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Hepato-nephro-toxicity Induced by Premium Fungicide and Protective Effect of Sesame Oil in Male Rats

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ABSTRACT

Background: Fungicides impact on agriculture is reflected by the wide spread use of Azoxystrobin (AZX) and metalxyl M, the active ingredients of premium fungicide used on more than 80 different crops.
Objective: The present study aimed to investigate the hepato-renal-toxicity of premium fungicide on adult albino rats and focused on benefits of sesame oil in detoxification of toxicological effects of fungicides.
Materials and methods: Thirty male albino rats allocated into five groups, -ve, +ve control and sesame oil groups. While the two other groups were treated with sesame oil before and after fungicide exposure daily by gavage for (28 days).
Results: Exposure of male albino rats to Azoxystrobin fungicide resulted in liver and kidney injury as evidenced by increased liver and renal function bioindicator (ALT, AST, ALP, urea and creatinine)..Also, glutathione (GSH) was significantly decreases and malondialdhyde (MDA) was increased as oxidative stress biomarkers. In addition, degeneration of some tubular epithelial cells and hemorrhage in kidney and severe inflammatory cell infiltration in liver.
Conclusion: The present study concluded that premium had induced functional and histological changes in liver and kidney male rats. Sesame oil successfully ameliorated the oxidative stress, hepatotoxicity and nephrotoxicity of fungicide.
Keywords: Liver function, Renal function, Oxidative stress, Sesame oil, Azoxystrobin, Metalxyl.

INTRODUCTION

To avoid crop losses, pesticides are widely used to sustain yields of economically valuable crops, to satisfy the growing demands of world food supply [(1)]. The commercialization of pesticides is subject to stringent regulatory limits because of improper disposal and inappropriate usage, accidental leaks and leakages pose a great danger to the atmosphere and human health [(2)]. Pesticides are a very significant group of environmental contaminants used to guard against pathogens and pests in intensive agriculture but nearly 1-2% of used pesticides reach the targeted pests [(3)].

Azoxystrobin (AZX) belongs to strobilurin fungicide which generated fungicidal activity by binding site of cytochrome b blocking the electron transfer [(4)]. Strobilurins are known an extraordinary new class of fungicides since they are broad-spectrum systemic fungicides after emergence, avoiding and/or curing foliar diseases caused by the main classes of pathogenic plant fungi. AZX residues equal to or below maximum residue levels (MRLs) were detected in fruit and vegetable samples [(5)]. AZX bioaccumulation potential or biomagnifications values have been scarce [(5)]. The other component of premium fungicide, Metalaxyl has a high water solubility that raises the likelihood of land and surface water reaching by agricultural run-offs and spillage, mild hydrolysis stability under physiological pH, and metalaxyl is also not highly biodegradable [(6)].

Several experiments in laboratory animals have shown the nephrotoxic and hepatotoxic effects of agro-pesticides [(7)]. A strong correlation between agro-pesticide toxicity and indicators of liver and kidney impairment [(8)]. Liver is the main detoxification organ, where most of metabolism takes place. Due to its role in the biotransformation of xenobiotics, the liver is at great risk of injury, when the pollutant can be reached at high intracellular concentrations [(9)].

It is well recognized that through the rapid generation of reactive oxygen species (ROS), biotic and abiotic stresses contributes to oxidative stress [(10)]. ROS induces imbalance, leading to membrane oxidation and DNA protein damage [(11)].

It will be logical to maximize the quantity of exogenous antioxidants (mainly by ingestion) to improve the protective properties of individuals against environmental oxidative stress, since we have no impact on the amounts of endogenous antioxidants [(12)]. Apparently due to their protective properties against both the toxicity of different toxins and pathogenic factors, more attention is being paid to bioactive compounds, especially where reactive oxygen species (ROS) are involved. [(13)]. A clear link between essential oil of some vegetables and plant products and the mitigation of harmful effects of different toxicants and environmental pollutants has been identified. Through scavenging free radicals and modulating the antioxidant protection mechanism, plant products are known to perform their defensive impact. [(14)]. Sesame oil is particularly useful for the treatment of liver and kidney toxicity caused by lead and iron and has no harmful effects by inhibiting xanthine oxidase, nitric oxide, superoxide anion, and hydroxyl radical generation. Sesame oil inhibits lipid peroxidation induced by iron and inhibits proinflammatory mediator's reactions [(15)].

The present study was therefore undertaken to investigate the effects of premium fungicide exposure on the liver and kidney function of male albino rats and

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through light on maximization antioxidant capacity of male albino rats by sesame oil supplementation before and after exposure to the fungicide.

MATERIALS AND METHODS

Pesticide Used:

Premium fungicide (39% SC).was obtained from Mammalian Toxicology Dept., Central Agricultural Pesticides Lab., Agriculture Research Center, Dokki, Giza, Egypt.

Experimental Animals:

This study used male albino rats aged 12 weeks. Animals were fed on an *ad libitum* basal diet and tap water. The animals were kept in an environmental condition kept at 23 ± 3 C temperatures and 50 ± 5 percent relative humidity with lighting12-hour.

Experimental Design:

After the acclimation time (2 weeks), the animals were structured into five groups, each of 6 animals.The first group was used as control; hence these animals were orally given tap water (5mL/kg). The second group was orally treated with sesame oil (5 ml/kg b.w.). The third group was orally treated with 1/20 LD_{50} of the pesticides. The fourth group was treated by sesame oil (5ml/kg b.w.) for 14 days followed by 14 days pesticides exposure (117.25 mg/Kg as1/20 LD_{50}) of the fungicide. The fifth group was daily-administered pesticide (117.25 mg/Kg) for 14 days followed by 14 days sesame oil. Experiments lasted for 28-days. The study was conducted in accordance with the national guidelines for the care and use of laboratory animals.

Ethical approval: The study was conducted in accordance with the national guidelines for the care and use of laboratory animals.

Blood and Organs Sampling:

Rats were fasted for 12 h, water was not limited and then were anaesthetized by diethyl ether. Blood samples were obtained from the orbital venous plexus in centrifuge non-heparinized tubes. Serum collected from blood samples after centrifugation at 3600 rpm for 15-min at 4 C and then was stored at -20 C until biochemical analysis (MDA, GSH, AST, ALT, ALP, Urea and creatinine). The liver and kidney were excised from the animals and preserved in formalin saline buffer for histopathological analysis.

Biochemical markers of hepatotoxicity:

Biochemical liver function alanine and aspartate aminotransferase (ALT, AST) and alkaline phosphatase (ALP), in serum of rats were quantified [14, 15].

Biochemical markers of Nephrotoxicity:

Serum urea was estimated by using the diagnostic kit based on the method of **Fawcett and Scott[16].** While, creatinine in the serum was estimated using the diagnostic kit based on the methods of **Tietz[17]**.

Oxidative stress biomarkers:

Lipid peroxidation of serum was determined by reaction of TBA with MDA the end product of lipid peroxidation according to **Ohkawa *et al.*** [18]. The GSH content in gills and liver tissues was determined by the method of **Beutler *et al.*** [19].

Histopathological Assessment:

Liver and kidney tissues were collected for histopathological examination and fixed in 10% buffered formalin overnight. Then, embedded with paraffin. All paraffin-embedded tissue was sectioned at 4μm, deparaffinized in xylene, dehydrated by ethyl alcohol in a decreasing concentrations (100%, 95% & 70%), and stained with haematoxylin and eosin stain. These specimens were examined under bright field optical microscopy using a light microscope and 40×magnification powers. Corresponding digital images were captured for later analysis [20].

Statistical Analysis

Analysis of data was performed by using SPSS (Version 15) and the results are expressed as Mean ± S.D. Statistical differences were determined by t- test for comparison.

RESULTS

Liver Functions:

AST, ALT and ALP are liver function's biomarkers. The selected fungicides group exhibited highly elevation compared to the control group (Table 1). When compared to the pesticides treated group, the groups treated with sesame oils before or after the exposure to fungicide showed an improvement in liver function biomarkers.

Table (1): Changes in some biochemical parameters, related to liver functions in male rats induced by premium fungicide and the ameliorative effect of sesame oil supplementation

Treatments	Control	Sesame oil	Pesticide	Prophylactic	Postlactic
AST (U/L)	23.75^{a} ±2.63	24.44^{a} ±2.14	38.41^{b} ±4.21	25.14^{c} ±3.004	29.33^{c} ±1.78
ALT (U/L)	27.19^{a} ±3.19	26.44^{a} ±1.17	42.99^{b} ±3.91	24.63^{c} ±1.11	29.02^{c} ±2.9
ALP (U/L)	4.91^{a} ±0.16	4.75^{a} ±0.32	5.76^{b} ±0.27	5.46^{a} ±0.35	4.75^{a} ±0.324

a, b,c Different letter have a significant difference - Proph (14 days sesame oil before fungicide exposure) -Post (14 days sesame oil before fungicide exposure)

Nephrotoxicity Biomarkers:

Regarding, the nephrotoxicity biomarkers (urea and creatinine) of male rats that orally administered with fungicide, the data in Table (2) reveal a significant increase (P≤0.05) in urea and creatinine compared with negative and positive control. When compared to the pesticides (P≤0.05) treated group, the groups treated with sesame oils before or after the exposure to fungicide induced an improvement in nephrotic biomarkers.

Table (2): Changes in some biochemical parameters (urea and creatinine), related to kidney functions in male rats treated by premium fungicide and the ameliorative effect of sesame oil supplementation

Treatments	Control	Sesame oil	Pesticide	Prophylactic	Postlactic
Urea (mg/dl)	39.88 [a] ±0.86	39.56 [a] ±1.72	45.45[b] ±0.96	40.39 [a] ±0.58	42.24 [a] ±0.76
Creatinine (mg/dl)	0.14[a] ±0.024	0.15[a] ±0.02	0.24[b] ±0.02	0.17[a] ±0.02	0.20[b] ±0.006

a, b, c Different letter have a significant difference, Prophylactic (14 days sesame oil before fungicide exposure), Postlactic (14 days sesame oil after fungicide exposure.

Oxidative Stress Biomarkers:

The data presented in Table (3) show the effect of premium fungicide on oxidative stress biomarkers of male albino rats. The data indicate that fungicide exposure induced oxidative stress biomarkers as evidenced by the significant decrease GSH content($P \leq 0.05$) and a significant increase in MDA level ($P \leq 0.05$) as lipid peroxidation biomarkers as compared with the control group. When compared to the pesticides (P≤0.05) treated group, the groups treated with sesame oils before or after exposure fungicide exposure induced an improvement oxidative stress biomarkers.

Table (3): Changes in some biochemical parameters, related oxidative stress biomarkers in male rats induced by premium fungicide and the ameliorative effect of sesame oil supplementation

Treatments	Control	Sesame oil	Pesticide	Prophylactic	Postlactic
GSH (nmol/ml)	299.7[a] ±6.01	305.1[a] ±10.59	70.3[b] ±4.28	108.92[c] ±5.67	106.13[c] 5.95
MDA (nmol/ml)	28.27 [a] ±2.06	27.89[a] ±2.04	48.98[b] ±3.36	37.49[c] ±0.94	37.70[c] ±0.81

a, b, c Different letter have a significant difference, Prophylactic (14 days sesame oil before fungicide exposure), Postlactic (14 days sesame oil after fungicide exposure.

Histological Result:

Histological examination of liver:

Microscopically, rat's liver of control and sesame oil groups showed that, the hepatocytes are large and polyhedral with normal histological structure (Fig. 1 & 2). On the other hand, rat liver of fungicide-treated group revealed inflammatory cell infiltration in portal area with dilation of portal vein (Fig. 3) and degeneration of hepatocytes (Fig.4). Sections of liver in both groups treated by sesame oil fourteen day before or after pesticide-exposure showed degeneration of little number of hepatocytes around the central vein with normal architecture (Fig.5) and slight inflammatory cell infiltration in portal area (Fig.6).

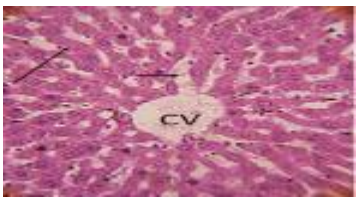

Fig. (1): Control

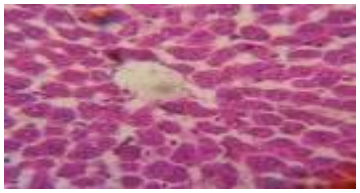

Fig. (2): Sesam oil

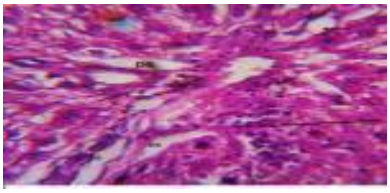

Fig.(3): Pesticide

Fig. (4): Pesticide

Fig. (5): Prophylactic

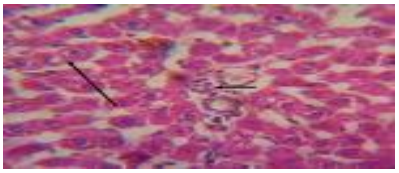

Fig. (6): Post-lactic

Fig.1: Liver section of control rat showed normal hepatic architecture represented by hepatocytes, the hepatic plates and hepatic sinusoids. Fig.2: Liver section of rat treated with sesame oil for 28 days showed normal histological structure. Sections of rats liver from group treated with premium for 28 days revealed severe inflammatory cell infiltration in portal area with dilation of portal vein (Fig. 3) and degeneration of hepatocytes (Fig.4). Fig. 5: Liver section of rats treated with sesame oil fourteen day before pesticide (prophylactic) showed degeneration of some hepatocytes around the central vein with normal architecture. Fig.6: Section of liver of rat treated with sesame oil fourteen day after pesticide (post-lactic) revealed slight inflammatory cell infiltration in portal area.

Histological examination of kidney:

Microscopic examination of rat's kidney of control and sesame oil groups shows normal histological structure (Fig. 7 & 8). While, examination of rat kidney administered the selected fungicide showed degeneration of tubular epithelial cells and hemorrhage in the intra tubular spaces (Fig. 9). Sections of kidney in both groups treated by sesame oil fourteen days before or after fungicide exposure showed degeneration of some tubular epithelial cells (Fig.10) and degeneration of glomerulus (Fig. 11).

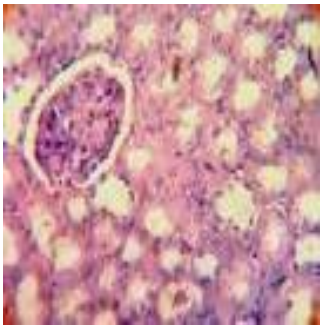

Fig. (7): -ve Control

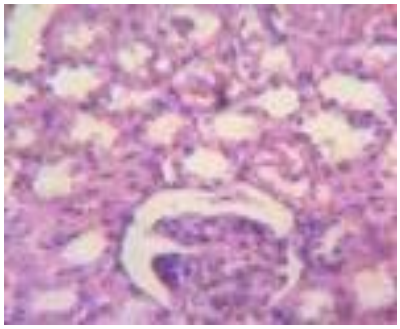

Fig. (8): Sesam oil

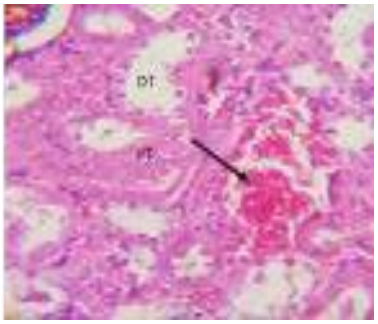

Fig.(9): Pesticide

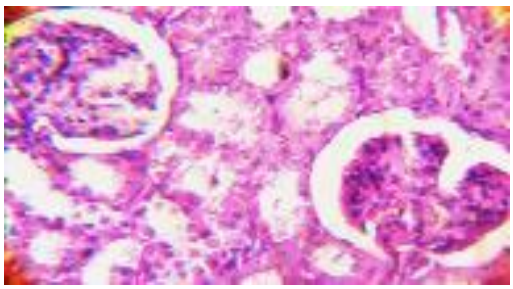

Fig. (10): Prophylactic

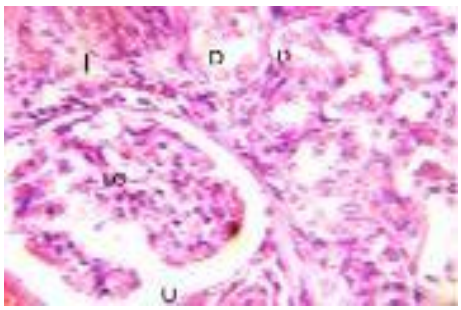

Fig. (11): Post-lactic

Fig.7: Showed normal renal architecture represented by renal tubules and renal corpuscles. Fig.8: Kidney section of rats treated with sesame oil for 28 days showed normal histological structure. Fig.9: Section of rat kidney treated with premium fungicide showed degeneration of some tubular epithelial cells and hemorrhage in the intra tubular spaces. Fig.10: Section of kidney of rat treated with sesame oil fourteen day before pesticide (prophylactic) showed degeneration of some tubular epithelial cells. Fig.11: Section of rat kidney treated with sesame oil fourteen day after pesticide (post-lactic) showed degeneration of some tubular epithelial cells and degeneration of glomerulus.

DISCUSSION

Pollutants, chemicals and inflammation in the body can probably Increases the production of ROS and/or reduce GSH content and probably cause a change in the balance of cellular redox level ultimately resulting in more oxidative damaged biomolecules. Changing the normal redox balance can alter the different enzymes activity and cell signaling pathways in tissues and can therefore be an important mechanism for exercising intoxication of different xenobiotics and mediating the pathogenesis of many diseases [21-23].

The liver plays a crucial role in the detoxification and digestion of xenobiotics and harmful compounds (e.g., pesticides). Thus, hepatotoxicity and health risks are caused by some form of change in its function. Liver biomarker enzymes are markers of liver disease and liver injury, including AST, ALT, ALP and LDH [24]. Biomarkers of effect can be used as an early warning in the development of diseases. Hepatic cells perform different roles in metabolism and contain many enzymes; AST, ALT and ALP are commonly employed biological markers for hepatic injury [25]. In the current study, the integrity of liver determined by serum ALT, and AST markers. Results revealed a significant increase in ALT and AST. ALT is a cytosolic enzyme predominantly expressed by hepatocytes, which entails liver cell lysis and release of the enzyme into the blood, thus allowing the agrochemicals to cytotoxically affect the liver [26].

The kidneys, which are predominantly engaged in the excretion of xenobiotics and associated metabolites, are highly sensitive to xenobiotics such as pesticides. Serum creatinine is a glomerular filtration rate indicator and is used in clinical research as an indicator of renal function [27]. The disturbance in renal functions noticed in this study was confirmed by degeneration of some tubular epithelial cells and glomerulus.

GSH and GSH-related enzymatic systems in cells drive benefit physiological roles in detoxification. GSH is a nucleophile that can interact with electrophilic species rendering the electrophilic molecules more solubility and unable to interact with cellular components [28]. The decrease in the content of GSH is attributed to its use to conjugate electrophilic metabolites to combat the overproduction of ROS and LPO In addition, excess Oxidative damage causes more damage to almost all bio-macromolecules resulting in cytotoxicity [29]. MDA adducts are clinically important as they can participate in secondary harmful effects (e.g. crosslinking) by facilitating intramolecular and intermolecular protein/DNA crosslinking that can cause modifications in cell biology properties and persist during aging and chronic diseases [30].

Marked hemorrhage were observed in tissues seemed to be a sort of inflammation induced by toxic agent such pesticides. Histamine librated from damaged cells is an important factor in the vascular response that follows injury, causing increased blood flow into the capillary bed and vessels which induced vasodilation. The inflammatory response includes migration and activation of both resident and circulating inflammatory cells and the production of cytokines. Activated inflammatory cells and released cytokines stimulate multiplication, migration, secretory activities and collagen production by fibroblasts [31].

Biologically active substances obtained from plants play a crucial role Due to its antioxidant functions; these natural antioxidants have various potential benefits on cancer, inflammation and cardiovascular disease [24]. Sesame oil has long been known for its health-promoting qualities and more recent times, tests have shown that its medicinal properties are due to the phenolic compounds present in sesame oil. Sesamol has been one of the most active and potent constituents in sesame oil, leading to its therapeutic effects [32]. Sesame lignans can defend body cells from damage caused by free radicals by ROS scavenging moiety [33]. Sesame oil increases contaminants' hepatic detoxification and defends against oxidative stress and expression of hepatic genes [34]. The noted defensive effects of sesame oil against exposure to fungicides can be due to the attenuation of the activity of xanthine oxidase and the production of nitric oxide, superoxide anion and hydroxyl radical, one of the main free radicals in lipid peroxidation Furthermore, sesame oil is a potent proinflammatory mediator inhibitor, and by inhibiting nitric oxide, tumor necrosis factor α [15].

It will be logical to maximize the quantity of exogenous antioxidants (mainly by ingestion) to improve the protective properties of individuals against environmental oxidative stress, since we have no impact on the amounts of endogenous antioxidants [12]. Current research indicates that improved the idea of dietary supplementation with natural antioxidant will greatly boost the safety against several different forms of toxicity induced by environmental pollutants.

CONCLUSION

The present study concluded that premium had induced functional and histological changes in liver and kidney male rats. Sesame oil successfully ameliorated the oxidative stress, hepatotoxicity and nephrotoxicity of fungicide.

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