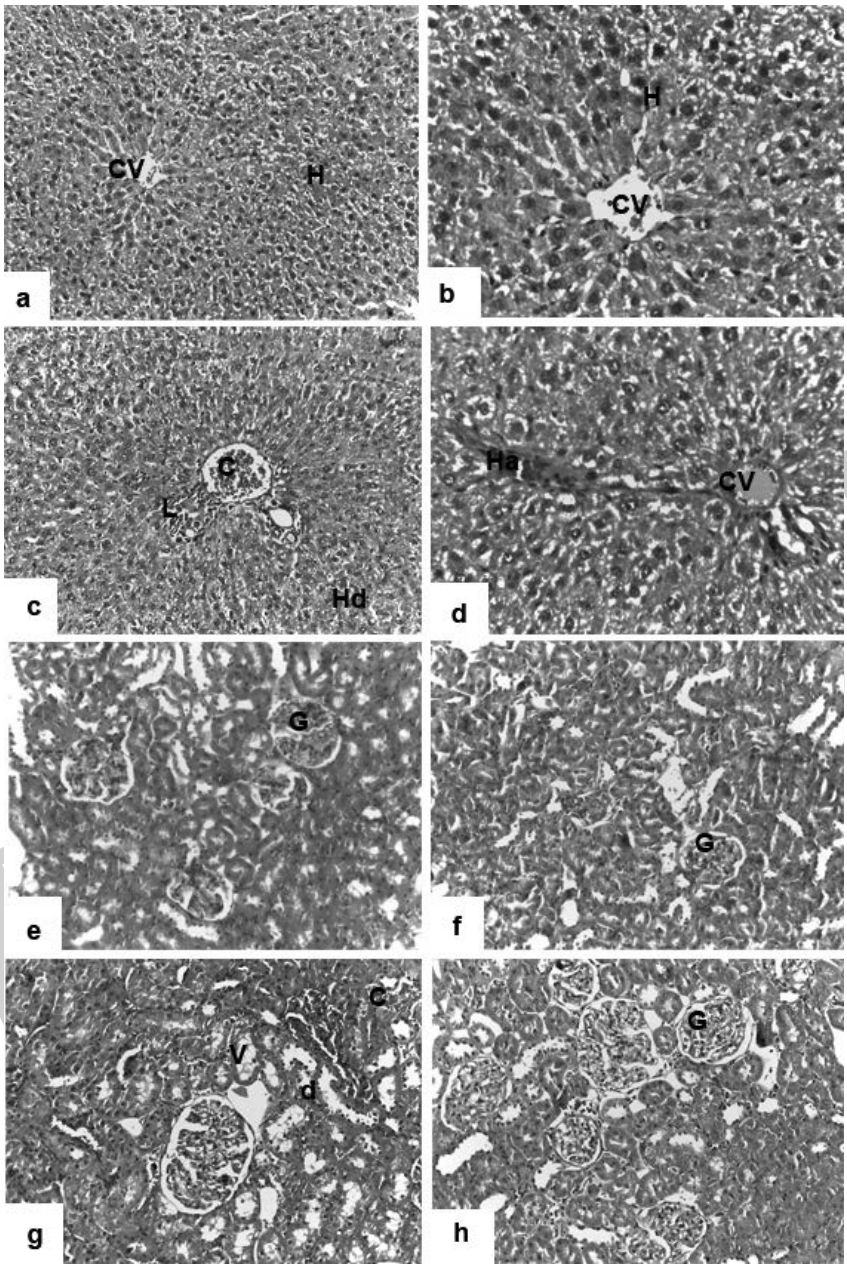

Fig. 5. ***a)*** *Photomicrograph of the liver of healthy control rats shows a normal hepatic architecture represented by hepatic lobule with a thin walled central vein (CV) and hepatic cords (H) radiating towards the periphery alternating with hepatic sinusoids. (H&E x 100).* ***b)*** *Photomicrograph 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 hepatocyte cytoplasm (arrows) and regeneration of hepatic parenchyma (H&E x100).* ***d)*** *photomicrograph of the liver of the treated group shows disappearence of most of the degenerative changes except edema in the central vein and few hepatic cells showing hydropic degeneration (HD) (H&E x 100).* ***e, 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).* ***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).* ***h)*** *Photomicrograph of the kidney of treated group shows recovery of the renal tissue to normal structure (G) (H&E x 100).*

range (Fig. 4 c and d).

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

The liver of the 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), the liver showed normal cell architecture. In the CYP group, the liver showed congestion of the central vein, lymphocytic infiltration and hydropic degeneration in the hepatocytes (Fig. 5c). The liver of the protective group (SO plus CYP) demonstrated disappearance of the degenerative changes in hepatocytes except odema 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 the 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 induces alterations in serum proteins, lipid profiles, and kidney and liver function parameters. Co-administration of SO with CYP ameliorated and normalized such alterations (Tables II and III). Antioxidant expression was decreased after CYP administration and up-regulated post SO co-administration (Fig. 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 normalizes changes in antioxidants expression and activity. PPAR-a is the main regulator of fatty acids b-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 increase 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 normalize changes in antioxidants expression and activity. PPAR-a is the main regulator of fatty acid b-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-a expression and SO coadministration ameliorated CYP alterations at the molecular levels. ROS scavenging moiety of sesame lignans can protect body cells from 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 irregular blood pressure and lipid peroxidation and increase 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-1b, IL-6, TNF-a 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 reflect 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 acid oxidation reported is the cause of the 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-1b, IL-6 and TNF-a is the cause of the increased 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 which occurred in the expression of AGT and its receptor AT-1 in the CYP-administered group were 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 the study of Abdou et al. (8) who 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. Taken together, these data confirm the protective role of SO on CYP-induced genetic and histopathological changes in the kidney and liver.

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.

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