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Assessment of the Preventive Effects of *Salvia officinalis* and *Ruta graveolens* Ethanolic Extracts on Chlorpyrifos- and Methomyl-induced Testicular and Cardiac Toxicities in Albino Rats

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Abstract Insecticides are believed to be the major factors behind the increase in agricultural productivity in the 20th century but their uses cause harmful effects on both animals and humans. Thus, the present work was designed to assess the preventive effect of *Salvia officinalis* and *Ruta graveolens* against chlorpyrifos- and methomyl-induced testicular and cardiac toxicities in rats. The rats orally administered 1/20 LD_{50} of either chlorpyrifos (13.8 mg/kg b.w.) or methomyl (1.6 mg/kg b.w.) 2 times for 4 weeks were co-treated with ethanolic extracts of *Salvia officinalis* and *Ruta graveolens* at dose level of 50 mg/kg b.w. six days/week by oral administration for 4 weeks. The administration of chlorpyrifos and methomyl to rats induced testicular and cardiac toxicities that were evidenced by the histological deteriorated changes and the significant decrease in serum testosterone, FSH and LH levels as well as the significant increase in CK-MB, LDH and AST activities. Chlorpyrifos- and methomyl-administered rats exhibited a significant increase in testes and heart lipid peroxidation as well as a significant decrease in reduced glutathione content and superoxide dismutase, glutathione peroxidase and glutathione-S-transferase activities. Concomitant supplementation with *Salvia officinalis* or *Ruta graveolens* markedly prevented chlorpyrifos- and methomyl-induced biochemical and histopathological alterations. These findings provide evidence that *Salvia officinalis* and *Ruta graveolens* ethanolic extracts could prevent chlorpyrifos- and methomyl-induced testicular and cardiac toxicities in rats through potentiation of the antioxidant defense system.

Keywords Cardiac toxicity, Testicular toxicity, Oxidative stress, *Salvia officinalis*, *Ruta graveolens*

1. Introduction

Pesticide drift from agricultural fields, exposure to pesticides during application and intentional or unintentional poisoning generally leads to the acute illness in humans [1-2]. Several symptoms such as headaches, body aches, skin rashes, poor concentration, nausea, dizziness, impaired vision, cramps, panic attacks and in severe cases coma and death could occur due to pesticide poisoning [3]. Pesticides have been shown to induce the production of reactive oxygen species (ROS) which ultimately lead to a condition of oxidative stress [4] resulting in the damage of macromolecules such as nucleic acids, lipids and proteins [5].

Problems and outbreaks have been reported to occur among animals and human from insecticide exposure [6]. Prolonged exposure to insecticides is known to cause chronic neurological syndrome, malignant tumors, immunosuppressive action, teratogenic effect, abortion and decreased fertility in experimental animals [7]. Pesticides may cause reproductive toxicity through direct damage to cells, interference with biochemical processes necessary for normal cell function and biotransformation resulting in toxic metabolites [8]. Exposure to pesticides is one of the most important occupational risks among farmers in developing countries [9-11]. Occupational exposure to pesticides is of great interest in order to identify the hazards of pesticide use and to establish safe methods of pesticide handling; this is because pesticide misuse in various sectors of the agriculture often has been associated with health problems and environmental contamination worldwide [12-14]. Misuse of highly toxic pesticides, coupled with a weak or a totally absent legislative framework in the use of pesticides, is one of the major reasons for the high incidence of pesticide poisoning in developing countries [10]. Low education levels of the rural population, lack of information and training on pesticide safety, poor spraying technology, and

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inadequate personal protection during pesticide use have been reported to play a major role in the intoxication scenario [15, 16].

Salvia is a genus belonging to the family Lamiaceae presenting approximately 900 species. Amongst these species, *Salvia officinalis* (*S. officinalis*) has been extensively used as a medicinal plant in treating many diseases [17-21]. The plant is reported to have multiple pharmacological effects, including antibacterial [22, 23], antiviral [24], antiinflammatory [25], hypoglycemic [26, 27], fungistatic [28], antimutagenic [29], anticancer [30], hepatoprotective [31] and antioxidative [19] effects.

Ruta graveolens (R. graveolens) has been used in the folklore medicine for long times to treat various conditions such as eye problems, rheumatism, dermatitis, pain and many inflammatory diseases [32]. A number of chemical constituents such as alkaloids, coumarins, volatile substances, terpenoids, flavonoids and furoquinolines have been isolated from different parts of the plant [33]. From current pharmaceutical studies, additional pharmaceutical applications of *R. graveolens* have revealed antioxidant, antiinflammatory [32], antinociceptive and antipyretic [34], antiulcerogenic [35], antidiabetic [36], antibacterial and antifungal [37] as well as antiandrogenic [38] effects.

The present work was designed to investigate the preventive effect of *S. officinalis* and *R. graveolens* ethanolic extracts on testicular and cardiac toxicities induced by two commonly used insecticides, chlorpyrifos and methomyl, in albino rats.

2. Material and Methods

2.1. Animals and Housing

Adult male albino rats, weighing about 120-130 g and aging 9-10 weeks, were used in the present study. The animals were obtained from the National Institute of Ophthalmology, Giza, Egypt. They were kept under observation for two weeks before the onset of experiment to exclude any intercurrent infection. The animals were housed in polypropylene cages with good aerated stainless steel covers in the Animal House of Zoology Department, Faculty of Science, Beni-Suef University, Egypt at normal temperature (20-25°C) and normal daily lighting cycle (10-12hr/day), and were given enough balanced standard diet and water *ad libitum*. All animal procedures are in accordance with the guidelines of Experimental Animal Ethics Committee of Faculty of Science, Beni-Suef University, Egypt. All efforts were done to minimize the suffering and pain of animals.

2.2. Chemicals

Both chlorpyrifos and methomyl were obtained from the Central Laboratories of Agricultural Pesticides, Doki, Egypt. *S. officinalis* and *R. graveolens* were obtained from Experimental Station of Medical Plants (ESMP), Faculty of Pharmacy, Cairo University.

Serum creatine kinase (CK-MB), alanine aminotransferase (AST) and lactate dehydrogenase (LDH) kits were obtained from Stanbio Laboratory Chemical Company (USA), Human Diagnostics Chemical Company (Germany) and Seppim S.A.S Chemical Company (France) respectively. Testosterone, LH and FSH kits were obtained from BioVendor (USA).

2.3. Dose Preparation of Chlorpyrifos and Methomyl

Chlorpyrifos was dissolved in 1% carboxymethylcellulose (CMC) and was given to animals by oral gavage at dose level of 1/20 LD_{50} (13.8mg/kg b. w.) 2 times a week for 4 weeks [39]. Methomyl was dissolved in1% CMC and orally given to animals by oral gavage at dose level of 1/20 LD_{50} (1.6mg/kg b. w.) 2 times a week for 4 weeks [40].

2.4. Preparation of Ethanolic Extracts of *S. officinalis* and *R. graveolens*

The leaves of *S. officinalis* and *R. graveolens* were washed and dried in good aerated shaded area. The dried leaves were powdered by an electric grinder. The powder of each plant was soaked in absolute ethyl alcohol for 72 hours and the mixture was filtered. The solvent in the filtrate was evaporated by rotatory evaporator at 50^{o}C and high pressure. The obtained viscous extract was kept in deep freezer until used.

The ethanolic extracts of *S. officinalis* and *R. graveolens* was given to the rats by oral gavage at dose level of 50mg/kg b. w. 6 days/week for 4 weeks. The dose of *S. officinalis* was chosen, according to Eidi *et al*. [41] while the dose of *R. graveolens* was chosen according to Pandey *et al*. [42].

2.5. Animal Grouping

The male albino rats included in the present study were allocated into seven groups, 6 rats in each, designed as follows:

Group 1 (Normal): The rats in this group were orally given the equivalent volume of 1% CMC (as vehicle), six days per week by oral gavage based on body weight (5 ml/kg b. w.).

Group 2 (Chlorpyrifos-administered group): The rats in this group were administered chlorpyrifos at a dose level of 13.8 mg/kg b.w. 2 times/week and the equivalent volume of 1% CMC 6 times/week by oral gavage both for 4 weeks.

Group 3 (Chlorpyrifos-administered group treated with *S. officinalis* leaf ethanolic extract): The rats in this group were administered ethanolic leaf extract of *S. officinalis* 6 days/week at dose level of 50 mg/kg b.w./day for 4 weeks along with chlorpyrifos oral administration.

Group 4 (Chlorpyrifos-administered group treated with *R. graveolens* leaf ethanolic extract): The rats in this group were administered ethanolic leaf extract of *R. graveolens* 6 days/week at dose level of 50 mg/kg b.w./day for 4 weeks along with chlorpyrifos oral administration.

Group 5 (Methomyl-administered group): The rats in this

group were administered methomyl at a dose level of 1.6 mg/kg b.w. 2 times/week and the equivalent volume of 1% CMC 6 times/week by oral gavage both for 4 weeks.

Group 6 (Methomyl-administered group treated with *S. officinalis* leaf ethanolic extract): The rats in this group were administered ethanolic leaf extract of *S. officinalis* 6 days/week at dose level of 50 mg/kg b.w./day for 4 weeks along with methomyl oral administration.

Group 7 (Methomyl-administered group treated with *R. graveolens* leaf ethanolic extract): The rats in this group were administered ethanolic leaf extract of *R. graveolens* 6 days/week at dose level of 50 mg/kg b. w./day for 4 weeks along with methomyl oral administration.

2.6. Blood and Tissue Samples

At the end of the experimental period, animals were weighed and sacrificed under mild diethyl ether anesthesia after 12 hours of food and water deprivation. Blood samples from jugular vein were collected. The blood was left to coagulate at room temperature then centrifuged at 3000 r.p.m. for 30 minutes. The clear nonhaemolysed supernatant sera were quickly removed for analysis of various biochemical parameters related to testes and heart functions. The obtained serum was kept at -30°C until used. Animals were dissected and testes and hearts were rapidly excised. A 0.5 g from testes and heart of each animal were homogenized in 5 ml 0.9% NaCl (10%w/v) using Teflon homogenizer (Glas-Col, Terre Haute, USA). The obtained homogenates were kept in deep freezer at -30°C to be used later for measurements of oxalate level. The homogenate supernatants for testes and heart samples were obtained by centrifuging the homogenates at 3000 r.p.m. for 10 minutes.

2.7. Biochemical Analysis

CK-MB, AST and LDH activities and testosterone concentration were determined according to the methods of Kachmar and Moss [43], Schumann and Klauke [44], Henderson and Moss [45] and Huang *et al*. [46] respectively. LH and FSH concentrations are determined using reagent kits purchased from BioVendor (USA). Glutathione content and lipid peroxidation were determined according to the procedure of Beutler *et al*. [47] and Preuss *et al*. [48] respectively. Glutathione peroxidase (GPx), glutathione-S-transferase (GST) and superoxide dismutase (SOD) activities were determined according to the methods of Matkovics *et al*. [49], Mannervik and Gutenberg [50] and Marklund and Marklund [51] respectively.

2.8. Statistical Analysis

Data are expressed as mean ± standard error (SE). The data were analyzed by one-way (ANOVA) [52] followed by Least Significant Difference (LSD) to compare various groups with each other. Values of $P>0.05$ were non-significantly different while values of $P<0.05$ and $P<0.01$ were significantly and highly significantly different respectively. F-probability of ANOVA expresses the general effect between groups.

3. Results

3.1. Effect on Testes Function Parameters in Serum

The chlorpyrifos-administered rats exhibited a highly significant decrease ($p<0.01$; LSD) in serum testosterone, LH and FSH concentrations as compared with normal ones. The treatment of chlorpyrifos-administered rats with *S. officinalis* and *R. graveolens* ethanolic extracts induced a highly significant increase ($p<0.01$; LSD) in testosterone, LH and FSH concentrations (Table 1).

At the same time, the methomyl-administered rats exhibited a highly significant decrease ($p<0.01$; LSD) in serum testosterone, LH and FSH concentrations as compared with normal ones. The methomyl-administered rats treated with *S. officinalis* and *R. graveolens* ethanolic induced a highly significant increase ($p<0.01$; LSD) in testosterone, LH and FSH concentrations (Table 1).

Table 1. Effect of *S. officinalis* and *R. graveolens* ethanolic extracts on serum testosterone, LH and FSH concentrations of chlorpyrifos- and methomyl-administered rats

Groups	Testosterone (ng/ml)	LH (ng/ml)	FSH (ng/ml)
Normal	1.28 ± 0.07^{a}	0.589 ± 0.008^{a}	0.364 ± 0.006^{a}
Chlorpyrifos	0.31 ± 0.04^{e}	0.265 ± 0.02^{g}	0.127 ± 0.003^{f}
Chlorpyrifos + *S. officinalis*	0.82 ± 0.03^{c}	0.427 ± 0.007^{d}	0.224 ± 0.002^{d}
Chlorpyrifos + *R. graveolens*	0.71 ± 0.02^{c}	0.389 ± 0.009^{e}	0.189 ± 0.005^{e}
Methomyl	0.54 ± 0.04^{d}	0.326 ± 0.004^{f}	0.184 ± 0.004^{e}
Methomyl + *S. officinalis*	1.09 ± 0.06^{b}	0.515 ± 0.01^{b}	0.311 ± 0.004^{b}
Methomyl + *R. graveolens*	0.96 ± 0.04^{b}	0.477 ± 0.004^{c}	0.279 ± 0.003^{c}
F- probability	P<0.001	P<0.001	P<0.001
LSD at the level 5%	0.139	0.022	0.012
LSD at the level 1%	0.188	0.029	0.016

-Data are expressed as Mean ± SE. Number of animals used in each group is six.
-Means, which share the same superscript symbol(s), are not significantly different.

It seems from the data presented in the table that chlorpyrifos produced higher toxicity than the methomyl in decreasing serum testosterone, LH and FSH levels. Moreover, *S. officinalis* seemed to be more potent in improving the impaired LH and FSH levels.

With regards to one way ANOVA, it was found that the effect between groups was very highly significant ($p<0.001$; F-probability) throughout the experiment.

3.2. Effect on Indices of Oxidative Stress and Antioxidant Defense System in the Testes

Data showing the effect of *S. officinalis* and *R. graveolens* ethanolic extracts on testes oxidative stress and antioxidant defense system markers of chlorpyrifos and methomyl-administered rats are represented in tables 2 and 3.

Table 2. Effect of *S. officinalis and R. graveolens* ethanolic extracts on testes lipid peroxidation and glutathione content of chlorpyrifos- and methomyl-administered rats

Groups	Lipid peroxidation (MDA nmole/g tissue/hr)	Glutathione (nmole/100mg tissue)
Normal	25.53±1.53^{f}	60.19±0.99^{a}
Chlorpyrifos	54.15±1.31^{a}	30.91±0.89^{e}
Chlorpyrifos + *S. officinalis*	36.48±1.51^{cd}	47.73±0.50^{c}
Chlorpyrifos + *R. graveolens*	39.79±1.75^{c}	46.52±0.61^{c}
Methomyl	46.68±1.82^{b}	34.83±0.47^{d}
Methomyl + *S. officinalis*	31.33±1.10^{e}	50.89±0.83^{b}
Methomyl + *R. graveolens*	35.15±1.19^{de}	49.83±0.53^{b}
F- probability	P<0.001	P<0.001
LSD at the level 5%	4.27	2.06
LSD at the level 1%	5.75	2.78

-Data are expressed as Mean ± SE. Number of animals used in each group is six.
-Means, which share the same superscript symbol(s), are not significantly different.

Table 3. Effect of *S. officinalis and R. graveolens* ethanolic extracts on testes GPx, GST and SOD activities of chlorpyrifos and methomyl-administered rats

Groups	GPx (mU/100mg tissue)	GST (U/100mg tissue)	SOD (U/g tissue)
Normal	153.82±1.47^{a}	88.75±1.94^{a}	144.55±1.58^{a}
Chlorpyrifos	61.24±1.77^{f}	45.85±1.42^{f}	75.58±3.76^{f}
Chlorpyrifos + *S. officinalis*	135.18±2.47^{cd}	72.93±2.37^{cd}	122.75±2.15^{cd}
Chlorpyrifos + *R. graveolens*	131.24±1.02^{d}	69.57±1.95^{d}	118.55±1.65^{d}
Methomyl	69.68±1.13^{e}	52.56±1.74^{e}	87.77±3.02^{e}
Methomyl + *S. officinalis*	141.48±1.67^{b}	79.36±1.59^{b}	132.77±1.38^{b}
Methomyl + *R. graveolens*	137.74±2.25^{bc}	77.27±2.10^{bc}	128.96±1.38^{bc}
F- probability	P<0.001	P<0.001	P<0.001
LSD at the level 5%	5.07	5.48	6.64
LSD at the level 1%	6.83	7.38	8.94

-Data are expressed as Mean ± SE. Number of animals used in each group is six.
-Means, which share the same superscript symbol(s), are not significantly different.

The administration of chlorpyrifos to albino rats produced a highly significant (P<0.01; LSD) decrease in testes glutathione content and GPx, GST and SOD activities, and a highly significant (P<0.01; LSD) increase in testes lipid peroxidation as compared to normal control. The treatment of chlorpyrifos-administered rats with *S. officinalis* and *R. graveolens* ethanolic extracts caused a highly significant increase in glutathione content and GPx, GST and SOD activities, and decrease in testes lipid peroxidation, when compared to chlorpyrifos control group.

Meanwhile, the administration of methomyl to albino rats produced a highly significant (P<0.01; LSD) decrease in testes glutathione content and GPx, GST and SOD activities, and a highly significant (P<0.01; LSD) increase in testes lipid peroxidation as compared to normal control. The treatment of methomyl-administered rats with *S. officinalis* and *R. graveolens* ethanolic extracts caused a highly significant increase in glutathione content and GPx, GST and SOD activities, and decrease in testes lipid peroxidation, when compared to methomyl control group.

The effect of administration of chlorpyrifos to normal rats seemed to be more deleterious on testes lipid peroxidation and glutathione content and GPx, GST and SOD activities than the administration of methomyl. Moreover, *S.*

officinalis seemed to be more effective in improving testes lipid peroxidation and glutathione peroxidase, GST and SOD activities.

With regards to one way ANOVA, it was found that the effect between groups on testes lipid peroxidation, glutathione content and antioxidant enzyme activities were very highly significant ($p<0.001$; F-probability) throughout the experiment.

3.3. Effect on Serum CK-MB, AST and LDH Activities

The data showing the effect on serum CK-MB, AST and LDH activities are represented in table 4.

The chlorpyrifos administration for 4 weeks produced a highly significant elevation ($p<0.01$; LSD) of CK-MB, AST and LDH activities as compared with normal rats. The treatment of chlorpyrifos-administered rats with *S. officinalis and R. graveolens* ethanolic extracts induced a highly significant amelioration ($p<0.01$; LSD) of the elevated CK-MB, AST and LDH activities as compared with the corresponding control group.

The administration of methomyl to normal rats produced a highly significant increase ($p<0.01$; LSD) in CK-MB, AST and LDH activities. The treatment of methomyl-administered rats with *S. officinalis* and *R. graveolens* ethanolic extracts produced a potential amelioration of the impaired CK-MB, AST and LDH activities recording a highly significance of $p<0.01$ as compared with the corresponding control group.

The administration of chlorpyrifos to normal rats seemed to produce more deleterious effects on CK-MB, AST and LDH activities than the administration of methomyl. Moreover, *S. officinalis* seemed to be more effective in improving CK-MB, AST and LDH activities.

With regards to one way ANOVA, it was found that the effect between groups was very highly significant ($p<0.001$; F-probability) throughout the experiment.

3.4. Effect on Indices of Oxidative Stress and Antioxidant Defense System in the Heart

Data regarding the effects of *S. officinalis and R. graveolens* ethanolic extracts on heart oxidative stress and antioxidant defense system markers are represented in tables 5 and 6.

Table 4. Effect of *S. officinalis and R. graveolens* ethanolic extracts on CK-MB, AST and LDH activities of chlorpyrifos- and methomyl-administered rats

Groups	CK-MB (mU/100mg tissue)	AST (U/L)	LDH (U/dl))
Normal	133.43 ± 1.04^{f}	26.33 ± 0.75^{e}	225.68 ± 1.78^{g}
Chlorpyrifos	239.74 ± 1.20^{a}	55.75 ± 1.24^{a}	586.24 ± 1.71^{a}
Chlorpyrifos + *S. officinalis*	168.73 ± 0.86^{d}	31.82 ± 1.14^{cd}	393.86 ± 2.93^{d}
Chlorpyrifos + *R. graveolens*	179.28 ± 1.02^{c}	34.37 ± 1.49^{c}	417.15 ± 1.66^{c}
Methomyl	215.74 ± 1.20^{b}	51.57 ± 1.13^{b}	541.83 ± 1.98^{b}
Methomyl + *S. officinalis*	153.38 ± 0.82^{e}	28.75 ± 1.11^{de}	315.40 ± 2.36^{f}
Methomyl + *R. graveolens*	166.46 ± 1.09^{d}	31.42 ± 1.01^{cd}	343.41 ± 3.25^{e}
F- probability	P<0.001	P<0.001	P<0.001
LSD at the level 5%	3.17	3.31	6.68
LSD at the level 1%	4.27	4.46	9.01

-Data are expressed as Mean ± SE. Number of animals used in each group is six.
-Means, which share the same superscript symbol(s), are not significantly different.

Table 5. Effect of *S. officinalis and R. graveolens* ethanolic extracts on heart lipid peroxidation and glutathione content of chlorpyrifos- and methomyl-administered rats

Groups	Lipid peroxidation (MDA nmole/g tissue/hr)	Glutathione (nmole/100mg tissue)
Normal	31.37 ± 1.42^{e}	60.82 ± 1.04^{a}
Chlorpyrifos	54.29 ± 1.15^{a}	34.15 ± 0.59^{e}
Chlorpyrifos + *S. officinalis*	42.58 ± 1.77^{bc}	50.96 ± 0.65^{bc}
Chlorpyrifos + *R. graveolens*	45.69 ± 1.33^{b}	49.49 ± 0.44^{c}
Methomyl	46.47 ± 2.16^{b}	36.59 ± 0.54^{d}
Methomyl + *S. officinalis*	35.50 ± 0.90^{de}	52.86 ± 0.86^{b}
Methomyl + *R. graveolens*	38.49 ± 2.63^{cd}	51.58 ± 1.15^{bc}
F- probability	P<0.001	P<0.001
LSD at the level 5%	4.96	2.29
LSD at the level 1%	6.68	3.09

-Data are expressed as Mean ± SE. Number of animals used in each group is six.
-Means, which share the same superscript symbol(s), are not significantly different.

Table 6. Effect of *S. officinalis and R. graveolens* ethanolic extracts on heart GPx, GST and SOD activities of chlorpyrifos- and methomyl-administered rats

Groups	GPx (mU/100mg tissue)	GST (U/100mg tissue)	SOD (U/g tissue)
Normal	161.71±1.72^{a}	74.53±1.61^{a}	138.28±1.66^{a}
Chlorpyrifos	71.92±1.32^{f}	37.28±0.83^{e}	68.22±2.27^{f}
Chlorpyrifos + *S. officinalis*	131.26±1.30^{cd}	63.62±2.05^{b}	121.24±1.32^{cd}
Chlorpyrifos + *R. graveolens*	126.68±2.06^{d}	57.17±2.86^{c}	118.67±2.20^{d}
Methomyl	78.75±2.28^{e}	44.52±2.32^{d}	77.64±1.69^{e}
Methomyl + *S. officinalis*	138.14±1.44^{b}	68.24±2.00^{b}	129.15±1.95^{b}
Methomyl + *R. graveolens*	134.65±2.09^{bc}	65.28±2.27^{b}	124.82±1.77^{bc}
F- probability	P<0.001	P<0.001	P<0.001
LSD at the level 5%	5.15	5.99	5.39
LSD at the level 1%	6.94	8.07	7.26

-Data are expressed as Mean ± SE. Number of animals used in each group is six.
-Means, which share the same superscript symbol(s), are not significantly different.

The administration of chlorpyrifos to albino rats produced a highly significant (P<0.01; LSD) decrease in heart glutathione content and GPx, GST and SOD activities and a highly significant (P<0.01; LSD) increase in heart lipid peroxidation as compared to normal control. The treatment of chlorpyrifos-administered rats with *S. officinalis* and *R. graveolens* ethanolic extracts caused a highly significant increase in glutathione content and GPx, GST and SOD activities and decrease in heart lipid peroxidation when compared to chlorpyrifos control group.

Also, the administration of methomyl to albino rats produced a highly significant (P<0.01; LSD) decrease in heart glutathione content and GPx, GST and SOD activities and a highly significant (P<0.01; LSD) increase in heart lipid peroxidation as compared to normal control. The treatment of methomyl-administered rats with *S. officinalis* and *R. graveolens* ethanolic extracts caused a highly significant increase in glutathione content and GPx, GST and SOD activities and decrease in heart lipid peroxidation when compared to methomyl control group.

The administration of chlorpyrifos to normal rats seemed to produce more deleterious effects on heart lipid peroxidation, glutathione content and GPx, GST and SOD activities than the administration of methomyl. Moreover, *S. officinalis* seemed to be more potent in alleviating heart lipid peroxidation, glutathione content and GPx, GST and SOD activities.

With regards to one way ANOVA, it was found that the effect between groups was very highly significant (p<0.001; F-probability) throughout the experiment.

3.5. Histological Changes

The testis section of normal rats illustrated normal organized histological architecture of the seminiferous tubules (Figure 1). Photomicrographs of testes sections of chlorpyrifos-administered rats depicted marked changes like necrosis, degeneration of spermatogoneal cells lining tubules and complete absence of spermatogoneal cells lining tubules (Figure 2).

The chlorpyrifos-administered rats treated with *S. officinalis* ethanolic extract exhibited desquamation of spermatogoneal cells lining seminiferous tubule (Figure 3; photomicrograph 3A) in some animals and nearly complete improvement of histopathological deleterious effects in others (Figure 3; photomicrograph 3B). The treatment of chlorpyrifos-administered rats with *R. graveolens* ethanolic extract exhibited degeneration of spermatogoneal cells lining tubules and interstitial oedema in some animals (Figure 4; photomicrograph 4A) and nearly complete improvement of histopathological deleterious effects to nearly normal structure of seminiferous tubules in others (Figure 4; photomicrograph 4B).

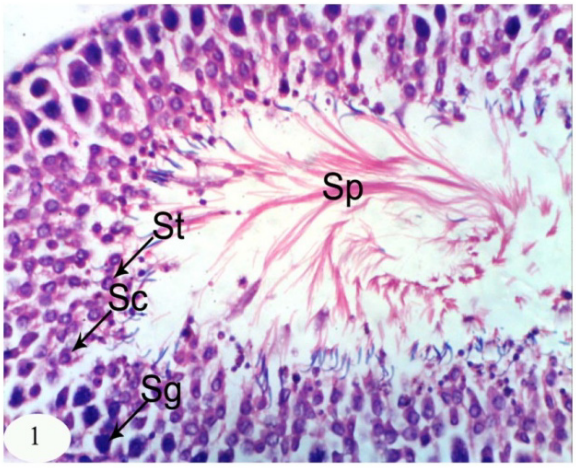

Figure 1. Photomicrograph of section of testis of normal rat showed normal organized histological architecture of seminiferous tubules that consist of spermatogonia (Sg), spermatocytes (Sc), spermatids (St) and spermatozoa (Sp). (X400)

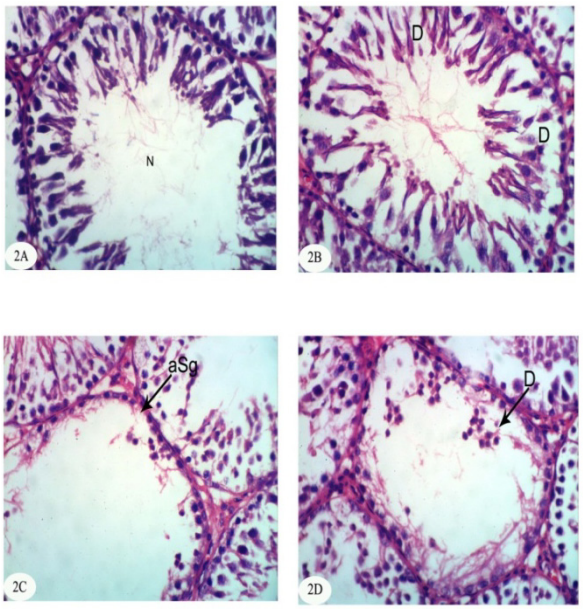

Figure 2. Photomicrographs (2A-2D) of sections of testes of chlorpyrifos-administered rats showed necrosis (N) (**2A**), degeneration of spermatogoneal cells lining tubules (D) (**2B** and **2D**) and complete absence of spermatogoneal cells lining tubules (aSg) (**2C**). (X400)

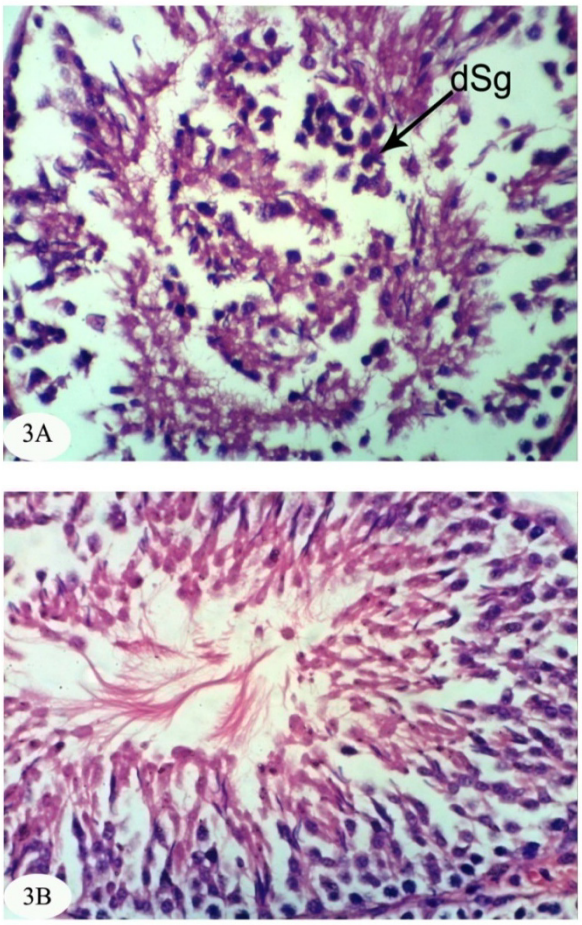

Figure 3. Photomicrographs of sections of testes of chlorpyrifos-administered rats treated with *S. officinalis* leaf ethanolic extract showed desquamation of spermatogoneal cells lining seminiferous tubule (**3A**) and nearly normal structure of seminiferous tubules (**3B**). (X400)

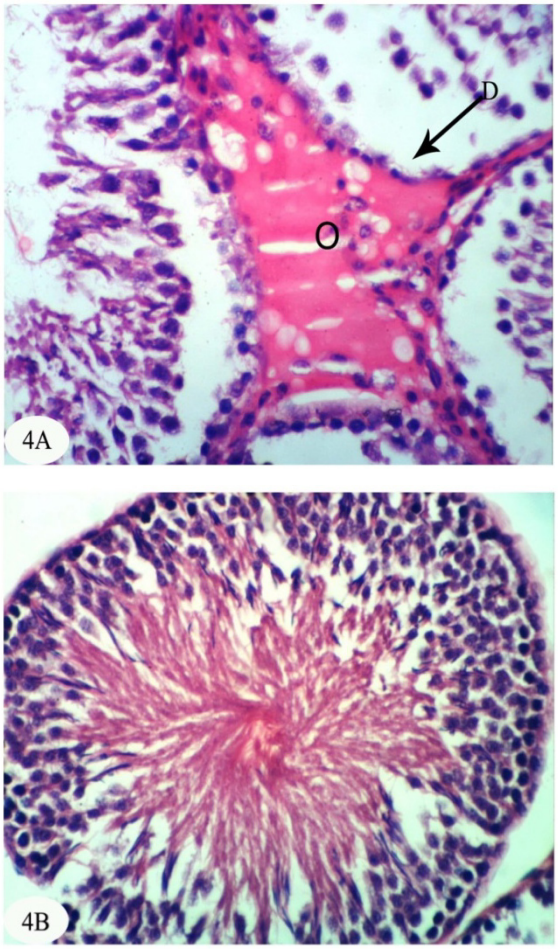

Figure 4. Photomicrographs of sections of testes of chlorpyrifos-administered rats treated with *R. graveolens* leaf ethanolic extract showed degeneration of spermatogoneal cells lining tubules and interstitial oedema (O) (**4A**) and nearly normal structure of seminiferous tubules as indicated in **4B**. (X400)

Photomicrographs of testes sections treated with methomyl-administered rats exhibited desquamated spermatogoneal cells in the lumen of tubule, presence of spermatid gaint cell in the lumen, necrosis and degeneration of spermatogoneal cells lining tubules (Figure 5). The methomyl-administered rats treated with *S. officinalis* ethanolic extract exhibited nearly normal structure of seminiferous tubules (Figure 6). The methomyl-administered rats treated with *R. graveolens* ethanolic extract induced interstitial odema in some animals (Figure 7; photomicrograph 7A) and also greatly improved the histopathological impacts to nearly normal structure of seminiferous tubules in others (Figure 7; photomicrograph 7B).

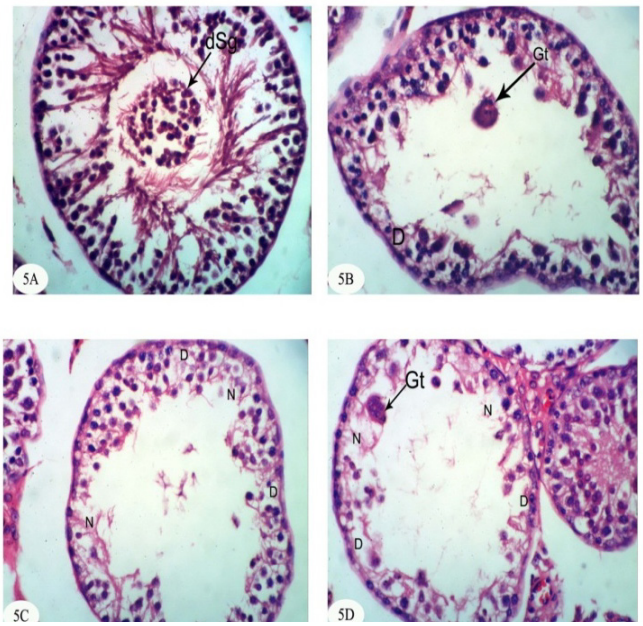

Figure 5. Photomicrographs (**5A-5D**) of sections of testes of methomyl-administered rats showed desquamated spermatogoneal cells (dSg) in the lumen of tubule (**5A**), presence of spermatid gaint cell in the lumen (Gt) (**5B** and **5D**), necrosis (N) and degeneration of spermatogoneal cells lining tubules (D) (**5C** and **5D**). (X400)

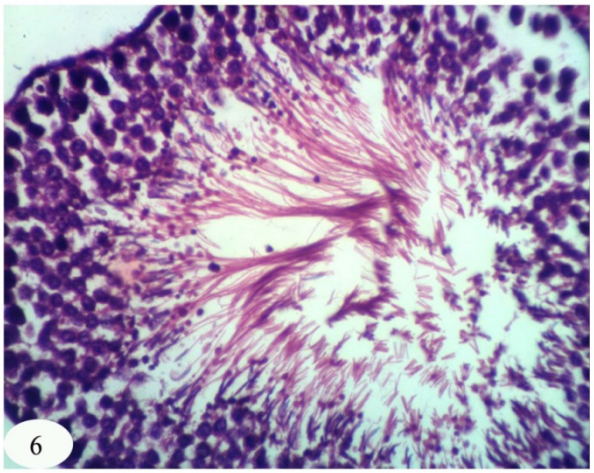

Figure 6. Photomicrograph of section of testis of methomyl-administered rats treated with *S. officinalis* leaf ethanolic extract showed nearly normal structure of seminiferous tubules. (X400)

The heart section of normal rats exhibited the normal organized histological structure of the cardiac myocytes (Figure 8). The heart of chlorpyrifos-administered rats exhibited perivasculitis and inflammatory cells infiltration (Figure 9). The chlorpyrifos-administered rats treated with *S. officinalis* ethanolic extract produced potential amelioration of the cardiac myocytes to almost the normal structure of cardiac myocytes (Figure 10). The chlorpyrifos-administered rats treated with *R. graveolens* ethanolic extract exhibited congestion of myocardial blood vessel in some animals (Figure 11; photomicrograph 11A) and potential amelioration of the cardiac myocytes to nearly the normal structure in others (Figure 11; photomicrograph 11B).

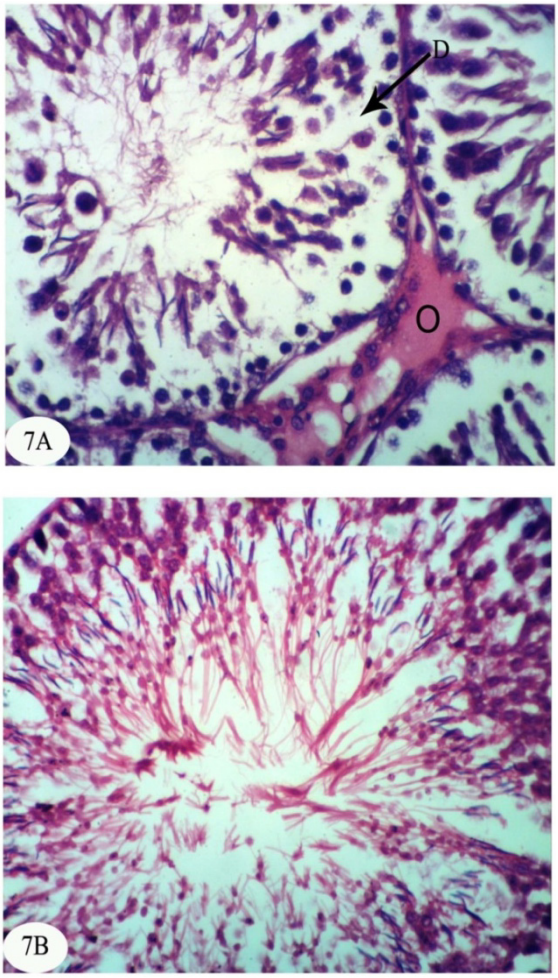

Figure 7. Photomicrographs of sections of testes of methomyl-administered rats treated with *R. graveolens* leaf ethanolic extract showed interstitial odema (O) (**7A**) and nearly normal structure of seminiferous tubules (**7B**). (X400)

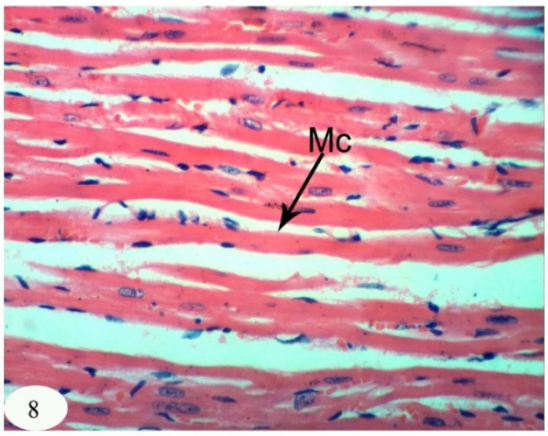

Figure 8. Photomicrograph of section of heart of normal rat showing the normal cardiac myocytes (Mc). (X400)

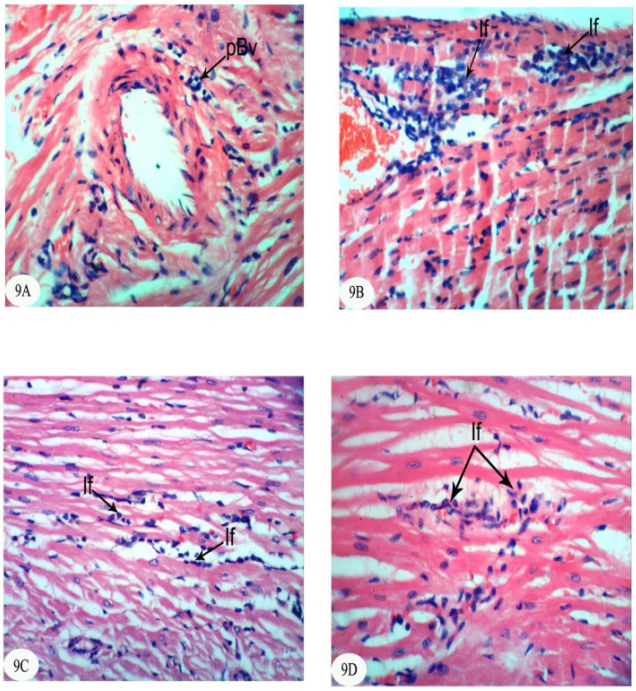

Figure 9. Photomicrographs (**9A-9D**) of sections of heart of chlorpyrifos-administered rats showed perivasculitis (pBv), inflammatory cells infiltration (If). (X400)

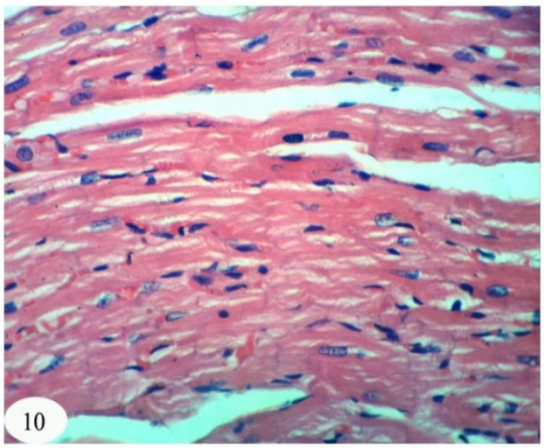

Figure 10. Photomicrograph of section of heart of chlorpyrifos-administered rats treated with *S. officinalis* leaf ethanolic extract showed nearly normal structure of cardiac myocytes. (X400)

The heart of methomyl-administered rats exhibited inflammatory cells infiltrations, necrosis of cardiac myocytes and fibroblasts proliferation between cardiac myocytes (Figure 12). The methomyl-administered rats treated with *S. officinalis* and *R. graveolens* ethanolic extracts produced potential amendment of the cardiac myocytes (Figures 13 and 14).

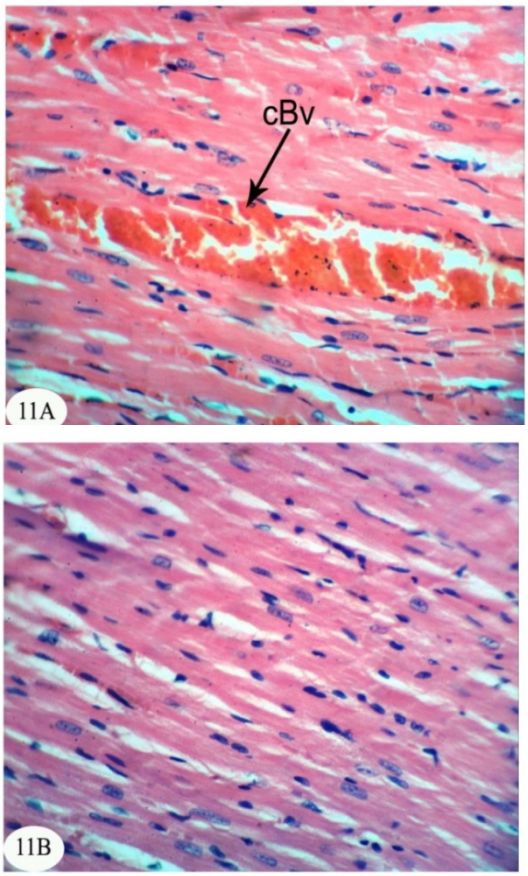

Figure 11. Photomicrographs of sections of heart of chlorpyrifos-administered rats treated with *R. graveolens* leaf ethanolic extract showed congestion of myocardial blood vessel (cBv) (**11A**) and nearly normal structure of cardiac myocytes (**11B**). (X400)

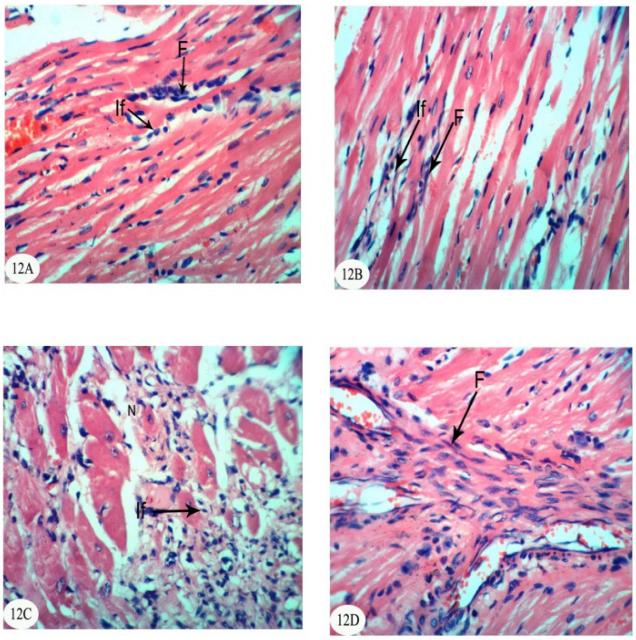

Figure 12. Photomicrographs (**12A-12D**) of sections of heart of methomyl-administered rats showed inflammatory cells infiltrations (If) (**12A-12C**), fibroblsts (F) profiliration (**12A, 12B** and **12D**). (X400)

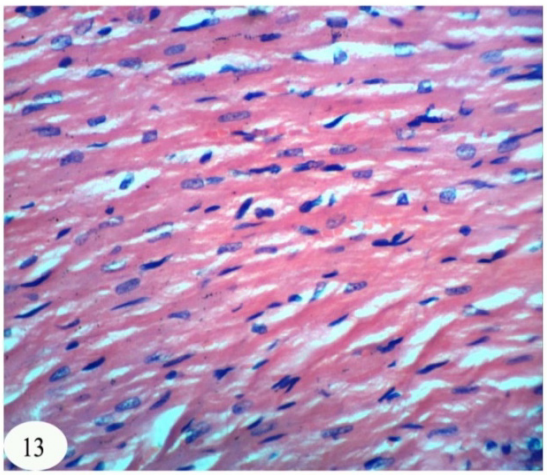

Figure 13. Photomicrograph of section of heart of methomyl-administered rats treated with *S. officinalis* leaf ethanolic extract showed nearly normal structure of cardiac myocytes. (X400)

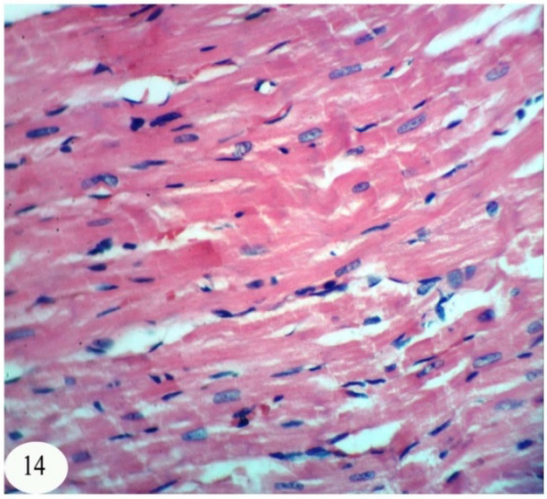

Figure 14. Photomicrograph of section of heart of methomyl-administered rats treated with *R. graveolens* leaf ethanolic extract showed nearly normal structure of cardiac myocytes. (X400)

4. Discussion

Concerning serum testosterone, LH and FSH, chlorpyrifos significantly decreased their levels. These results are in agreement with several publications [53-58]. It was reported that chlorpyrifos displayed an anti-androgen activity which was manifested as inhibition of testosterone stimulated increase in the weight of accessory sex organs [53].

On the other hand, the results of methomyl also go parallel with those of Mahgoub and Mednay [59] and Shalaby *et al*. [60] who recorded a significant decrease in the level testosterone in serum of methomyl intoxicated rats. Shalaby *et al*. [60] reported that methomyl had a dose-dependent adverse effect on male reproduction because the large dose (1/20 of LD_{50}) in comparison to the small dose (1/40 of LD_{50}) exhibited more pronounced deleterious effects on male fertility. In addition, Mahgouband and Mednay [59] reported the hormonal changes and testicular damage after chronic exposure of male rats to insecticide methomyl.

The decrease in serum testosterone, LH and FSH was ameliorated by administration of *S. officinalis and R. graveolens* leaf ethanolic extracts in the present study. Oxidative stress is a possible mechanism of action of chlorpyrifos and methomyl-induced toxicity in the male reproductive organs and thus *S. officinalis and R. graveolens* leaf ethanolic extracts by their ability to scavenge free radicals ameliorated this toxicity.

Chlorpyrifos produced histopathological alterations in the testes of the rats. The testes of the rats treated with chlorpyrifos depicted marked changes like degeneration of spermatogoneal cells lining tubules, necrosis and complete absence of spermatogoneal cells lining tubules.

Concomitant supplementation with *S. officinalis* and *R. graveolens* ethanolic extracts markedly prevented chlorpyrifos-induced deteriorations in sex hormones' levels in serum and histopathological alterations of the testes. The testes of chlorpyrifos-administered rats treated with *S. officinalis* ethanolic extract caused desquamation of spermatogoneal cells lining seminiferous tubule in some animals and greatly improved the histopathological impacts of chlorpyrifos administration in others. The testes of chlorpyrifos-administered rats treated with *R. graveolens* ethanolic extract showed degeneration of spermatogoneal cells lining tubules, interstitial oedema tubule in some animals and also greatly amended the histopathological impacts to nearly normal structure of seminiferous tubules in others.

Also, the testes of methomyl-administered rats showed marked histopathological alterations such as desquamated spermatogoneal cells in the lumen of tubule, presence of spermatid giant cell in the lumen, necrosis and degeneration of spermatogoneal cells lining tubules.

Concomitant supplementation with *S. officinalis* and *R. graveolens* ethanolic extracts markedly prevented methomyl-induced deleterious effects on sex hormones' levels in serum as well as histopathological alterations in the testes. The testes of methomyl-administered rats treated with *S. officinalis* leaf ethanolic extract exhibited nearly normal structure of seminiferous tubules. The treatment of methomyl-administered rats with *R. graveolens* ethanolic extract showed interstitial odema in some animals and also greatly improved the histopathological impacts to nearly normal structure of seminiferous tubules in others.

In reference to the biochemical markers of cardiac function, the present study revealed that serum CK-MB, AST and LDH activities was markedly increased in chlorpyrifos and methomyl-administered rats. On the other hand, the treatment of these intoxicated rats with *S. officinalis and R. graveolens* induced a profound decrease in these elevated enzyme activities. In concurrence with these results, several researchers reported that the exposure to pesticides led to cardiotoxicity in experimental animals [61-66]. It was also revealed that the leaves of *S. officinalis* L. (sage) are well known for their antioxidative properties [67]. There is increasing evidence to suggest that many

degenerative diseases, such as brain dysfunction, cancer, heart diseases, and weakened immune system, could be the result of cellular damage caused by free radicals, and antioxidants present in human diet may play an important role in disease prevention [68-70].

Rutin and quercetin are the main active flavonoids of *R. graveolens*. Keli *et al*. [71] reported that stroke incidence were inversely associated with dietary flavonoids mainly. Quercetin cardiovascular effects are attributed at least in part to its antioxidant and anti-inflammatory activity, and its ability to inhibit platelet aggregation *ex vivo* [72]. Moreover, Chopra and Singh [73] stated that rutin has a cardioprotective effect and decreases the infract size in a dose dependant manner due to its free radical scavenging power.

In the current study, chlorpyrifos-administered rats exhibited histopathological alterations in the heart of the rats. The heart of chlorpyrifos-administered rats depicted marked changes like perivasculitis and inflammatory cells infiltration. Concomitant supplementation with *S. officinalis* and *R. graveolens* ethanolic extracts markedly prevented chlorpyrifos-induced biochemical and histopathological alterations in the heart. The chlorpyrifos-administered rats treated with *S. officinalis* ethanolic extract produced a potential amelioration of the cardiac myocytes to nearly the normal structure. The chlorpyrifos-administered rats treated with *R. graveolens* leaf ethanolic extract exhibited congestion of myocardial blood vessel in some animals and potential amelioration of the cardiac myocytes to nearly normal structure in others.

Meanwhile, the heart of methomyl-administered rats showed marked histopathological alterations such as inflammatory cells infiltrations, necrosis of cardiac myocytes and fibroblasts proliferation between cardiac myocytes. The treatment of methomyl-administered rats with either *S. officinalis* and *R. graveolens* ethanolic extracts produced potential amelioration of the cardiac myocytes.

Defense against oxidative stress are maintained by using several mechanisms which include antioxidant machinery [74]. It has been indicated that lipid peroxidation was significantly increased in rat testicular tissue treated with methomyl and chlorpyrifos. The toxic manifestations induced by chlorpyrifos pesticide may be associated with the enhanced production of ROS or the increase in MDA levels which is induced by the pesticide itself (degradation of phospholipids and ultimately result in cellular deterioration) or by a possible increase in free radicals caused by chlorpyrifos [75].

Among the antioxidant enzymes, SOD, GPx and GST are crucial in the antioxidant defense system against oxidative injury. SOD is the primary step of the defense mechanism in the antioxidant system against oxidative stress by catalyzing the dismutation of 2 superoxide radicals (O^{2-}) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) [76].

GPx is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biological function of GPx is to reduce lipid hydroperoxides conversion to their corresponding alcohols and to reduce free H_2O_2 reaction [77]. In our experiment, GPx activity was decreased in testicular tissue of rats-administered methomyl and chlorpyrifos. This result is in contrast with other authors who found that GPx activity was not altered in rats exposed to chlorpyrifos [78-80]. Another studies observed that GPx activity was increased [81, 82]. These discrepancies could be related to the differences in the animal species, doses and tissues. However, it has been also reported that organophosphate pesticides caused a decrease in GPx activity both *in vivo* and *in vitro* [83, 84]. In our present study, a significant fall in GSH level and GPx activity was observed in methomyl and chlorpyrifos--administered animals. This observation may be due to enhanced free radical production (as evidenced by the increase of LPO) and apart from GPx, also involved in the removal of H_2O_2. H_2O_2 generated due to chlorpyrifos and methomyl toxicity, engage more GSH, which thereby get converted to oxidized glutathione (GSSG) in presence of GPx, hence, the GSH, and GPx level decreased on chlorpyrifos and methomyl administration.

GST is a family of phase II detoxifying enzymes with broad substrate specificities that catalyzes the conjugation of a variety of electrophilic substrates to the thiol group of GSH, producing less toxic forms [85, 86]. A decrease of GST activity in rats-administered chlorpyrifos and methomyl was observed. This decrease may be due to the decrease in GSH and glutathione dependent enzyme systems that provide major protection against the toxic agents. A pronounced decrease of GSH level was found in testis of the rats intoxicated with chlorpyrifos; this may be responsible for enhancement of LPO. Several studies observed depletion of GSH in chlorpyrifos-intoxicated animals in different tissues [87, 88]. GSH is an important naturally occurring antioxidant, which prevents free radical damage and helps detoxification by conjugating with chemicals. In addition, GSH is central to the cellular antioxidant defenses and acts as an essential cofactor for antioxidant enzymes including GPx and GST [89]. GSH in the testis acts either directly by scavenging the free radicals or by acting as a substrate to GPx and GST during the detoxification of hydrogen peroxides, lipid peroxides and electrophiles as well as by preventing oxidation of –SH groups of proteins [89].

Antioxidants such as glutathione, GPx, GST and SOD are very important systems which protect the cell against free radicals. It is well known that endogenous antioxidant enzymes are responsible for preventing and neutralizing the free radical-induced oxidative damage.

In the present investigation, a striking decrease in these antioxidants in both chlorpyrifos- and methomyl-administered rats elicits strong evidence for the involvement of oxidative damage in the testes and heart.

Several reports indicated that the compounds responsible for antioxidative activity of *S. officinalis* ethanolic extract are mainly phenolic acids and flavonoids, namely rosmarinic acid, caffeic acid, carnosol, and carnosic acid [90, 91]. The antioxidant activity and suppression of metabolic activation

could be mechanisms through which sage or some of its components act as desmutagens [92]. The restoration of oxidative stress by improving the antioxidant defense system might be ascribed to the free radical scavenging/antioxidant properties of the phytochemical constituents present in *S. officinalis* [93].

The treatment of chlorpyrifos or methomyl-administered rats with *R. graveolens* ethanolic extract produced a significant decrease in the elevated heart lipid peroxidation. The reduction seen in the glutathione content and GST, GPx and SOD in the testes and heart of chlorpyrifos- or methomyl-administered rats was significantly improved as a result of *R. graveolens* ethanolic extract treatment.

Considering treatment, the flavonoids, a group of polyphenolic compounds found mainly in plants of the Rutaceae family, have been shown to exhibit a series of biological effects among which stand out the inhibition of lipid peroxidation due to their antioxidant properties and their ability of removing free radicals and chelating divalent cations [94, 95]. Quercetin and rutin, a flavone glycoside and its aglycone, are the flavonoids most widely and abundantly present in herbs and plant foods. Quercetin has been reported to exert numerous pharmacological activities, such as free radical scavenging [96], TNF-α inhibition [97], and anti-carcinogenic effects [98, 99]. Rutin has been reported to scavenge free radicals, to lower hepatic blood cholesterol levels, and showed antiplatelet activity [100, 101]. Based on these findings, it is worth to mention that *R. graveolens* has free radicals scavenging activity and this activity may be due to its phenolic and flavonoid compounds, which significantly increased the antioxidant status and decreased lipid peroxidation.

In conclusion, chlorpyrifos and methomyl toxicities increase the formation of oxygen free radicals, reduce antioxidants and increase lipid peroxidation which leads to organ damage. *S. officinalis and R. graveolens* ethanolic extracts have potential chemopreventive effects against chlorpyrifos- and methomyl-induced testicular and cardiac toxicities. These ameliorative chemopreventive effects may be mediated *via* improving the antioxidant defense system and suppressing the oxidative stress.

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