

Biochemical and Ultra Structure Studies of the Antioxidant Effect of Aqueous Extract of Hibiscus Sabdariffa on the Nephrotoxicity Induced by Organophosphorous Pesticide (Malathion) on the Adult Albino Rats

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Abstract: Organophosphorous (OP) pesticide is applied to numerous crops, including wheat and corn. Residual amounts of organophosphorous pesticides have been detected in soil, vegetables, grains and other food products. Several mechanisms of the OP toxicity have been proposed, including the induction of cellular proliferation, oxidative stress and immune-toxicity. Roselle (ROS, *Hibiscus sabdariffa* L., family Malvaceae) is an annual shrub commonly used to make jellies, jams and beverages. Many biological activities have been recorded for ROS, such as anti-atherosclerosis, anti-carcinogenic, hepato-protective and anti-oxidative properties. **The Aim:** this study was set to evaluate the possible protective effect of Roselle on nephro-toxicity induced by sub-lethal dose of Malathion in rats. **Material and Methods:** 24 adult male albino rats were used and divided into four groups of 6 rats/each. Group I: animals were given corn oil at a dose of 0.2 ml per animal via gavage once a day for one month and served as a control. Group II: animal received only aqueous extracts of Roselle at a daily dose of (500 mg /kg/day). Group III: animal were given Malathion at a sub lethal dose of 27mg/kg/day. Groups IV: animal were given both of aqueous extracts of Roselle as the same dose of group II three hours before the administration of Malathion. At the end of the experimental period the kidney function and markers of oxidative stress were investigated. Moreover, histopathological examination of the renal tissue was carried by light and electron microscopes. **The results** of the present study showed that treatment with Malathion alone caused increase in the kidney weight (P=0.000), cellular degeneration, necrosis of the renal tissues and increase in the serum urea and creatinine (P=0.000 for both). However, administration of aqueous extract of Roselle prior to Malathion resulted in a significant alleviation of the kidney injuries evidenced by a decrease of the kidney weights when compared to the Malathion-treated (P=0.000) and biochemical indices; urea and creatinine (P=0.000; P>0.05) for both when compared to the Malathion-treated and control groups respectively. Furthermore, there was significant improvement of the histological picture toward the normal among the Malathion+ROS-treated group. All these effects may be due to the antioxidant effect of the Roselle as treatment with the extract of *Roselle* significantly elevated (P=0.000) the decreased CAT activity observed with Malathion treated rats. Moreover, treatment with the extract of *Roselle* significantly elevated the SOD levels when compared to the Malathion-treated animals (P=0.000). Furthermore, the GSH level reduced significantly (P=0.000) along with increased in MDA concentration (P=0.000) in the Malathion treated group as compared to the control group. However on treatment with *Roselle* extract, the GSH level was found to be enhanced significantly (P=0.000) and the MDA contents were reduced (P=0.000) when compared to the Malathion treated group. **Conclusion,** the results of the current study showed that the aqueous extracts from *Hibiscus sabdariffa* possess a potent protective effect from the oxidative stress induced by sub lethal dose of Malathion on the kidney.

[Hala H. Mossalam -Olfat A. Abd-El Aty. Enas N. Morgan - Sahar M. S. Youssaf - Amal M H. Mackawy. **Biochemical and Ultra Structure Studies of the Antioxidant Effect of Aqueous Extract of Hibiscus Sabdariffa on the Nephrotoxicity Induced by Organophosphorous Pesticide (Malathion) on the Adult Albino Rats.** Journal of American Science 2011;7(12):407-421]. (ISSN: 1545-1003). <http://www.americanscience.org>.

Keywords: kidney, Malathion, Roselle, ultra structure, anti oxidant protective effect.

Introduction:

In agricultural settings, Malathion an organophosphorous (OP) pesticide is applied to numerous crops, including wheat and corn. It is also used for home and garden applications, mosquito control, Mediterranean fruit fly eradication and as a topical treatment for head lice (*Gervais et al., 2009*). Malathion is also an ingredient in shampoos regulated by the United States Food and Drug Administration (FDA) to control head lice (*Frankowski, 2004*).

The exposure to Malathion became an ongoing basis as there are residual amounts of organophosphorous pesticides have been detected in soil, vegetables, grains and other food products (*Jarc, 1983*).

Several mechanisms of the OP toxicity have been proposed, including the induction of cellular proliferation (*Cabello et al., 2001*), oxidative stress (*John et al., 2001 and Abdollahi et al., 2004*) and immunotoxicity (*Galloway and Handy, 2003*). OP

pesticides are known to induce toxicity in mammals by inhibiting acetyl cholinesterase which leads to accumulation of acetylcholine and the subsequent activation of cholinergic muscarinic and nicotinic receptors (*Hazarika et al., 2003*). Acetylcholinesterase enzyme (AChE) is found in mammals, amphibians, fish, reptiles, and birds (*Massoulie and Bon, 1982*). In these organisms, the binding of AChE with Malathion allows the accumulation of ACh at the nerve junction. This accumulation of ACh leads to over stimulation of glandular cells, autonomic ganglia, the central nervous system, and both smooth and skeletal muscles (*Reigart and Roberts, 1999*).

Researchers administered Malathion orally reported that more than 90% of the dose was excreted in urine within 24 hours. The remaining Malathion was found in feces, blood, intestines, liver, and kidneys. Also, four hours after oral administration, 75% of the Malathion was still in the stomach, while 8% was in the small intestines, and 7% in the saliva (*Zeid et al., 1993*). Researchers noted suppression of thyroid secretory function in young adult rats that were fed 0.06 mg/rat/day of Malathion for 21 days. They also noticed an increase in thyroid stimulating hormone (TSH), suggesting that the pituitary gland was compensating to restore normal levels of thyroid hormones (*Akhtar et al., 1996*).

Recently, much attention has been focused on the protective effects of anti-oxidants and naturally occurring substances against oxidative stress damage. Roselle is a nature's generosity which providing mankind with cheap and natural bioactive materials. Thus the exploitation of this natural gift is necessary to overcome the unwanted side effects of some essentially used medications. Roselle (ROS, *Hibiscus sabdariffa* L., family Malvaceae) is an annual shrub commonly used to make jellies, jams and beverages. In folk medicine, Ros has commonly been used for its antihypertension properties (*Herrera-Arellano et al., 2004*). The anthocyanin pigments that confer Ros's color make it a valuable food product (*Tsai and Huang, 2004*). Many biological activities, such as anti-atherosclerosis, anticarcinogenic (*Tsai et al., 2002*), hepatoprotective (*Amin and Hamza, 2005*) and anti-oxidative properties (*Prenesti et al., 2007*), have been reported in Ros and its anthocyanin.

Botanically, it is described as thick red fleshy, and cup shaped calyxes plant. The calyxes are rich in phenolic compounds with marked physiological activities (*Rosendiz-Lopez et al., 1998*). Hibiscus flowers contains gossypetin, glucoside, bibiscin, hibiscus anthocyanin and protocatechuic acid, which may have diuretic and choleric effects, decreasing the viscosity of the blood, reducing blood pressure,

and stimulating intestinal peristalsis (*Alli and Salih, 1991*). Hibiscus protocatechuic acid (PCA) was shown to significantly decrease the leakage of lactate dehydrogenase (LDH) and alanine transaminase (ALT) and the formation of malondialdehyde (MDA) induced by tert-butylhydroperoxide (t-BHP) in rat primary hepatocytes (*Tseng et al., 1997a*).

The anti-inflammatory activity of this plant has been traced partially to its phenolic acid composition. Another potent antioxidant and cytoprotective agent characterized in *H. sabdariffa* is betaine, which can also account for its antioxidant property (*Rossi et al., 2003*).

Hibiscus sabdariffa L. has been reported to be antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, refrigerant, sedative, stomachic and tonic (*Morton, 1998*).

Numerous in vitro experiments have evaluated the effects of hibiscus flower or anthocyanin extracts against various cancer cell lines. Proposed mechanisms of action focus on antioxidant activity and the ability to induce apoptosis (*Lin et al., 2007; Lo et al., 2007; Olvera-García et al., 2007*). Studies in rats have evaluated effects against liver, oral, colon, bladder, and stomach cancers (*Ali et al., 2005*).

Now a day the exposure to residual of OP compounds is an imperative process because its wide use in agriculture and this chronic exposure may be the cause of wide spread of nephro-toxicity. Based on these, this study was designated to evaluate the possible protective effect of Roselle on nephro-toxicity induced by sub-lethal dose of Malathion in rats in trial to found save natural, potent and effective protective procedure.

Material and Methods:

Animals:

24 adult male albino rats (weight 185-200 gm) were housed at the animal house in the faculty of medicine for girls Al-Azhar – University at 21 °C–22 °C in a 12 hr/12 hr light/dark cycle, fed standard rat chow, and given free access to water. Rats accommodated to the laboratory conditions for 2 weeks before starting the experiment.

Experimental design:

Rats were divided into four groups of 6 rats/each:

Group I: animals were given corn oil at a dose of 0.2 ml per animal via gavage once a day for one month and served as a control group.

Group II: animal received only aqueous extracts of Roselle at a daily dose of (500mg/kg/day) for one month according to *Amr et al. (2008)*.

Group III: animal were given Malathion at a dose of 27mg/kg/day in corn oil via gavages for one month .This dose equal to 1/50 of the LD50 for an oral dose according to *Gallo and Lawryk, (1991) and Kamrin, (1997)*.

Groups IV: animal were given both of aqueous extracts of Roselle as the same dose of group II (500 mg/kg/day) three hours before the administration of Malathion as the same dose of group III (27mg/kg/day) for one month.

At the end of the experimental period (one month) and after overnight fasting, at 8:00 a.m, blood samples were obtained from sinus orbitus vein of each rat after ether inhalation (*Yang et al., 2006*). The blood samples were allowed to clot at room temperature before centrifuging at approximately 3000 rpm for 15 minutes. The serum was stored at -20 °C until assayed for the biochemical parameters. Then, all studied animals were sacrificed; the two kidneys of each rat were excised and weighed then prepared for estimation of the markers for oxidative stress and histopathological study. One kidney for estimation of oxidative stress markers and light microscope and the other one prepared for transmission electron microscopical study.

Preparation of renal homogenate:

The kidneys were removed and dissected free from the surrounding fat and connective tissue. Each kidney was longitudinally sectioned, and renal cortex was separated and kept at -8°C. Subsequently, renal cortex was homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The renal cortical homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was used for the determination of malondialdehyde (MDA) content, reduced glutathione (GSH) levels and antioxidant enzyme levels such as superoxide dismutase (SOD), and catalase (CAT), using colorimetric assay.

Histopathological preparation for light microscopic examination:

3 Pieces of kidneys tissues from each group were fixed in Bouin's solution for 48 h. Later, they were dehydrated in graded levels of ethanol, cleared in xylene, and embedded in paraffin wax for sectioning. The 4-µm thick sections were cut, mounted on glass slides, and stained with hematoxylin and eosin stain (*Bancroft and Steven, 1996*).

Preparation for Transmission Electron Microscopy (TEM):

3 renal tissues samples were immediately placed in 5% gluteraldehyde buffered at pH 7.4with Milloning phosphate for 4 hours and subsequently

fixed in 1% osmium tetroxide for two hours .The samples were dehydrated in graded ethanol and embedded in araldite. Thin sections were stained with lead citrate and uranyl acetate, and examined with a JEOL 1010 Transmission Electron Microscope at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University Cairo.

Biochemical analysis

1-Urea and creatinine assessment in serum:

By using a commercially available spectrophotometric enzymatic kit (Thermo Trace BECGMAN, Germany) according to *Young, (2000)*.

2-Biochemical estimation of markers of oxidative stress:

MDA content was measured according to the earlier method reported (*Zhang, 1992*). Reduced Glutathione was assayed according to the previous reports (*Carlberg and Mannervik, 1975; Mohandas et al., 1984*) and *Jollow et al. (1974)*: A 1.0mL supernatant of renal homogenate was mixed with 1.0mL of sulfosalicylic acid (4%). The samples were incubated at 4 °C for at least 1 h and then centrifuged at 1200x g for 15 min at 4 °C. The reaction mixture contained 0.4mL of the filtered sample, 2.2mL phosphate buffer (0.1M, pH 7.4), and 0.4mL DTNB (4 mg=mL) in a total volume of 3.0mL. The yellow color developed was read immediately at 412 nm on a spectrophotometer.

SOD activity was determined according to the previous report (*Rai et al., 2006*) CAT activity was determined from the rate of decomposition of H₂O₂ by the reported method (*Bergmeyer et al., 1974*). Protein content in the tissue was determined by the method reported earlier (*Lowry et al., 1951*) using bovine serum albumin (BSA) as the standard.

Statistical Analysis:

The data were analyzed by using SPSS 11.0 for Windows. The significance of differences was calculated by using one-way analysis of variance (ANOVA) followed by LSD procedure for multiple comparisons. P<0.05 was considered statistically significant.

Aqueous extract preparation: A corrodng to *Abdul (1990) and Bako et al., (2010)*. 100 g of Hibiscus sabdariffa leaves and seeds were washed thoroughly, sun dried and ground into powder. Then 2500 ml of distilled water was added. The mixtures were then shaken for ten hours with mechanical shaker and left over night. The supernatant liquid (extract) was filtered through a Whatman's No.1 filter paper. The process was repeated for complete extraction. The extract was then poured into evaporating dish to

evaporate the solvent in the extract over the water bath at the temperature of 40 °C - 45 °C. A solid extract weighing 8.4 gm was obtained (Okasha *et al.*, 2008). The extract stored in the refrigerator at 4 °C for further use.

Results

1- Light microscopic findings:

Light microscopic examination of the kidney of the control albino rat and Roselle treated rat showed that the normal renal corpuscle consisted of glomerulus and Bowman's capsule. The glomerulus was a globular network of densely packed anastomosing capillaries. The numerous nuclei in the glomerulus were those of the capillary endothelial cells, mesangial cells and podocytes. The Bowman's capsule was formed of the parietal layer characterized by its flat nuclei of the squamous cells lining it, while the visceral layer was formed of podocytes. The Bowman's space was the space between the parietal layer and the glomerular tuft (Fig.1&2).

The proximal convoluted tubules (PCT) appeared rounded, and were lined by a single layer of short columnar cells with indistinct cell boundaries, and spherical nuclei. The free ends of these cells had well-developed brush borders that almost fill most of the lumen (Figs.1&2).

The distal convoluted tubules (DCT) were lined with simple cuboidal epithelial cells that possess distinct cell boundaries and a granular cytoplasm, and spherical centrally located nuclei. The lumen of distal convoluted tubule was wider with more defined lumen (Figs. 1&2).

In one-month Malathion-dosed rats, revealed distinct pathological lesions. Some glomeruli were manifested by their hypertrophy probably due to their congestion and the mild proliferation of their constituent cells (Fig. 3). In addition other glomeruli had lost their normal circular shape and converted into shrunken, abnormally cellular and relatively vascular structures having few red blood cells and leaving rather wide urinary space (Fig. 4).

Intertubular extensive hemorrhagic areas were obviously noticed among the tubules (Figs.3&4). Also areas of cavitations with tissue loss were easily noticed (Fig. 5). Moreover the lining cells of many tubules had ill-defined boundaries and contained lightly stained large vesicular nuclei or dark pyknotic nuclei. (Figs. 3 & 4). Also, the cytoplasm of the lining cells were completely degenerated and replaced by vacuolar spaces and acidophilic masses (Figs. 3, 4 & 5).

As regard the proximal convoluted tubules, they were greatly affected if compared to distal convoluted tubules. The outer borders of the cells which lining

the convoluted tubules were deteriorated, their lumina contained faintly stained casts and the brush borders of some proximal convoluted tubules were destructed (Figs.4&5). Furthermore some tubules were replaced by homogenous acidophilic material or vacuoles and the lumina of these tubules became relatively small (Fig. 4).

Examination of longitudinal sections of the kidneys of group VI, the Malathion plus Roselle treated rat, showed that the glomerulus, the proximal and distal convoluted tubules were nearly similar to the control group except the persistent presence of an area of cellular infiltration (Fig.6).

2- Electron microscopic findings

Examination of ultrathin sections of the kidney of the control group revealed the usual component of the glomeruli where part of capillary loop was recognized by its fenestration (Fig.7). The capillaries were lined by a thin layer of fenestrated endothelium. The podocyte nucleus and its primary process gave rise to numerous secondary foot process rested on glomerular basement membrane (Figs. 7& 8). Also, the thickness of basement membrane was uniform (Fig.8).

As regard the proximal convoluted tubules (PCTs), were characterized by its narrow lumen. The large cubical cells that lined it had prominent apical microvilli. The cytoplasm beneath the microvilli contained many pyknotic vesicles. The nucleus of each cell was rounded and basally located. Numerous scattered mitochondria were observed (Fig. 9).

In addition the distal convoluted tubule (DCTs) had many ultrastructural features in common with the proximal tubule the most striking difference were that the distal tubule lacked the prominent microvilli and the nuclei of its cells tend to bulge into the lumen with fewer mitochondria (Fig. 10).

Examination of ultrathin sections of the kidney of one- month Malathion dosed rats revealed obvious signs of degeneration. Extremely narrowing of capillary spaces with deposition of electron dense material in the podocyte (Fig. 11). Moreover a slight increase in the thickness of the glomerular basement membrane with deposition of electron dense material was focally seen (Fig.12). In addition, the mesangial cells appeared well defined with scattered electron dense fibrils were noticed (Fig.11). Furthermore, there was vacuolation of the podocyte cytoplasm with fusion of its foot processes (Figs.11&12).

As regard the ultra structures of the cells of renal proximal tubules, there were partial loss of microvilli with electron pale material in lumen and the membranous structures were observed, as well as

there were swollen and pleomorphic mitochondria were a dominant feature (Fig.13). The Cytoplasmic bulges were seen in some distal convoluted tubular cells (Fig.14).

Also, there was an increase in the cytoplasmic density of some of the distal convoluted tubule cells. Another prominent signs were the extension in the length of some cells and cytoplasmic bulges toward the lumen from the apical cytoplasm. Moreover the lumen contained electron dense material (Fig.15).

In the other hand , ultrathin sections of the kidney of Malathion pulse Roselle confirmed the

light microscopic finding whereas it relieved normal renal ultra structures, normal glomeruli with normal capillary lumen ,normal podocyte and mesangial cells. Moreover the foot processes of podocyte were rested on the glomerular basement membrane similar to the control group (Figs.15&16). Furthermore the proximal convoluted tubules had normal nucleus, microvilli and mitochondria. Also the distal convoluted tubules appeared with clear lumen and normal nucleus (Fig.17).In spite of these signs of improvement the fenestrations could not be detected (Figs.15&16).

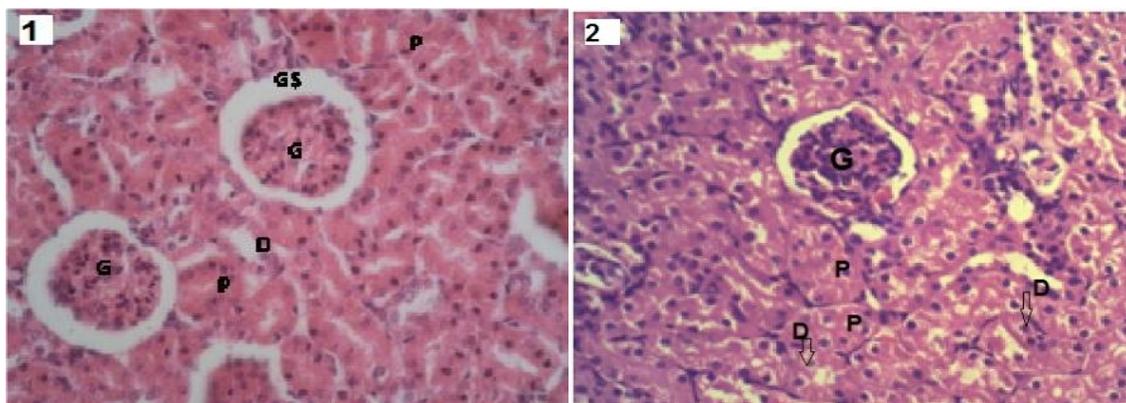


Fig. (1): Photomicrograph of longitudinal section of the kidney of the control albino rat shows the normal structure of glomerulus (G), glomerular space (GS), proximal convoluted tubules (P) and distal convoluted tubules (D). (Hx. & E.; X400).

Fig.(2): Photomicrograph of longitudinal section of the kidney of the albino rat exposed to Roselle only shows the glomerulus (G), proximal convoluted tubules (P) and distal convoluted tubules (D) are normal similar to the control group . (Hx. & E.; X400).

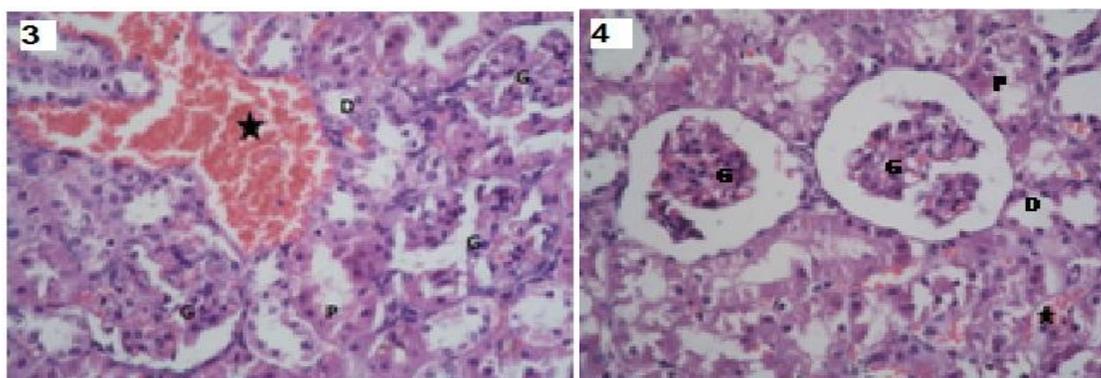


Fig.(3): Photomicrograph of longitudinal section of the kidney of the Malathion treated rat shows: presence of glomerulus hypertrophy (G) , vacuolation of cytoplasm of the lining cells of both the proximal convoluted tubules (P) and distal convoluted tubules (D).Notice the presence of area of hemorrhage (*). (Hx. & E.; X400).

Fig. (4): Photomicrograph of longitudinal section of the kidney of the Malathion treated rat shows the presence of: glomerular atrophy (G) , the proximal convoluted tubules (P) contained large vesicular nuclei and the distal convoluted tubules (D) contained dark pyknotic nuclei. Notice the presence of an area of hemorrhage (*). (Hx. & E.; X400).

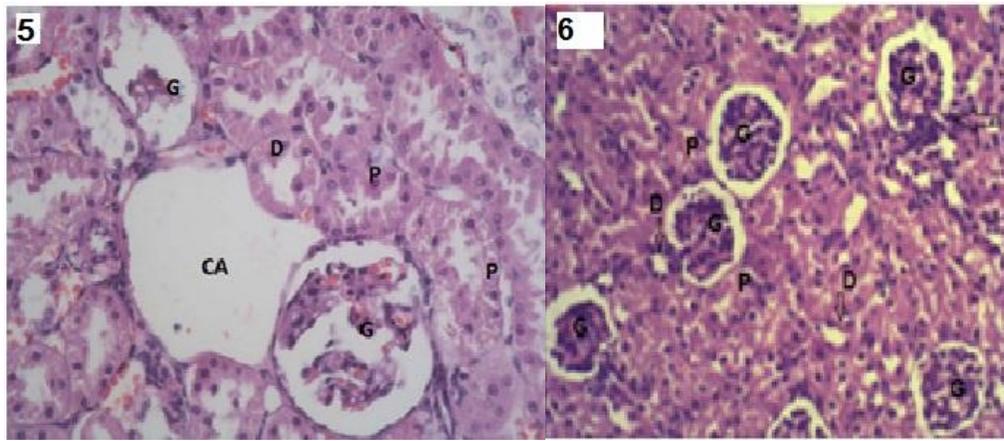


Fig. (5): Photomicrograph of longitudinal section of the kidney of the Malathion treated rat shows the atrophied glomerulus (G) and the proximal convoluted tubules lumen (P) contain casts . Notice the presence of an area of cavitation (CA) .
(Hx. & E.; X400).

Fig. (6) :Photomicrograph of longitudinal section of the kidney of the Malathion plus Roselle treated rat shows that the glomerulus (G) , the proximal convoluted tubules (P) and distal tubule are normal in shape except for the presence of an area of cellular infiltration (arrow)
(Hx. & E.; X400).

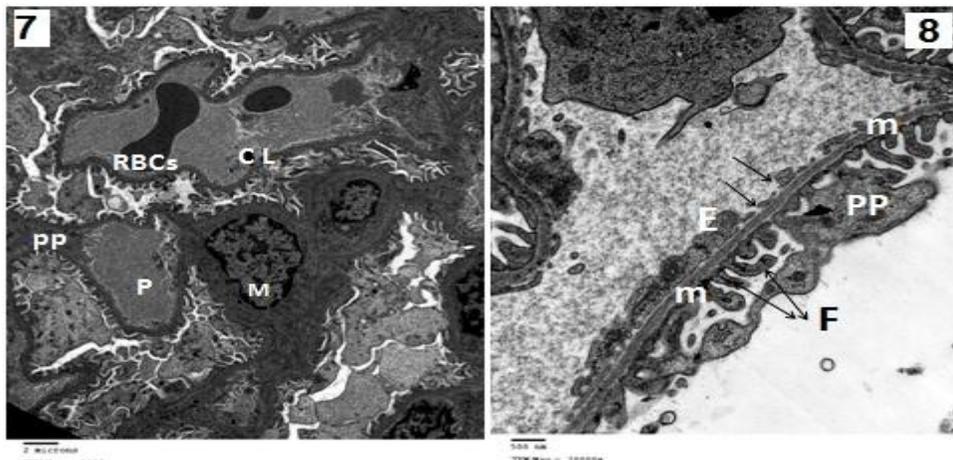


Fig. (7): Electron micrograph of ultrathin section of the control group revealed the usual component of the glomeruli showing: normal part of capillary lumen (CL) which contains red blood cells (RBCs). Notice the normal podocyte (P) and its primary process (pp). (TEM. Mag =5000)

Fig. (8): Electron micrograph of ultrathin section of the control group of the previous figure shows normal foot processes of podocyte process (pp) rest on the glomerular basement membrane (m) which lined by capillary endothelium . Notice the fenestrations (arrows) and foot processes (F). (TEM Mag = 20000).

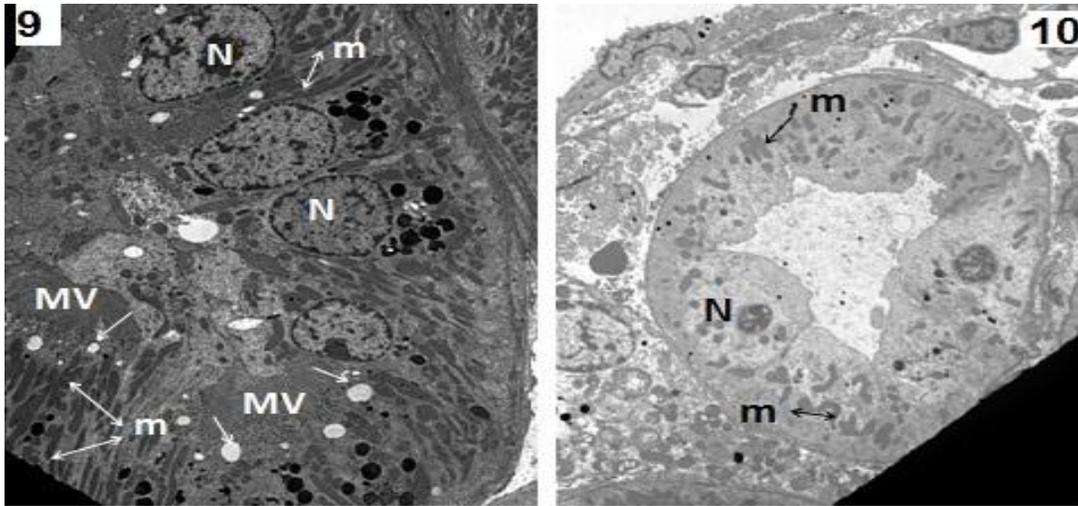


Fig. (9): Electron micrograph of ultrathin section of the control group shows normal proximal convoluted tubules shows normal nucleus (N), microvilli (Mv) ,mitochondria (m) and pinocytic vesicles (arrows). (TEM Mag = 4 000)

Fig.(10): Electron micrograph of ultrathin section of the control group shows normal distal convoluted tubules shows normal shaped nucleus (N) with few mitochondria (m). (TEM Mag = 4 000)

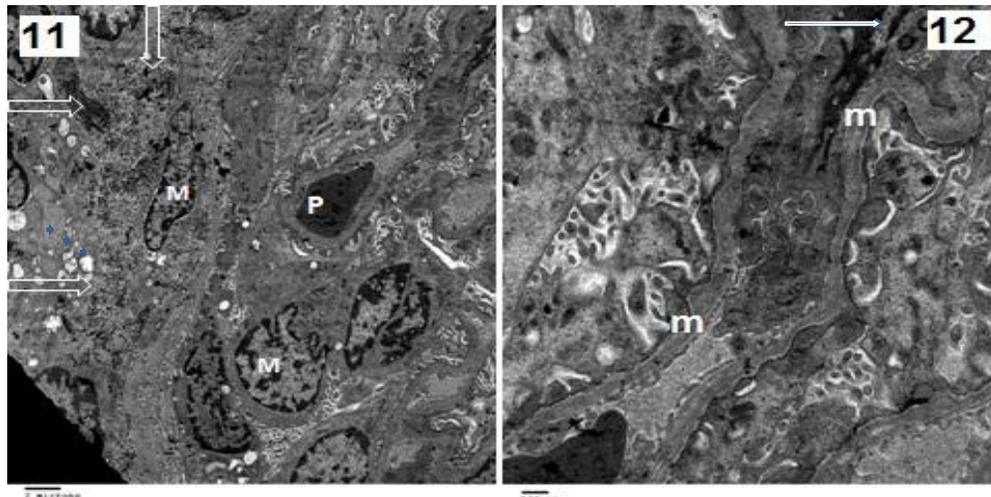


Fig.(11) : Electron micrograph of ultrathin section of the kidney of the Malathion treated rat shows extrem narrowing of capillary spaces with presence of electron dense material in podocyte (P) and scattered electron dense fibrils (arrows). (TEM Mag = 4000)

Fig.(12) : Electron micrograph of ultrathin section of the previous figure shows thickning of basment membrane (m) with deposition of electron dense material (arrow). (TEM Mag=15000).

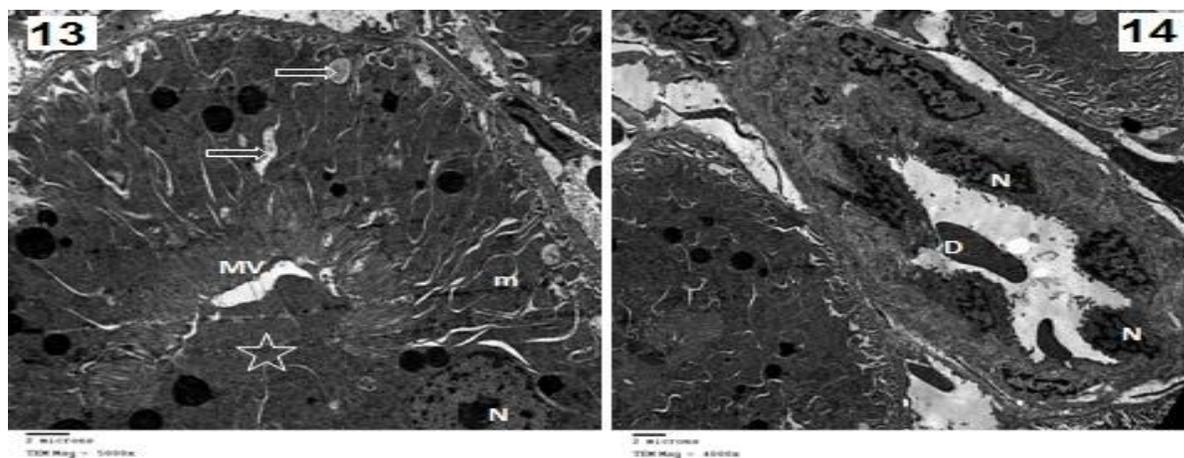


Fig. (13): Electron micrograph of ultrathin section of PCT of Malathion dosed rat kidney shows: the presence of normal nucleus (N) and swollen mitochondria (m). Notice the presence of membranous structures (arrows) and electron pale material in the lumen (*) (TEM mag = 5 000).

Fig. (14): Electron micrograph of ultrathin section of (DCT) of Malathion dosed rat kidney shows the normal nucleus (N) and electron dense material (D) in its lumen. (TEM Mag = 4000).

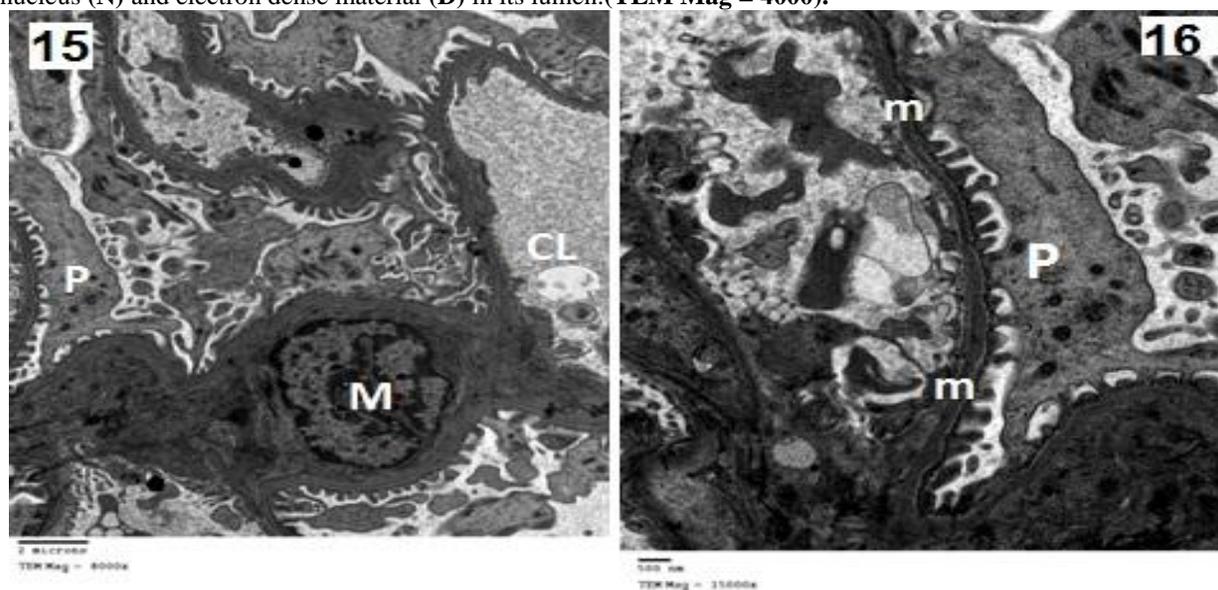


Fig.(15) :Electron micrograph of ultrathin section of of Malathion plus Roselle shows the usual component of the glomeruli showing: normal part of capillary lumen (CL) Notice the normal podocyte (P) and mesangial cells (M). (TEM Mag = 3000).

Fig. (16) : Electron micrograph of ultrathin section of Malathion plus Roselle shows normal foot processes of podocyte(P) rest on the glomerular basement membrane (m). (TEM Mag =15000)

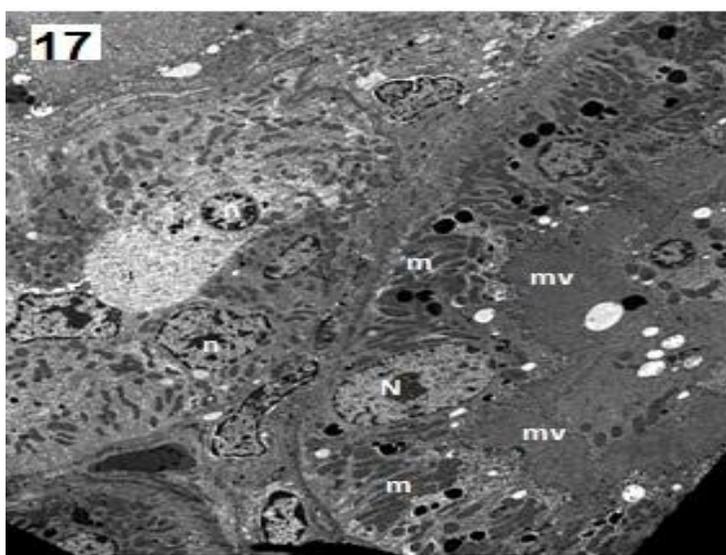


Fig. (17): Electron micrograph of ultrathin section of Malathion plus Roselle shows part of (PCT) with normal nucleus (N), microvilli (mv) and mitochondria (to the right side of the picture). Also part of (DCT) with normal nucleus (n) and clear lumen (to the left side of the picture). (TEM mag =15000).

3-Evaluation of the organ Weights:

No statistically significant difference in the absolute kidney weight has been observed between the control and ROS-treated group ($P > 0.05$). However, absolute kidney weight showed significant increase in Malathion-treated groups compared to control group ($P = 0.000$). Moreover there was significant decrease in the absolute kidney weight for Malathion+ Ros-treated group when compared to Malathion treated group ($P = 0.000$). When the absolute kidney weight for Malathion+ Ros-treated group was compared to control group, no statistically significant changes were observed ($P > 0.05$). **Table, (18).**

4- Evaluation of Biochemical Results:

1-Results of the oxidative stress markers:

The activity of CAT in the Malathion-treated group was significantly ($P = 0.000$) decreased when compared to the control animals. Treatment with the extract of *Roselle* significantly prevented decrease in the level of CAT activity compared to the Malathion treated rats ($p = 0.000$). Renal SOD activity was decreased significantly ($p = 0.000$) in the Malathion treated-animals compared to control group. Treatment with the extract of *Roselle* significantly

elevated ($P = 0.000$) the SOD levels as compared to the malathion-treated animals. The GSH level reduced significantly ($P = 0.000$) along with increased in MDA concentration in the Malathion treated group as compared to the control group ($p = 0.000$). However on treatment with *Roselle* extract, the GSH level was found to be enhanced significantly ($p = 0.000$) and the MDA contents were reduced in ($P = 0.000$) as compared to the Malathion treated group. These data were represented in **Table (1)**.

2- The Urea and creatinine levels:

At the end of the experiments, when the Malathion-treated group was compared with the control group, there was a significant increase in the serum urea and creatinine levels ($P = 0.000$ for both) (Figs.19 and 20). In the *Roselle* + Malathion treated group there was a statistically decrease in the serum urea level and creatinine levels when compared to the Malathion-treated group ($P = 0.000$ for both). There were statistically insignificant differences between the levels of urea and creatinine in *Roselle*+ Malathion- treated group when compared to the control group ($p > 0.05$), **Table(1)**.

Table (1): the Renal function, Oxidative Status & Antioxidant Enzymes in Control, ROS-treated, Malathion treated and Malathion+ROS-treated groups

Parameters	Group (n=6)	Mean \pm SD	P value		
			Vs control	Vs ROS-treated	Vs Malathion - treated
Absolute Kidney weights (g)	Control	19.85 \pm 0.86			
	ROS-treated	20.17 \pm 0.45	P>0.05		
	Malathion-treated	22.05 \pm 0.54	P=0.000	P=0.000*	
	Malathion+ROS treated	20.67 \pm 0.66	P>0.05	P=0.000*	P=0.000*
Urea (mg/dl)	Control	38.3 \pm 0.675			
	ROS-treated	37.9 \pm 0.876	P>0.05		
	Malathion-treated	44.2 \pm 1.751	P=0.000*	P=0.000*	
	Malathion+ROS treated	37.9 \pm 0.994	P>0.05	P>0.05	P=0.000*
Creatinine (mg/dl)	Control	1.29 \pm 0.057			
	ROS-treated	1.28 \pm 0.034	P>0.05		
	Malathion-treated	1.43 \pm 0.048	P=0.000*	P=0.000*	
	Malathion+ROS treated	1.28 \pm 0.063	P>0.05	P>0.05	P=0.000*
Tissue MDA (nm/g of tissue)	Control	1.81 \pm 0.025			
	ROS-treated	1.61 \pm 152	P=0.000*		
	Malathion-treated	2.13 \pm 0.131	P=0.000*	P=0.000*	
	Malathion+ROS treated	1.60 \pm 0.063	P=0.000*	P>0.05	P=0.000*
Tissue Catalase (mmol/ g of tissue)	Control	0.53 \pm 0.008			
	ROS-treated	0.53 \pm 0.067	P>0.05-		
	Malathion-treated	0.26 \pm 0.014	P=0.000*	P=0.000*	
	Malathion+ROS treated	0.50 \pm 0.063	P>0.05-	P>0.05-	P=0.000*
Tissue SOD (U/ g of tissue)	Control	28.62 \pm 0.261			
	ROS-treated	29.23 \pm 0.281	P=.015*		
	Malathion-treated	19.83 \pm 0.117	P=0.000*	P=0.000*	
	Malathion+ROS treated	23.96 \pm 0.996	P=0.000*	P=0.000*	P=0.000*
Tissue GSH (U/ g of tissue)	Control	4.28 \pm 0.071			
	ROS-treated	4.17 \pm 0.307	P>0.05		
	Malathion-treated	1.54 \pm 0.092	P=0.000*	P=0.000*	
	Malathion+ROS treated	3.85 \pm 0.128	P=0.000*	P=0.000*	P=0.000*

* The mean difference is significant at the 0.05 level.

Discussion

The Kidney is a target organ of the experimental animals attacked by OP compounds (*Mansour et al., 2010*). Malathion is used in public health, agriculture and household purposes (*Lasram et al., 2008*). Several studies showed that Malathion caused hepatotoxicity (*Kalender et al., 2010*), testicular toxicity (*Uzun et al., 2009*), hematotoxicity (*Durak et al., 2009; Al-Attar, 2010*), genotoxicity (*Giri et al., 2002*) and nephrotoxicity (*Al-Attar, 2010*).

In the current study, all the examined parameters in group II (Roselle alone) were similar to the control group except that Roselle administration significantly decreased the tissue MDA activity and increased the SOD activity indicating that Roselle is safe herbal to the kidney with significant anti-oxidative stress effect. These results were in line with the results of *Abbas et al., (2011)* who concluded that *H. Sabdariffa* is probably a safe medicinal plant as there wasn't significant harmful change in cholesterol, triglyceride, BUN, serum creatinine and Na and K levels were observed. In addition, *Amin and Hamza, (2006)* noticed insignificant changes in the weights of testes and epididymis in rats treated with *H. Sabdariffa*. Moreover, many studies indicated that

Roselle is an interesting herb ingredient because its petals consist of anthocyanin pigment which has many properties corresponding to biological activities such as antioxidant activity (*Tseng et al., 1997b; Wang et al., 2000; Liu et al., 2002; Tsai et al., 2002; Ali et al., 2003*).

In the current study, light microscopic examination of the kidneys of the group III, one-month treated rat with Malathion, revealed that the glomerular tufts showed marked alterations including shrinkage or hypertrophy and congestion. Also areas of hemorrhage were obvious. These results were in consistence with the results of *Abdel Rahman and Zaki (1992) and Afshar et al., (2008)* who noticed deformation of the structure of the glomeruli of the cortical region in mice kidney treated with Malathion or sevin. Furthermore, *Enan et al., (1983)* noticed that pesticides caused degeneration followed by dilation of the proximal and distal convoluted tubules which contained hyaline casts.

Moreover, by the electron microscopic examination the results revealed that the degenerative changes appeared clearer indicated pronounced changes in the renal corpuscles. These changes including swelling appearances, decrease of urinary

spaces, and highly degeneration and fibrosis of podocyte. Also, there were distortion of Bowman's capsules and vacuolar degeneration of associated tubules.

The glomerular structure was affected markedly by administration of Malathion. The presence of prominent degeneration in the cytoplasm of podocytes and fusion of pedicels with fibrosis were striking. Fusion of pedicels may indicate a toxic effect of Malathion while degeneration in glomerules may be related with the interaction of the biological membrane with Malathion during its passage through the filtration barrier. *Bertani et al. (1982)* reported that fusion of pedicels and proteinuria in kidney after administration of adriamycin in rat were suggested to be developed as a result of loss of electrical load in podocyte pedicels.

In the current study, an increase in the thickness of the glomerular basal membrane with deposition of electron dense material may be due to the fact that all the organophosphorous pesticides (OP) are lipophilic and are known to have a strong affinity for interaction with membrane phospholipids (*Antunes and Madeira, 1987; Datta et al., 1994*).

Many studies reported that organo-phosphorous compounds; Malathion, may induce oxidative stress leading to the generation of free radicals and alterations in antioxidant and scavengers of oxygen-free radicals which alter structural and functional integrity of cell membrane (*Bagchi et al., 1995; Poovala et al., 1999; Ahmed et al., 2000; Possamaia et al., 2007; Franco et al., 2009*).

The effect of Malathion on the PCT revealed swelling of the mitochondrial membrane. It may be due to the effect of Malathion metabolites that can produce oxygen free radicals and affect configuration and active transport of the cell membrane. Moreover, the lipophilic metabolites of Malathion may impair the membrane of the mitochondria and play a role in the mitochondrial dysfunction. *Verma et al. (1978)* reported that pesticides caused alteration of the membrane configuration and inhibition of Mg, Na, K and ATPase in concomitant with a high concentration of aldrin and dieldrin in homogenate of kidney tissue. The membranes of mitochondria are rich with unsaturated fatty acids which are sensitive to free radicals (*Mc Cord et al., 1978*).

In the current study, there were also many tubular degenerative signs like casts in the proximal convoluted tubular lumen and the presence of cytoplasmic bulges which were seen in some degenerative distal convoluted tubular cells. These signs suggested the presence of lipid peroxidation and production of free radicals which destruct the lipid and protein structure of intracellular membranes

and lyses of cytoplasm (*Tos-luty et al., 2003*) and (*Saadi et al., 2008*).

All the histopathological changes in the current study were in concomitant with biochemical changes as, there were significant increase in the serum urea and creatinine levels in Malathion treated group. These results were in line with the results of *Kerem et al. (2007)* and *Al-Attar (2010)* who reported significant changes of kidney hemato-biochemical indices including statistically increased levels of creatinine, urea and uric acid, and decreased levels of total protein and albumin concentrations in all animals treated by Malathion. Also, *Husain (2004)* and *Mansour et al. (2010)* reported that pesticides can alter plasma urea, uric acid and creatinine levels as a result of the impairment of the glomerular function and tubular damage in the kidneys.

Mora et al. (2003) and *Yearout et al. (2008)* considered the creatinine level as a good risk marker for chronic renal insufficiency.

This impairment in the kidney functions may be due to the tubular degeneration as a result of oxidative stress which induced by Malathion and evidenced by decreased CAT and SOD activities with significant reduction in the GSH level along with increased MDA concentration in the renal tissues. Furthermore, in the present study, the presence of oblivious injuries to the membranous structures in the cytoplasm of some cells of the proximal convoluted tubule may reflect the probable injury caused by oxidative radicals.

Administration of aqueous extract of Roselle prior to Malathion resulted in a significant alleviation of the kidney injuries evidenced by a reduction of the absolute kidney weights, biochemical indices and improvement of the histological picture toward the normal. There was a statistically significant decrease in the weight of the kidney indicating improvement of the sign of degeneration and inflammation of the kidney. Moreover, the serum urea and creatinine levels were decreased toward the normal control levels. Furthermore, the glomeruli appeared within normal size with normal capillary lumen and podocyte. The mesangial cells showed normal foot processes of podocyte which rested on the normal glomerular basement membrane nearly similar to the control group. In addition, the proximal convoluted tubules had normal nucleus, microvilli and mitochondria. The distal convoluted tubules had a normal nucleus and clear lumen.

These improvements attributed to the antioxidant protective effect of Roselle and its scavenger effect. Similar histopathological findings, by using aqueous extract of Roselle, were reported by *Kalyan et al. (2009)* and *Josiah et al. (2010)*. Also these

improvement are evident in the current study by increased CAT and SOD activities along with decreased MDA and increased GSH levels in the renal tissues. These results were in consistence with the results of many study that revealed that the extract of Roselle ignificantly decreased the leakage of lactate dehydrogenase, formation of MDA and liver damage induced by t-BHP (Tseng et al., 1997b; Wang et al., 2000) and paracetamol (Ali et al., 2003) and increased glutathione (Wang et al., 2000) in rats. The antioxidant activity of Roslle could be attributed to its phenolic contents, namely protocatechuic acid as cited by Liu et al. (2002), Tsai and Huang (2004) and Prenesti et al. (2007). Moreover, Amin and Hamza (2005) reported that Roselle could prevent or attenuate the decrease in tissue anti-oxidant enzymes in different animal models and to provide cellular protection against oxidative stress.

Also similar results were reported by Tebekeme and Ibiba (2008) who reported that reduction in the levels of serum creatinine, urea and the elevation of the levels of kidney GSH and catalase in rats treated by cytotoxic drug, cisplatin, with roselle indicate that Roselle extract can reduced cisplatin induced kidney dysfunction. From their results, they suggested that the various phytochemicals extract acted synergistically to sequester the free filterable platinum hence making it less available for cellular damage.

Fatma Gökçe and Yusuf (2011) indicated that a low dose of Malathion caused sub acute nephrotoxicity that could not be ameliorated by the antioxidant vitamins; vitamin C and E, these results may indicate more potent anti-oxidant effect of Roselle over these vitamins. Furthermore, Farombi and Fakoya (2005) observed that the Roselle extracts has antimutagenic activity (60% to 70%) greater than that of vitamin C.

In conclusion, the results of the current study revealed that the aqueous extracts from Hibiscus sabdariffa is considered as a safe, natural and potent antioxidant that protects the kidney from the oxidative stress induced by chronic exposure to sub-lethal dose of Malathion.

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W

11/25/2011

دراسة كيميائية والبيكرونية للتأثير المضاد للأكسدة لخلصة الكركديه المائي على سمية الكلى المعالجة بالمبيد العضوي الفوسفاتي (مالاثيون)

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1² من قسم التشريح - كلية الطب - جامعة الأزهر (بنات) - 3 من قسم الفسيولوجي - 5 من قسم الكيمياء الحيوية - كلية الطب - جامعة الزقازيق

تستخدم المبيدات الفوسفورية العضوية على محاصيل كثيرة ، بما في ذلك القمح والذرة . وقد تم الكشف عن بقايا للمبيدات الفوسفات العضوية في التربة والحبوب والخضروات والمنتجات الغذائية الأخرى . وقد اقترحت عدة آليات لسمية المبيدات الفوسفورية العضوية ، بما في ذلك تحريض الانقسام الخلوي والأكسدة والتسمم المناعي . ومن المعروف أن المبيدات الفوسفورية العضوية تحت على السمية في الثدييات عن طريق تثبيط إنزيم الاستيل كولين استيريز مما يؤدي إلى تراكم الأستيل كولين وتفعيل المستقبلات المسكارينية والنيكوتينية . وهذا التراكم يؤدي إلى فرط الخلايا الغدية ، والعقد العصبية ، والنظام العصبي المركزي والعضلات الهيكلية على حد سواء . الكركديه هو شجيرة سنوية تستخدم عادة للصناعة . وقد شاع استخدامها في الطب الشعبي لخصائصها المقاومة لإرتفاع ضغط الدم وقد اكتشفت لها حديثاً تأثيرات مكافحة لتصلب الشرايين ومضادة للسرطان ويعتبر أيضا مستخلص الكركديه مضاد قوي للأكسدة .

الهدف من البحث : يعد التعرض لبقايا مبيدات الفسفور العضوي عملية حتمية وذلك لاستخدامه على نطاق واسع في مجال الزراعة وهذا التعرض المزمن قد يكون من أهم أسباب انتشار مرض الفشل الكلوي . ولهذا قد صممت هذه الدراسة لتقييم الدور المحتمل لخلصة الكركديه المائية في الحماية من السمية الفاجمة عن جرعة دون القاتلة من المالاثيون في الفئران كمحاولة لإيجاد طرق حماية طبيعية آمنة قوية وفعالة

مواد والطرق : تم استخدام 24 من الفئران البيضاء الذكور البالغة مقسمة الى أربع مجموعات متساوية 6 / لكل مجموعة : المجموعة الأولى: أعطيت الحيوانات 0.2 مل زيت الذرة لكل حيوان عبر الفم مرة واحدة يوميا لمدة شهر واحد ، وكانت بمثابة مجموعة ضابطة . المجموعة الثانية: أعطيت خلاصة الكركديه المائي بجرعة يومية (500 ملغ / كغ / يوم) لمدة شهر واحد . المجموعة الثالثة: فقد أعطيت الحيوانات مالاثيون في جرعة دون القاتلة 27 مج / كغ / يوم . لمدة شهر .

المجموعة الرابعة: أعطيت خلاصة الكركديه المائي كما في المجموعة الثانية بليدة بفارق ثلاث ساعات مالاثيون بالجرعة دون القاتلة في نهاية الفترة التجريبية (شهر واحد) تم سحب عينات من دم من كل الفئران في كل المجموعات لقياس مستوي البولينيا والكرياتينين . وتجهيز أنسجة الكلية لإجراء كلا من

1- قياس مؤشرات الأكسدة و2- فحص النسيج الكلوي بواسطة المجهر الضوئي والإلكتروني
النتائج : أظهرت نتائج الدراسة الحالية أن مالاثيون تسبب في زيادة وزن الكلى وأحدث تنكس في الخلايا والأنسجة ونخر كلوي وزيادة في مستوي بولينيا الدم والكرياتينين ونقص مضادات الأكسدة الطبيعية مع زيادة مؤشرات الأكسدة . كما أدى تناول المستخلص المائي للكركديه قبل المالاثيون في التخفيف من وطأة إصابات الكلى واتضح ذلك من ارتداد وزن الكلى للمؤشرات الطبيعية ، وانخفاض مستوي بولينيا الدم والكرياتينين ومؤشرات الأكسدة وتحسن في الصورة النسيجية لتصبح شبة طبيعية . وبناءً على ذلك أظهرت النتائج أن خلاصة الكركديه المائي لها تأثير واقى على الكلى ضد الأكسدة التي تحدثها جرعات المالاثيون