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Gene Expression, DNA and Kidney Damage Induced by Pirimiphos-methyl in Male Mice: Chemical Composition and Ameliorative Role of *Origanum majorana* Leaves Extract

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Abstract

The mechanism of renal toxicity after exposure to pesticides is not completed known until now. Some scientists suggested that the accumulation of pesticide residues in kidney tissue is one theory for explaining how pesticides alter kidney function and cause cell injury. This study is, therefore, the first one to explain the mechanism of pirimiphos-methyl (PM) induce kidney dysfunction and damage in mice. The ameliorative effect of *Origanum majorana* leaves extract against renal dysfunction and damage. The effect of pirimiphos-methyl (PM) on gene expression, DNA damage, oxidant/antioxidant status and renal toxicity of male mice were studied. Chemical fingerprint by HPLC and GC/MS of *O. majorana* leave extract and their ameliorative role was investigated. Mice received PM orally at dose 12.0 mg/kg b. wt. (1/10 LD_{50}) for four weeks with or without plant extracts at dose 150 and 300 mg/kg. b. wt. Phenolic and flavonoid compounds e.g., gallic acid, protocatechuic and cateachin while beta-terpineol (25.71%), gamma-terpineol (15.03%), (-)-spathulenol (10.79%), terpinen-4-ol (10.59%), terpineol (10.08%) and trans-caryophyllene by GC-MS analysis. Pirimiphos methyl increased molecule-1 gene (KIM-1) relative expression and increased percentage of the cell with DNA damage in kidney tissue of mice. It changed antioxidant biomarkers such as glutathione-s-transferees (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (LPO) in the kidney. They enhanced the concentration of uric acid and creatinine in serum and caused histopathological alterations in the kidney tissue. Co-administration of plant extract restored serum kidney function and oxidative stress biomarkers within the normal range especially at a high dose of plant extracts. It can be concluded that the mechanism of renal damage induced by PM could be due to the effect on molecule-1 gene (KIM-1) expression and damage DNA in the renal cells. The mechanism of change in kidney function and injure could be due to the imbalance between oxidant/antioxidant status because of producing reactive oxygen species. Administration of *O. majorana* extract ameliorates KIM-1 gene expiration, reduced DNA damage, and improved kidney function and protect renal cells via their scavenger ability to free radicals. These findings indicated that leave extract of *O. majorana* could be used as a tea to protect agricultural workers and pesticide sprayers against oxidative damage and kidney injury induced by pesticides.

Keywords: Gene expression, DNA, Comet assay, pirimiphos-methyl, oxidative stress, HPLC, GC-MS, *Origanum majorana*, kidney, mice.

1. Introduction

Due to the beneficial role, pesticides are extensively used worldwide for control pests in both agriculture and public health sectors. The illogical and unsafe use of pesticides led to the accumulation of safe use of pesticides has led to the accumulation of pesticide residues in food, water, air, and environment [1]. Pesticides can induce acute and chronic toxicity, which leads to human health illness and deaths. For example, the World Health Organization [2] reported that exposure to pesticides caused poisoning to 3 million cases that lead to 2,000 deaths globally. Chronic exposure to pesticides at

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low doses is of particular interest by researchers because of its association with changes at the cell level without signs of toxicity. Several studies have shown the correlation between exposure to pesticides and change in oxidant/antioxidant status in experimental animals and humans. Pesticides can induce oxidative stress and lipid peroxidation (LPO) because of increases in the production of reactive oxygen species (ROS) which cause damage in cell micro-molecule, tissue dysfunction and damage [3-7].

Pirimiphos-methyl is one of the important organophosphorus insecticide (OPIs) which has a broad-spectrum use for control insect pests and victor bore diseases in agriculture and public health. PM has contact and fumigant action and effect on the center nervous system by inhibiting acetylcholinesterase [8]. Other studies also, reported organ toxicity of PM at different doses with different exposure time on experimental animals [3,6,7].

Currently, the role of exposure to pesticides in kidney dysfunction and disease, both in experimental animals and human was reported. Nevertheless, the mechanism of how pesticides effect on kidney function and injure is not completed known until now. An important theory to explain this mechanism is the accumulation of pesticide residues in kidney tissue and generate ROS that leads to oxidative stress and damage [8-11]. Change in oxidative stress biomarkers reflected to imbalance between oxidant/antioxidant status in kidney and enzymatic and non-enzymatic antioxidant in cell work together to convert ROS such as hydroxyl radical to hydrogen peroxide and then to water [5,6,12,13].

The use of antioxidants especially natural is important for scavenging free radicals such as ROS and protect kidney cells from damage. In traditional medicine, family Lamiaceae is one of the important family of medicinal plants and Marjoram, *Origanum majorana* L, is a member is member of this family that has therapeutic properties. This plant contains many antioxidant compounds such as 4-terpineol, γ-terpinene and trans-sabinene and has high antioxidant activity [14].

For our knowledge, the mechanism of renal toxicity after exposure to pesticides is not completed known until now. Some scientist suggested that the accumulation of pesticide residues in kidney tissue is one theory for explaining how pesticides alter kidney function and cause cell injury [6,7,15]. Several researchers reported that the molecule-1 gene (KIM-1) is a specific and useful biomarker and a good indicator for renal injury resulting from chemical toxicity [16,17].

There is no available information regarding the mechanism of renal toxicity and damage induced by pirimiphos-methyl and the protective effect of O. majorana leave extracts in male mice. This study is, therefore, the first to explain the mechanism of pirimiphos-methyl induce kidney dysfunction and damage in mice. Also, evaluate the ameliorative effect of *O. majorana* extract against renal dysfunction and damage.

2. Materials and methods

2.1. Plant material and extraction Organization of the manuscript

Origanum majorana leaves were collected from the local market of Cairo, Egypt and identified according to the taxonomic characters of Tackholm [18]. After dried, the leaves were powdered and soaked in aqueous methanol (80%) and re-extracted three times. Then the supernatants were collected and concentrated in a rotary evaporator and the dry extract stored under the refrigerator.

2.2. Insecticide

The insecticide used in this study pirimiphos-methyl (95%), (0, 2-diethylamino-6-methylpirimidin-4-yl O, O-dimethyl phosphorothioate) was obtained from Taegeuk Corporation, China.

2.3. Chemicals and kits

All kits used for biochemical studies such as catalase (EC 1.11.1.6), superoxide dismutase (EC 1.15.1.1), glutathione peroxidase (EC 1.11.1.9), glutathione reduced, lipid peroxidation, total protein, uric acid and creatinine were purchased from Biodiagnostic Company, Dokki, Giza, Egypt. All other chemicals were of reagent grades and obtained from Sigma-Aldrich and local scientific distributors in Egypt.

2.4. Chemical fingerprint

2.4.1. HPLC analysis

HPLC (Agilent Technologies 1100 series liquid chromatograph) equipped with an auto sampler and a diode-array detector and Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA) were used for chemical analysis. Both acetonitrile and 2% acetic acid in water (v/v) were used as solvent A and B,

respectively. Flow rate was kept at 0.8 ml/min for 70 min as total run time then the gradient programme as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µl and peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively. All samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards.

2.4.2. Gas chromatography–mass spectrometry analysis (GC-MS)

The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a splitless mode, injection volume of 1 µl and the following temperature program: 40 °C for 1 min; rising at 4 °C /min to 150 °C and held for 6 min; rising at 4 °C/min to 210 °C and held for 1 min. The injector and detector were held at 280 °C and 220 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 40-550 and solvent delay 5 min. The identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

2.5. Experimental animals

Experimental animal (Swiss albino male mice, weighing 29 ± 2 g) were obtained from Animal Breeding House (ABH), of the National Research Centre (NRC), Dokki, Cairo, Egypt. Mice were fed on a standard pellet diet and tap water *ad libitum* with 12 h light/dark cycle, 20±2 °C, 46% a minimum relative humidity. All experimental procedures and protocols involving animals were done according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals and conducted within the provisions of the Animals (Scientific Procedures) Act 1986 (ASPA, Ref 70/8710). The Local Ethics Committee at the National Research Centre (NRC) approved the ABH.

2.6. Groups and treatments

Mice were acclimated for one week and divided into six groups (six mice each). Control group (I) received corn oil (0.1 ml/kg b.wt.). Plant extract groups (II and III) received aqueous extract of leaves of *O. majorana* at doses of 150 and 300 mg/kg b.wt, respectively. PM group (IV) administered PM in corn oil at a dose of 12.0 mg/kg b.wt, 1/10 LD_{50}. Plant extract co-administered to PM group (V and VI) which received the same dose of PM (12.0 mg/kg b.wt) and the extracts at doses of 150 and 300 mg/kg b.wt for V and VI groups, respectively. The extracts were given thirty minutes before the administration of PM. Dosages of PM and *O. majorana* leaves extract were freshly prepared, adjusted weekly for body weight changes and given via oral route for 28 consecutive days. The groups and experimental conditions of this study were selected based on a previous study [6] that reported high antioxidant and hepatoprotective effects of *O. majorana*.

2.7. Blood collection

Mice were fasting overnights and blood samples were withdrawn by puncturing the retero-orbital venous plexus with a fine sterilized glass capillary. The blood was collected in heparinized tubes. from the animals. The samples were centrifuged at 3000 rpm (600 g) for 10 minutes using Heraeus Labofuge 400R, Kendro Laboratory Products GmbH, Germany, to separate sera. The serum was kept in a deep freezer at -20°C until used.

2.8. Renal samples

Kidney tissues were collected after mice killing, then dissected, washed, cleaned and divided into two pieces. The first was transferred into 10% neutral formalin solution for histological studies. The second portions was homogenized in 10% (w/v) ice-cold phosphate buffer (0.1M pH 7.4) and centrifugation at 10,000 ×g for 15 minutes at 4°C using Heraeus Labofuge 400R, Kendro Laboratory Products GmbH, Germany. The supernatants were used for oxidative stress determinations such as SOD, CAT, GPx, LPO, GSH and total protein.

2.9. Renal function biomarkers

Serum uric acid and creatinine as specific kidney biomarkers were determined according to methods of Barham and Trinder [19] done according to the details given in the kit's instructions. All spectrophotometric measurements were performed by using a Shimadzu UV–VIS Recording 2401 PC (Japan).

2.10. Oxidative damage biomarkers in kidney tissue

2.10.1. Malondialdehyde (MDA) content

Malondialdehyde (MDA) as biomarkers of lipid peroxidation (LPO) was determined by a colorimetric method of Satoh [20] according to the details given in the kit's instructions. A thiobarbituric acid reactive substance (TBARS) was used for the estimation of LPO and expressed in terms of malondialdehyde (MDA) content. The MDA values were expressed as nmoles of nmol/g protein.

2.10.2. Renal antioxidant enzymes

Oxidant/ antioxidant statues in kidney tissue were determined by using antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reduced (GSH). All biomarkers were performed according to the details given in Biodiagnostic kit's instructions and the principals below of different methods are given for each concerned biochemical parameter. Total protein also, was determined in kidney homogenate [21].

2.11. Expression of kidney injury molecule-1 gene

2.11.1. Isolation of total RNA

Total RNA was extracted by TRIzol® reagent (Invitrogen, Germany) from kidney tissues and isolation according to the standard procedure. The pellet of RNA was stored in DEPC treated water and digest the DNA residues of isolated RNA by treated with RNAse-free DNAse kit (Invitrogen, Germany) according to kits instruction. RNA aliquots were stored at -20°C or utilized immediately for reverse transcription [22].

2.11.2. Reverse transcription (RT) reaction

Poly(A)+ RNA isolated from kidney was reverse transcribed into cDNA using RevertAidTM First Strand cDNA Synthesis Kit (MBI Fermentas, Germany) via reverse transcription reaction (RT). Reverse transcription (RT) reaction was carried out at 25°C for 10 min, followed by 1 h at 42 °C, and the reaction was stopped by heating for 5 min at 99 °C. Afterwards the reaction tubes containing RT

histopathological changes (x160) under light microscope [26]. All experiments and biochemical analysis were performed at the end of the 2019.

Statistical analysis

Results of biochemical and oxidative stress biomarkers were presented as means ± S.E. Results preparations were flash-cooled in an ice chamber until being used for DNA amplification through Real Time polymerase chain reaction (RT-PCR) [23].

2.11.3. Real Time-Polymerase Chain Reaction (RT-PCR)

Poly(A)+ RNA isolated from kidney was reverse transcribed into cDNA using RevertAidTM First Strand cDNA Synthesis Kit (MBI Fermentas, Germany) via reverse transcription reaction (RT). Reverse transcription (RT) reaction was carried out at 25°C for 10 min, followed by 1 h at 42 °C, and the reaction was stopped by heating for 5 min at 99 °C. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for DNA amplification through Real Time polymerase chain reaction (RT-PCR) [23]. The primers sequence used for RT-qPCR was showed in Table (1).

2.12. DNA damage using comet assay

2.12.1. Comet assay

Collected kidney samples of control and animal treated groups of male rats were subjected to the modified single-cell gel electrophoresis or comet assay by using standard protocol as cited by Augustyniak *et al.* [24]. For each animal about 100 cells were examined to determine the percentage of cells with DNA damage that appear like comets. The non-overlapping cells were randomly selected and were visually assigned a score on an arbitrary scale of 0–3 (i.e., class 0 = no detectable DNA damage and no tail; class 1 = tail with a length less than the diameter of the nucleus; class 2 = tail with length between 1× and 2× the nuclear diameter; and class 3 = tail longer than 2× the diameter of the nucleus) based on perceived comet tail length migration and relative proportion of DNA in the nucleus [25].

2.13. Histological Study

Kidney samples of all mice were excised, washed and fixed in neutral formalin (10%), dehydrated and imbedded in paraffin wax. Sections of kidney tissues were taken (5 µm) and stained with haematoxylin and eosin (H&E). The sections were examined for were analysis by Statistical Package for Social Sciences (SPSS 17.0 for windows) using one way analysis of variance (ANOVA) followed by Duncan's test for comparison between different treatment groups. Statistical significance was set at $p < 0.05$.

3. Results and discussion

HPLC analysis of *O. marjoram* ethanolic extract revealed the presence of several peaks, which indicated the presence of present of 18 phenolic, and flavonoid components (Table 2 and Fig.1). HPLC analysis showed the major phenolic and flavonoid compounds are gallic acid, protocatechuic, cateachin, chlorogenic, caffeic, vanillic, ferulic, sinapic, rutin, apigenin-7-glucoside, cinnamic, quercetin, and the miner compounds were p-hydroxybenzoic, syringic, rosmarinic and kaempferol. Chemical analysis of methanolic extract *O. marjoram* showed the major active compound were phenolic acids such as caffeic acid, chlorogenic acid, rosmarinic acid and flavonoids such as apigenin, luteolin, quercetin and rutin [27]. Other studies reported that marjoram methanolic extracts content phenolic derivatives such as phenolic acids and flavonoids such as luteolin, quercetin and their glycosides as rutin or isovitexin [28,29]. Result of HPLC analysis of *O. marjoram* extract showed that the phenolic and flavonoid profiles were similar to the results of previously studies [27-31]. GC–MS is one of the best methods for the determination of the volatile constituents [32,33]. As shown in Table 3 and Figure 2, GC-MS analysis of *O. marjoram* revealed the presence of several peaks that indicated the presence of 12 volatile compounds. These compounds are beta-terpineol (25.71%), gamma-terpineol (15.03%), (-)-spathulenol (10.79%), terpinen-4-ol (10.59%), terpineol (10.08%), trans-caryophyllene (7.33%), 2-pentadecanone, 6,10,14-trimethyl- (6.26%), sabinene (3.09%), pinocarvone (2.81%), 4-terpinenyl acetate (1.03%) and. pentane, 2,4-dimethyl- (0.33%).

Extensive uses of OPIs for control insects caused many adverse health effects on animals, human and their ecosystem. Pirimiphos-methyl (PM) is widely used in both agriculture and public health sectors to control insects on fruits, vegetables, stored grain in agriculture and control vector borne diseases in public health. Pirimiphos-methyl is a work on the nerves system and inhibits acetylcholinesterase similar to other OPIs [8]. In the present study, PM caused reduces in body weight gains and kidney weights in mice (Untabulated data). The decrease in weights in PM-treated animals could be due to the neurotoxic effect of PM as an OPI [26]. Co-administration of plant extract (*O. majorana*) at high dose (300 mg/kg b.wt.) improved the body and kidney weights of PM-treated animals to normal values of untreated animals. The ameliorative effect of *O. majorana* extract could be due to the high content of phenolic and flavonoids compounds that show high antioxidant activity. Other studies reported that PM induced loss in body weight and organ weights in experimental animals [3-7].

Kidneys are an important organ and playing an essential role in eliminating and excretion the waste products, drugs, and toxins into the urine. The nephron as an important functional unit in kidney works for excretion the body wastes and injurious chemicals e.g. pesticides. In the present work, PM reduced the relative kidney weights and increase uric acid and creatinine levels in treated animals. The increased in uric acid and creatinine account 9.43±0.62 mg/dl and 2.08±0.03 mg/dl compared to 6.12±0.24 mg/dl and 0.54±0.01 mg/dl of control (Table 4). Co-administration of plant extract of O. majorana at high and low doses (150 & 300 mg/kg b.wt) ameliorate the toxic effect and decrease kidney functions in PM-treated mice in dose dependent manner. The high dose of *O. majorana* extract (300 mg/kg b.wt) restored uric acid and creatinine to normal levels and around to 6.58±0.25 mg/dl and 0.72±0.04 mg/dl, respectively.

Results in the current study demonstrated a significant decrease in antioxidant enzymes in kidney tissues. Compared to control the changes in oxidative stress biomarkers of PM treated mice account 4.02 vs. 5.22 U/mg protein, 9.32 vs. 14.10 µmol/ mg protein, 2.87 vs. 7.53 U/mg protein and 0.042 vs. 0.091 µmol/ mg protein of SOD, CAT, GPx and GSH, respectively. While MP induced a significant increase in LPO (117.80 vs. 68.32 nmol/g protein) level in kidney tissue compared to the control group (Table 5). Administration of *O. majorana* extract to PM-treated mice improved kidney function, increase antioxidant enzymes and decrease LPO in a dose dependent mater. The activity of antioxidant biomarkers and lipid peroxidation were restored to normal values in PM treated mice administered plant extract at dose 300 mg/kg. b.wt. PM causes histopathological alteration in kidney tissue (Figure 3).

Table 1. Primers sequence used for RT-qPCR.

Gene	Forward	NCBI Reference
KIM1	F: GTGGTTGTCACCAGGTACATCAT R: GTTGTCTTCAGCTCGGGGAT	NM_173149.2
GAPDH	F: GGATGCAGGGATGATGTTCT R: GAAGGGCTCATTGACCACAGTT	NM_017008.3

GAPDH; glyceraldehyde-3-phosphate dehydrogenase, KIM1; kidney injury molecule-1

Table 2. Phytochemical analysis by HPLC of *O. majorana* leaves extract.

No	compound	sam conc (ug/g)	%
1	Gallic acid	11.770	0.54
2	Protocatechuic	32.699	1.50
3	*p*-hydroxybenzoic	1.988	0.09
4	Gentisic	0.000	0.00
5	Cateachin	10.251	0.47
6	Chlorogenic acid	13.684	0.63
7	Caffeic acid	15.201	0.70
8	Syringic	1.059	0.05
9	Vanillic	10.279	0.47
10	Ferulic	10.352	0.48
11	Sinapic	48.465	2.23
12	*p*-coumaric	0.000	0.00
13	Rutin	286.394	13.18
14	Apigenin-7-glucoside	1691.975	77.85
15	Rosmarinic	0.000	0.00
16	Cinnamic	12.259	0.56
17	quercetin	23.866	1.10
18	Kaempferol	3.224	0.15
19	Chrysin	0.000	

Retention time (RT)

Table 3. GC/MS analysis of *O. majorana* leaves extract

Peak	RT	Name	Formula	Area Sum %
1	13.556	Sabinene	$C_{10}H_{16}$	3.09
2	16.547	Pentane, 2,4-dimethyl-	C_7H_{16}	0.33
3	17.316	Cis-Sabinene hydrate	$C_{10}H_{18}O$	3.75
4	18.499	beta-Terpineol	$C_{10}H_{18}O$	25.71
5	21.402	Terpinen-4-ol	$C_{10}H_{18}O$	10.59
6	22.002	Terpineol	$C_{10}H_{18}O$	10.08
7	23.704	gamma-Terpineol	$C_{10}H_{18}O$	15.03
8	25.365	4-Terpinenyl acetate	$C_{12}H_{20}O_2$	1.03
9	29.457	trans-Caryophyllene	$C_{15}H_{24}$	7.33
10	36.772	(-)-Spathulenol	$C_{15}H_{24}O$	10.79
11	46.151	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	6.26
12	46.559	--	---	3.22
13	46.961	Pinocarvone	$C_{10}H_{14}O$	2.81

Retention time (RT).

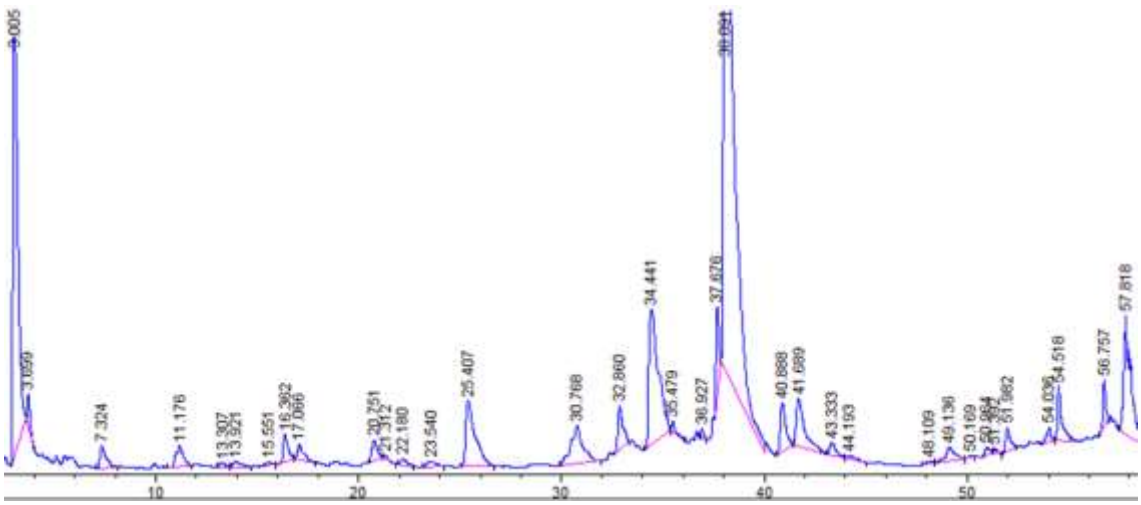
In contrast, our result reported the potential protective effect of *O. majorana* extract and reduce the renal histopathological alteration induced by PM in male mice.

The mechanism of renal toxicity induced by pesticides is not completed known until now. One theory for explains how pesticides effect on kidney function and cell injure is accumulate of pesticide residues and their metabolites in tissue especial fatty tissue and generate free radicals especially reactive oxygen species (ROS). ROC can induced cell injure throw the effect on protein, lipid and genetic materials [6,7,15]. Several researchers reported that the molecule-1 gene (KIM-1) is a specific and useful biomarker and a good indicator for renal injury resulting from chemical toxicity [16,17]. It has been

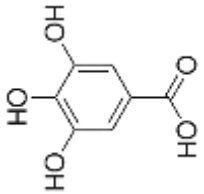
reported that KIM-1 is the first gen identified as an accepted cell adhesion molecule that can recognize and induce phagocytosis of dead cells in the tubular lumen of the kidney [34]. KIM-1 has been suggested to be an ideal biomarker in many chemical and pathological nephrotoxicity models due to its robust and marked expression in the injured state. Moreover, there is a total lack of its expression in healthy kidneys [35]. Results in the present study showed that pirimiphos-methyl increased KIM-1 relative expression in kidney tissue of mice (Figure 4). Co administration of *O. majorana* to PM- treated mice improved kidney function by decrease KIM-1 gen expiration in a dose-dependent mater. For our knowledge, there are no available data about the effect of pirimiphos methyl on KIM-1 gen in the kidney of mice.

Previous studies have used cytogenetic analyses to assess the possible genotoxicity of pesticides [36]. Currently, the alkaline single cell gel electrophoresis (SCGE) assay, also known as the Comet assay, has increasingly been used for studying the DNA damage both in animals and in human. Comet assay is a good, fast, rapid and sensitive tool to determine the damaging of pesticides and other toxic chemicals on DNA on individual cells [37]. Cells with damaged DNA display increased migration of DNA fragments from the nucleus, generating a comet shape [25,38]. In the present study, pirimiphos-methyl increase percentage of the cell with DNA damage to 24.25% compared to the control group that accounts 10.5% (Table 6 and Figure 5). Administration of *O. majorana* extract to PM-treated mice decrease cells with DNA damage in a dose-dependent mater. The results suggest that pirimiphos-methyl could act as clastogens on the DNA of kidney cells of mice. The detected damage in DNA by comet assay in the present study could be due to the cytotoxic and or genotoxic effect of pirimiphos-methyl.

The correlation between renal disease and exposure to pesticides were reported both in human and experimental animals [9-11, 39]. In these studies, patients that have kidney disease had a high concentration of pesticides in their blood compared to healthy peoples. In addition, people's participation in the application of pesticides or pesticides sprayer has abnormal kidney function. However, the mechanism of how pesticides effect on kidney function and injure is not completed known until now. Other researchers suggested that pesticides could be induced oxidative stress in kidney tissue that leads to kidney dysfunction and injury [5-7].



HPLC



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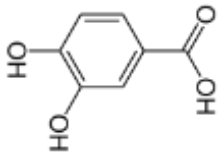

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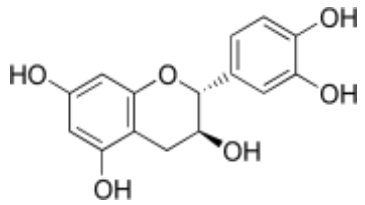

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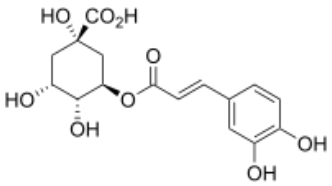

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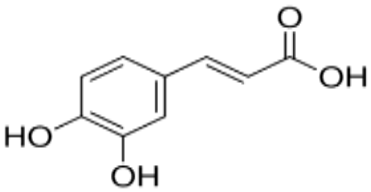

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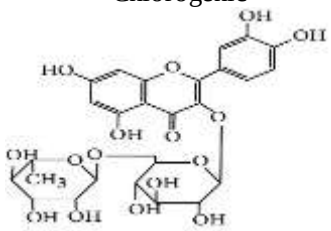

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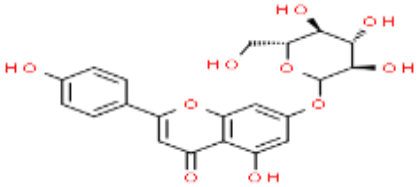

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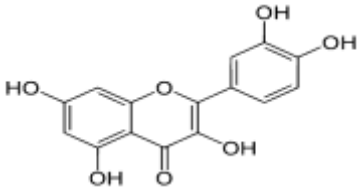
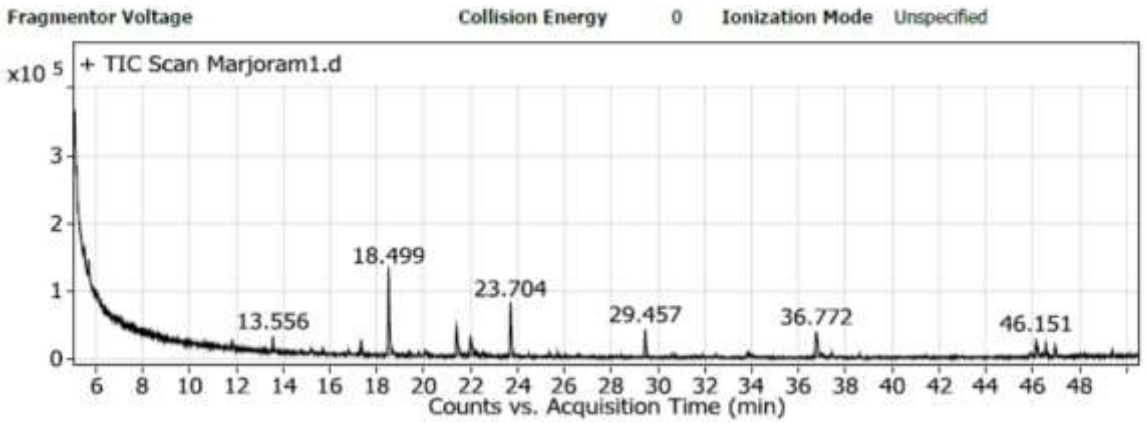


quercetin

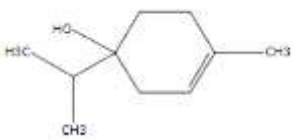
Figure 1. HPLC chromatogram of *O. majorana* leaves extract and chemical structure of major compounds.



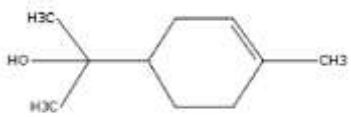
GC/MS

Sabinene

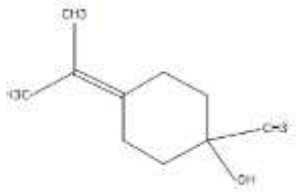
beta-Terpineol



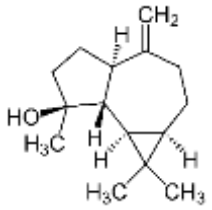
Terpinen-4-ol



Terpineol



gamma-Terpineol



(-)-Spathulenol

Figure 2. GC-MS spectrum and chemical structure of major volatile compounds of *O. majorana* leaves extract.

In the present study, a decrease in the activity of antioxidant enzymes (SOD, CAT, GPx) and GSH level along with an increase in LPO level could be due to the overproduction of reactive oxygen species (ROS) in the kidney of PM-treated mice. These changes in oxidative stress biomarkers reflected an imbalance between oxidant/antioxidant statuses in the kidney. As we have known GSH have antioxidant activity while SOD, CAT and GPx work together to convert hydroxyl radical to hydrogen peroxide and then to water. Therefore, these oxidative stress biomarkers work to limit the damaging effect via scavenging free radical (ROS) and balance between the production and elimination of ROS in tissue [5,6,13]. However, there are many by-products resulting from lipid peroxidation especially in samples with high protein (such as animal tissues) also, protein-MDA complexes or oxidized lipids therefore, using the trichloroacetic acid (TCA) is very important to resolve this issue.

Previous studies supported these findings regarding the induction of ROS and alteration enzymatic and non-enzymatic biomarkers by pesticides in experimental animals [7, 12]. However, the protective effect of *O. majorana* could be due to the free radical scavenger properties of this extract because of the high content of phenolic compounds with high antioxidant activity [6]. In the present work, the protective effect could be due to the present of phenolic and flavonoid compounds such as gallic acid, protocatechuic, cateachin, chlorogenic, caffeic, vanillic, ferulic, sinapic, rutin, apigenin-7-glucoside, cinnamic, quercetin as major compounds and the miner such as p-hydroxybenzoic, syringic, rosmarinic and kaempferol as shown in HPLC analysis. Also, the present terpenes, trans-caryophyllene, and sabinene as shown by GC/MS analysis. Other studies reported high activity of *O. marjoram* extract is due to the high contain of phenolic and flavonoid [6, 27-33]. These compounds showed several beneficial health effects. They have high antioxidant, anti-inflammatory, hypoglycemic, neuroprotective and involved in the prevention and treatment of various diseases [40-43].

Table 4. Effect of exposure to pirimiphos-methyl on kidney of male mice and the protective role of *O. majorana* leaves extract.

Treatment	Uric acid (mg/dl)	Creatinine (mg/dl)
I	6.12 ± 0.24^{c}	0.54 ± 0.01^{d}
II	5.99 ± 0.14^{c}	0.53 ± 0.01^{d}
III	6.10 ± 0.11^{c}	0.50 ± 0.01^{d}
IV	9.43 ± 0.62^{a}	2.08 ± 0.03^{a}
V	7.01 ± 0.41^{b}	1.24 ± 0.04^{b}
VI	6.58 ± 0.25^{c}	0.72 ± 0.04^{c}

Each value is a mean of 6 mice ± S.E.; $^{a, b, c}$ values are not sharing superscripts letters (a, b, c) differ significantly at $p < 0.05$; I, control group; II and III, extract groups (150 and 300 mg/kg b.wt, respectively); IV, pirimiphos methyl group; V and VI, extract (150 and 300 mg/kg b.wt) along with pirimiphos methyl groups.

Table 5. Effect of exposure to pirimiphos-methyl on oxidative stress biomarkers in kidney of male mice and the protective role of *O. majorana* leaves extract.

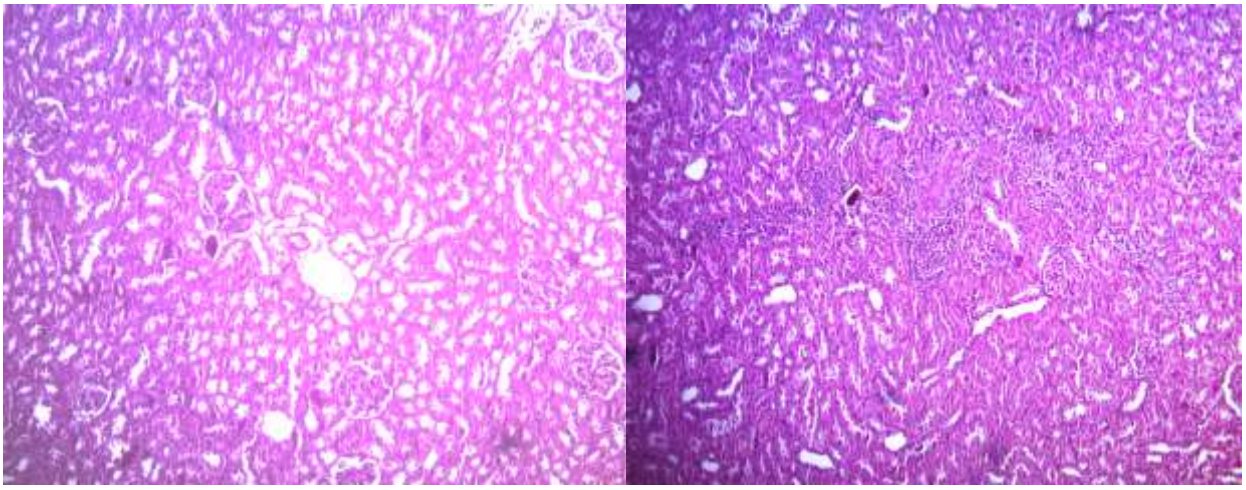
Treatment	Oxidative stress biomarker				
	SOD (U/mg protein)	CAT (µmol/ mg protein)	GPx (U/ mg protein)	GSH (µmol/ mg protein)	LPO (nmol/g protein)
I	5.22±0.108^{b}	14.10±0.11^{b}	7.53±0.31^{d}	0.091±0.00^{2b}	68.32± 1.05^{a}
II	5.14±0.11^{b}	15.05±0.84^{b}	7.23±0.54^{cd}	0.072±0.001^{b}	66.96± 148.^{a}
III	5.34±0.09^{b}	14.54±1.32^{b}	7.36±0.51^{d}	0.89±0.005^{b}	68.28± 1.71^{a}
IV	4.02±0.10^{a}	9.32±0.43^{a}	2.87±0.46^{a}	0.042±0.001^{a}	117.80± 1.95 ^{b}
V	4.52±0.12^{a}	12.31±0.18^{b}	4.17±0.21^{b}	0.049±0.001^{a}	74.52± 1.92^{a}
VI	5.20±0.11^{b}	13.15±0.19^{b}	5.04±0.51^{c}	0.074±0.001^{b}	72.25± 2.08^{a}

Each value is a mean of 6 mice ± S.E.; $^{a, b, c}$ values are not sharing superscripts letters (a, b, c) differ significantly at $p < 0.05$; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; GSH: glutathione reduced; LPO: lipid peroxidation. I, control group; II and III, extract groups (150 and 300 mg/kg b.wt, respectively); IV, pirimiphos methyl group; V and VI, extract (150 and 300 mg/kg b.wt) along with pirimiphos methyl groups.

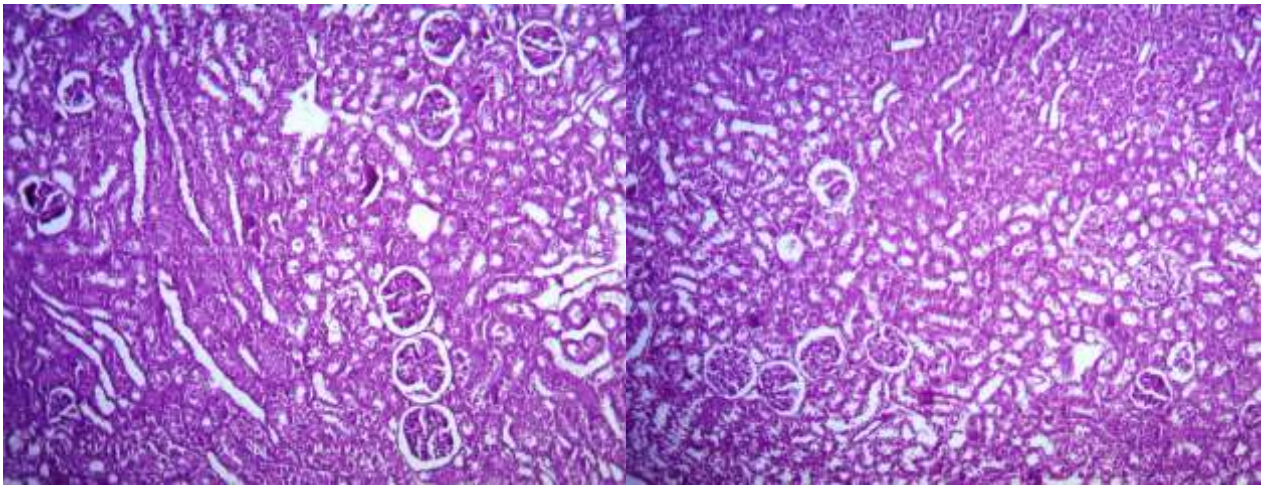
Table 6. Visual score of DNA damage in kidney of mice exposure to pirimiphos-methyl and the protective role of *O. majorana* leaves extract.

Treatment	No. of cells		Class**				DNA damaged cells % (Mean ± SE)
	Analyzed*	Comets	0	1	2	3	
I	400	42	358	32	7	3	10.50±0.33
II	400	39	361	18	14	7	9.75±0.56
III	400	41	359	21	11	9	10.25±0.82
IV	400	97	303	53	21	23	24.25±1.12
V	400	83	317	56	22	5	20.75±0.61
VI	400	58	342	39	15	4	14.50±0.42

*: Number of cells examined per a group, **: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus. Each value is a mean of 4 respects ± S.E.; $^{a, b, c}$ values are not sharing superscripts letters (a, b, c) differ significantly at $p < 0.05$; I, control group; II and III, extract groups (150 and 300 mg/kg b.wt, respectively); IV, pirimiphos methyl group; V and VI, extract (150 and 300 mg/kg b.wt) along with pirimiphos methyl groups.



Control Pirimiphos-methyl group



PM+ extract 150 PM+ extract 300

Figure 3. Photomicrograph of kidney sections stained by haematoxylin and eosin (H&E) for histopathological changes. Group 1 showing (A) the normal histological structure of kidney and no detectable abnormality and group PM treated mice showing focal interstitial mononuclear cellular infiltration (H&E X100). Other groups show normal histopathological changes as shown in control (H&E X100).

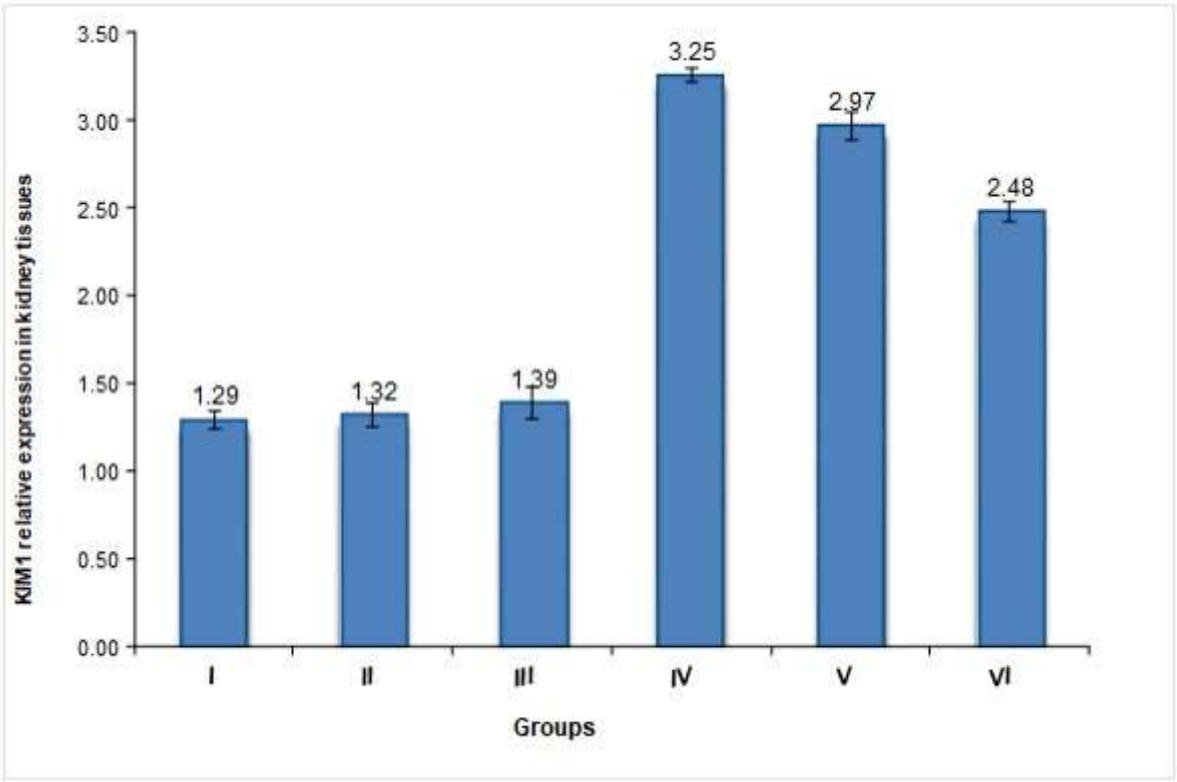


Figure 4. The expression alterations of COMT gene in kidney samples. Data are presented as mean ± SD. Each value is a mean of 6 mice ± S.E.; $^{a, b, c}$ values are not sharing superscripts letters (a, b, c) differ significantly at $p < 0.05$. Control group, I; II and III, extract groups (150 and 300 mg/kg b.wt, respectively); IV, pirimiphos methyl group; V and VI, extract (150 and 300 mg/kg b.wt) along with pirimiphos methyl groups.

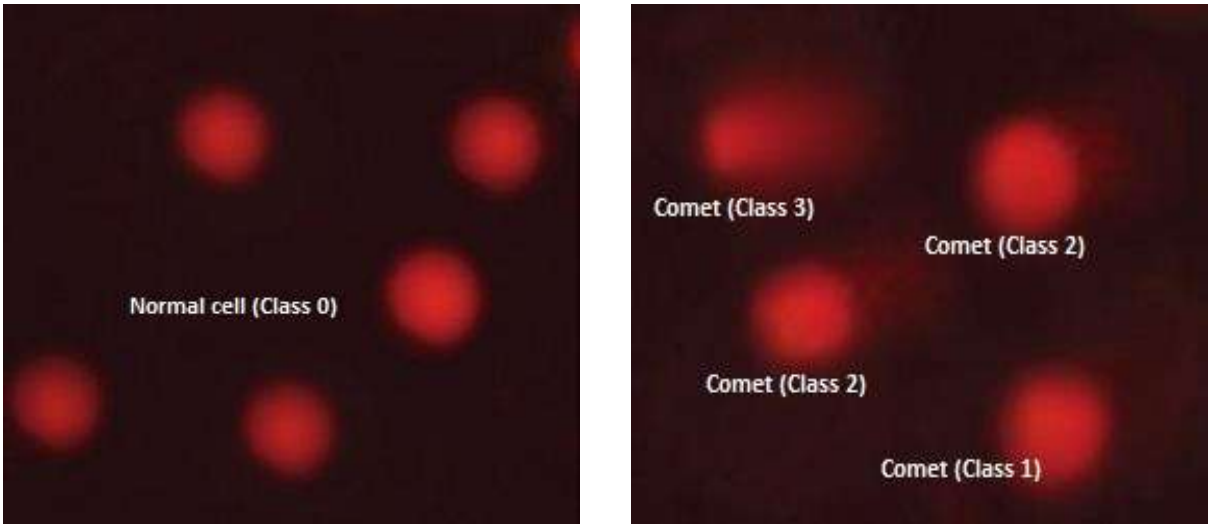


Figure 5. Visual score of normal DNA (class 0) and DNA damage (classes 1, 2 and 3) using comet assay in kidney samples.

4. Conclusion

It can be concluded that the mechanism of renal damage induced by PM could be due to the effect on molecule-1 gene (KIM-1) expression, damage DNA in the renal cells. The mechanism of change in kidney function and injure could be due to the imbalance between oxidant/antioxidant status because of producing reactive oxygen species. Administration of *O. majorana* extract ameliorates KIM-1 gene expiration, reduced DNA damage and improved kidney function and protect renal cells via their scavenger ability to free radicals. These findings indicated that leave extract of *O. majorana* could be used as a tea to protect agricultural workers and pesticide sprayers against oxidative damage and kidney injury induced by pesticides.

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6. Conflict of interests

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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التعبير الجيني وتلف الحامض النووي والكلي الناتج عن مبيد برميفوس ميثيل في ذكور الفئران: المحتوي الكيميائي والدور المحسن لمستخلص أوراق البردقوش

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ميكانيكية التأثير السام للمبيدات على الكلي غير معروف حتى الأن ويقترح بعض العلماء أن تراكم متبقيات المبيدات في أنسجة الكلي أحد أهم النظريات التي تفسر الخلل الوظيفي للكلي وحدوث الضرر ونجد أن الدراسة الحالية هي أول دراسة لتفسير ميكانيكية حدوث خلل في وظائف الكلي عن طريق مبيد البريميفوس- ميثيل في ذكور الفئران. بالإضافة إلى دراسة الدور الوقائي لمستخلص أوراق البردقوش كما تتضمن الدراسة تأثير مبيد البريميفوس- ميثيل على التعبير الجيني Gene expression وتلف المادة الوراثية DNA والتأثير علي مستوي مضادات الأكسدة وسمية الكلي. تم إجراء التحليلات باستخدام HPLC وGC-MS لمعرفة المواد الفعالة الموجودة في مستخلص أوراق البردقوش. تم توزيع الفئران إلي مجاميع مختلفة والمعاملة بالمبيد عن طريق الفم بجرعة 12 mg/kg في عدم وجود أو وجود مستخلص نبات البردقوش بجرعات 150-300 mg/kg لمده ٤ أسابيع. أظهرت نتائج تحليل ال HPLC وGC-MS وجود مركبات فينوليه وفلافينويدات مثل (Gallic acid). أحدث مبيد البريميفوس- ميثيل زيادة في التعبير الجيني وتلف المادة الوراثية داخل نسيج الكلي، وسبب خفض في وزن الجسم والوزن النسبي للكلي وزيادة الضغوط التأكسدية وتكوين الدهون المؤكسدة في نسيج الكلي. كما أحدث تغيرات غير مرغوبة في الصفة التشريحية في نسيج الكلي. أدت المعاملة بمستخلص النبات إلي تعديل التعبير الجيني وتقليل الضرر علي المادة وراثية. وزيادة مضادات الأكسدة داخل جسم الحيوان وتحسين الصفة التشريحية لنسيج الكلي تظهر الدراسة الحالية إن ميكانيكية التأثير السام للبيريموفوس- ميثيل علي الكلي ممكن أن ترجع إلي التأثير علي التعبير الجيني (KIM-1) وتلف المادة الوراثية DNA والتي تحدث بسبب تكوين شوارد حرة وضغوط تأكسدية داخل نسيج الكلي كنتيجة من التأثير السام للمبيد. مقدرة مستخلص نبات البردقوش على تعديل الضغوط التأكسدية بما يجعله مهم في الجانب التطبيقي لاستخدامه كمشروب وقائي لحماية عمال الرش وعمال الزراعة المتعرضين للمبيدات.