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

Hala H. Mossalam [1] Olfat A. Abd-El Aty [*1]. Enas N. Morgan **[2] - Sahar M. S.Youssaf [1] and Amal M H. Mackawy[3]

[1]Department of Anatomy, Faculty of Medicine, Al-Azhar –University (girls)
Departments of [2]Physiology and [3]Biochemistry, Faculty of Medicine, Zagazig, University
[*]Olfat_fair@yahoo.com **omarpubmed@yahoo.com

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: animals received only aqueous extracts of Roselle at a daily dose of (500 mg /kg b. wt./day). Group III: animals were given Malathion at a sub lethal dose of 27mg/kg b. wt./day. Groups IV: animals 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 ($P<0.001$), cellular degeneration, necrosis of the renal tissues and increase in the serum urea and creatinine ($P<0.001$ 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 ($P<0.001$) and biochemical indices; urea and creatinine ($P<0.001$; $P>0.05$, respect.) 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 ($P<0.001$) 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 ($P<0.001$). Furthermore, the GSH level reduced significantly ($P<0.001$) along with increased in MDA concentration ($P<0.001$) 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 ($P<0.001$) and the MDA contents were reduced ($P<0.001$) 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 against the oxidative stress induced by sub lethal dose of Malathion on the rat kidney.

[Hala H. Mossalam Olfat A. Abd-El Aty . Enas N. Morgan Sahar M. S.Youssaf and 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]** Life Science Journal. 2011; 8(4):561-574] (ISSN: 1097-8135). http://www.lifesciencesite.com.

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, Mediterran-ean 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 (***Iarc, 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*).**

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 fleshly, and cup shaped calyxes plant. The calyxes are rich in phenolic compounds with marked physiological activities (***Rosendiz-Lopez et al., 1998***). Hibiscus flowers contains gossypectin, glucoside, bibiscin, hibiscus anthocyann and protocatechuic acid, which may have diuretic and choleretic 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 malondialdhyde (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*).**

Nowadays 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.

2. Material and Methods:

Animals:

Twenty four adult male albino rats (weight 185-200 g) were housed at the animal house in the Faculty of Medicine for Girls Al-Azhar – University at 21–22°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 gavages once a day for one month and served as a control group.

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

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

G**roups IV**: animals 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 b. wt. / 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:

Three pieces of kidneys tissues from each group were fixed in Bouin's solution for 48 hrs. 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):

Three renal tissues samples were immediately placed in 5% gluteraldehyde buffered at pH 7.4 with Milloning phosphate for 4 hours and subsequently fixed in 1% osmium tetraoxide 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) and 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 (***Jollow et al. 1974, Carlberg and Mannervik, 1975;*** and ***Mohandas et al., 1984***): A 1.0mL supernatant of renal homogenate was mixed with 1.0mL of sulfosalicylic acid (4%). The samples were incubated at 4ºC for at least 1 h and then centrifuged at 1200x g for 15 min at 4º 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:

According 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 40-45ºC. A sold extract weighing 8.4 g was obtained (***Okasha et al., 2008***). The extract stored in the refrigerator at 4 ºC for further use.

3. 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 anastmosing 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 **(Figs.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. 3-5).**

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 ultastrucural 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 narrwoing of capillary spaces with deposition of elecrtron 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).**

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<0.001). Moreover there was significant decrease in the absolute kidney weight for Malathion+ Ros–treated group when compared to Malathion treated group (P<0.001). 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).***

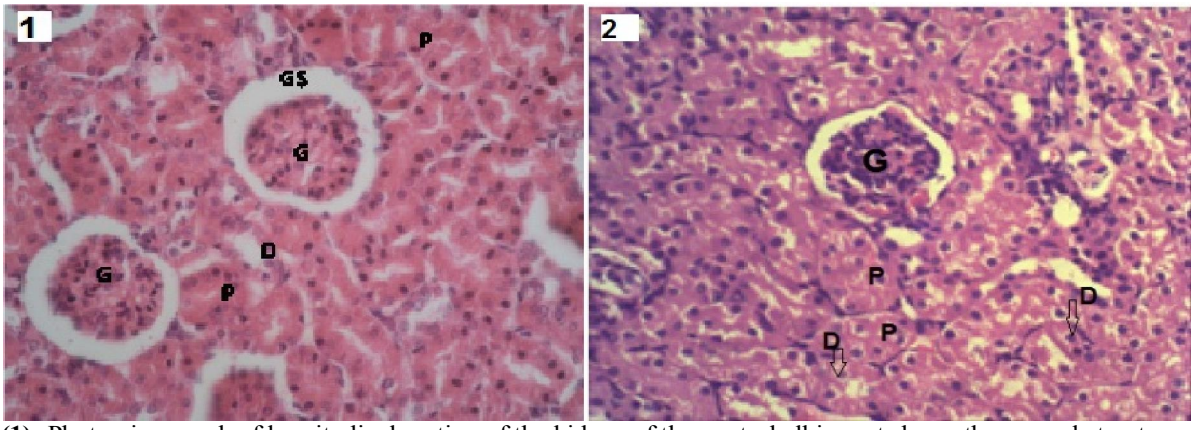


Fig. (1): Photomicrograph of longitudinal section of the kidney of the control albino rat shows the normal structure of glomerulus **(G),** glomerular space **(GS)**, proximal convoluted tubules **(P)** and distal convoluted tubules **(D). (Hx. & E.; X400).**

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).**

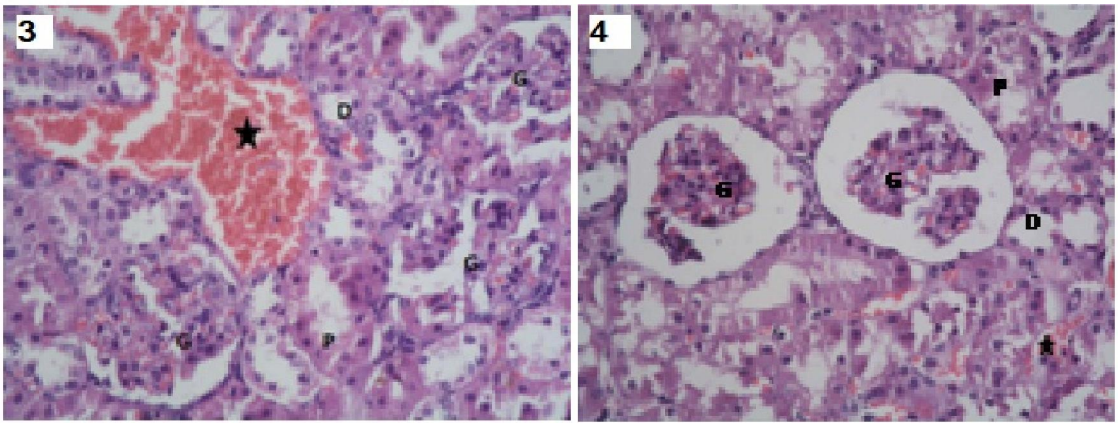
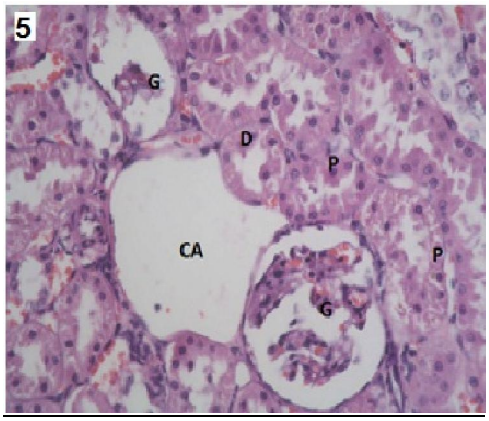


Fig.(3): Photomicrograph of longitudinal section of the kidney of the Malathion treated rat shows: presence of glomerulus hypertrophy (G), vacuolation of cytoplasm of the lining cells of both the proximal convoluted tubules (P) and distal convoluted tubules (D).Notice the presence of area of hemorrhage **(*). (Hx. & E.; X=400).**

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.; X=400).**



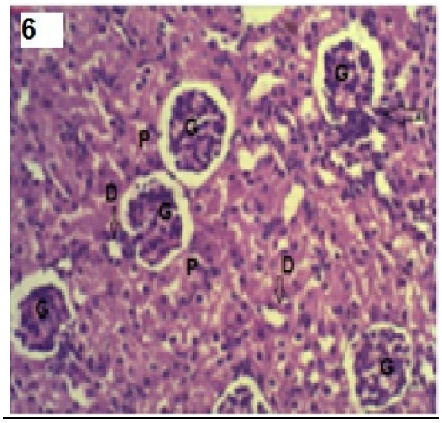


Fig .(5): Photomicrograph of longitudinal section of the kidney of the Malathion treated rat shows the atrophied glomerulus **(G)** and the proximal convoluted tubules lumen **(P)** contain casts . Notice the presence of an area of cavitation (CA). **(Hx. & E.; X=400).**

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.; X=400).**

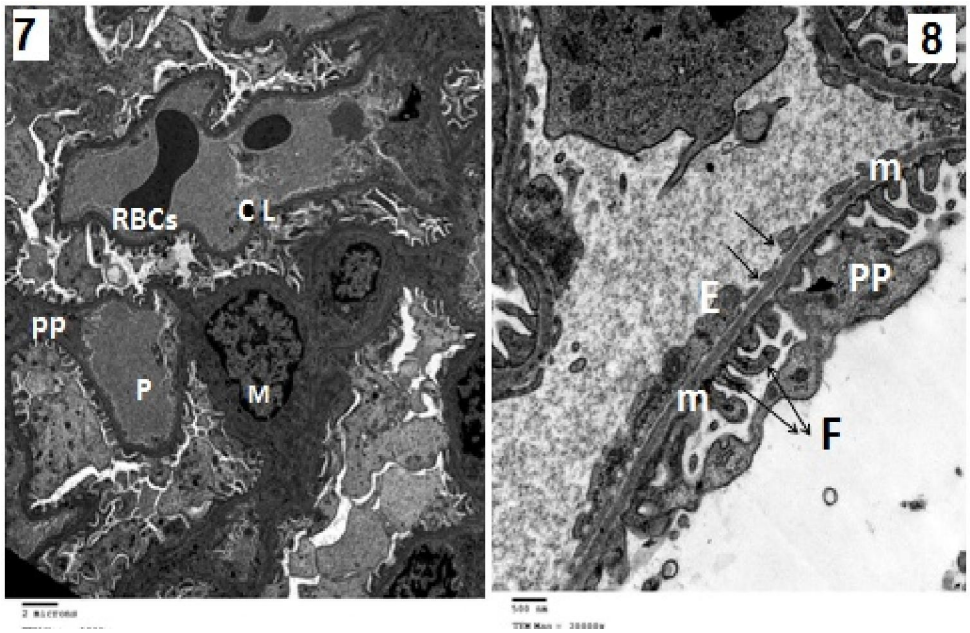


Fig. (7): Electron micrograph of ultrathin section of the control group revealed the usual component of the glomeruli showing: normal part of capillary lumen **(CL)** which contains red blood cells **(RBCs)**. Notice the normal podocyte **(P)** and its primary process **(pp). (TEM. Mag =5000)**

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).**

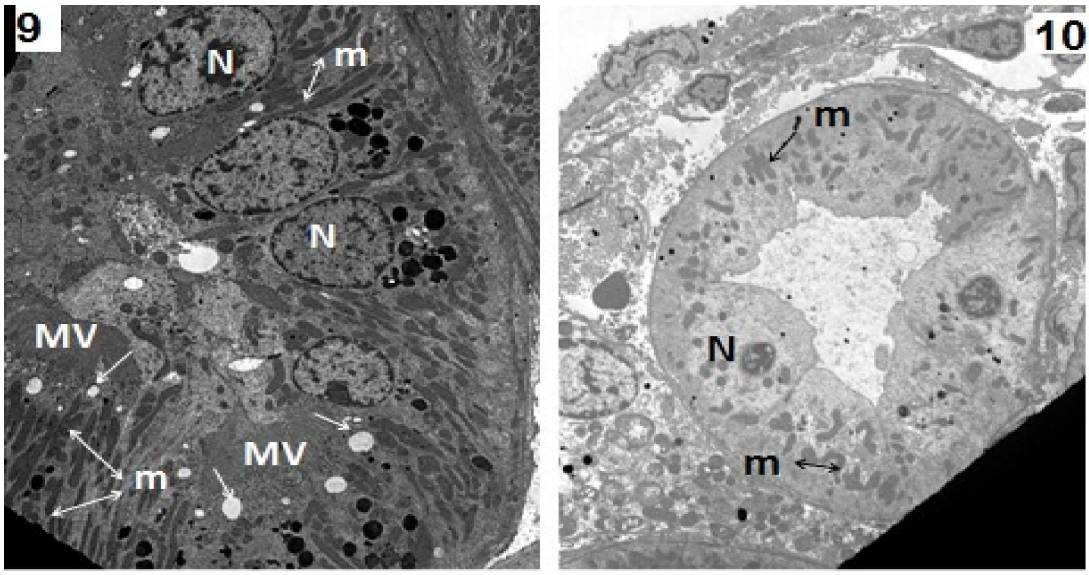


Fig. (9): Electron micrograph of ultrathin section of the control group shows normal proximal convoluted tubules shows normal nucleus **(N)**, microvilli **(Mv)** ,mitochondria **(m)** and pinocytic vesicles **(arrows). (TEM Mag =4 000**)
Fig.(10): Electron micrograph of ultrathin section of the control group shows normal distal convoluted tubules shows normal shaped nucleus **(N)** with few mitochondria **(m). (TEM Mag =4 000**)

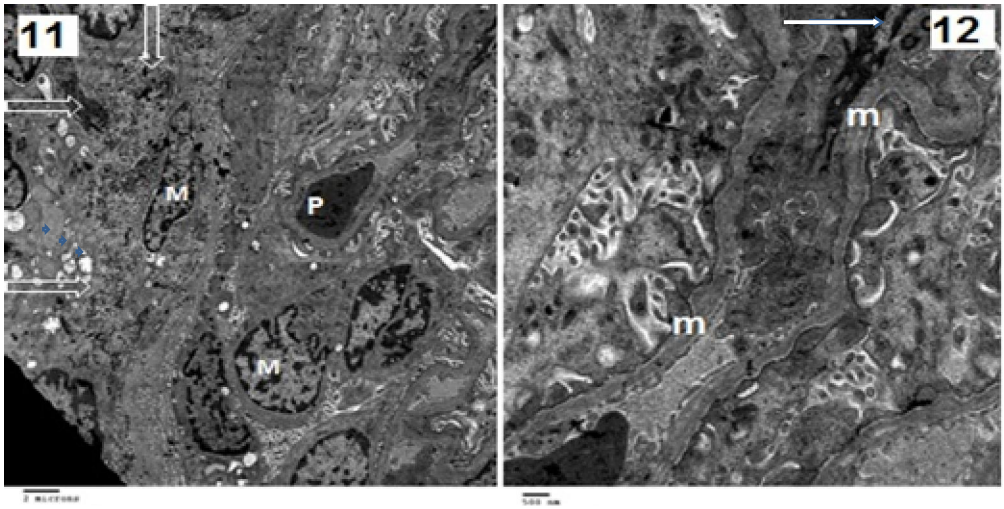


Fig.(11) : Electron micrograph of ultrathin section of the kidney of the Malathion treated rat shows extrem narrowing of capillary spaces with presence of elecrtron dense material in podocyte **(P)** and scattered electron dense fibrils **(arrows). (TEM Mag = 4000)**
Fig.(12) : Electron micrograph of ultrathin section of the previous figure shows thickning of basment membrane **(m)** with deposition of elecrtron dense material (arrow). **(TEM Mag=15000).**

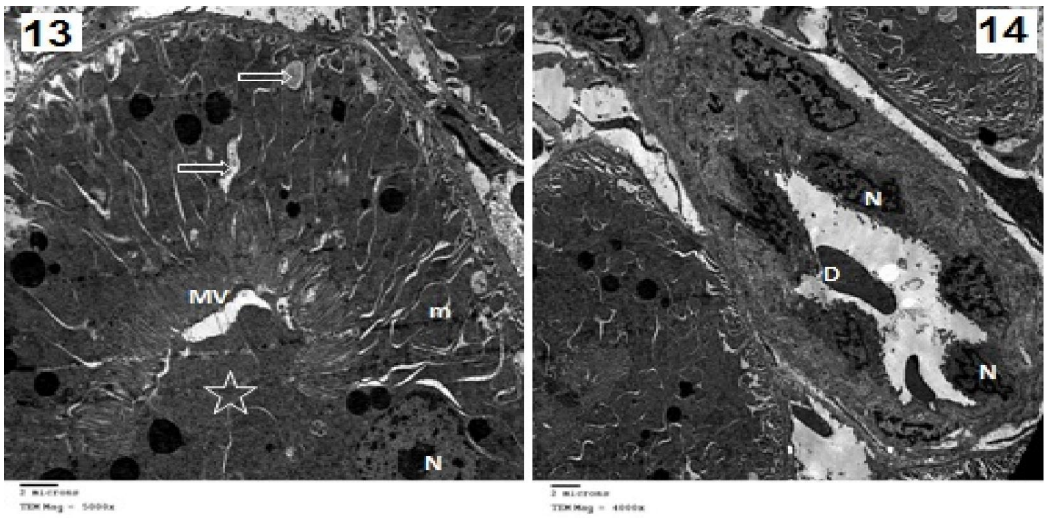


Fig. (13): Electron micrograph of ultrathin section of PCT of Malathion dosed rat kidney shows: the presence of normal nucleus **(N)** and swollen mitochondria **(m) .**Notice the presence of membranous structures **(arrows)** and electron pale material in the lumen (*) **(TEM Mag = 5 000).**
Fig. (14): 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).**

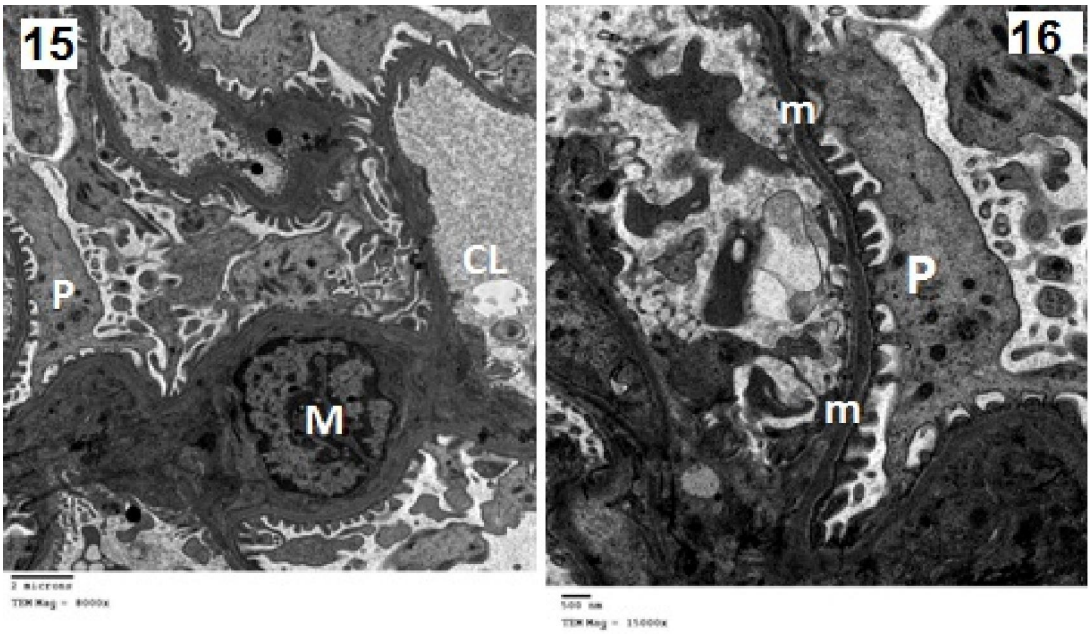


Fig.(15) :Electron micrograph of ultrathin section of of Malathion plus Roselle shows the usual component of the glomeruli showing: normal part of capillary lumen **(CL)** Notice the normal podocyte **(P)** and mesangial cells **(M)**. **TEM Mag = 3000).**
Fig. (16): Electron micrograph of ultrathin section of Malathion plus Roselle shows normal foot processes of podocyte**(P)** rest on the glomerular basement membrane **(m). (TEM Mag =15000)**

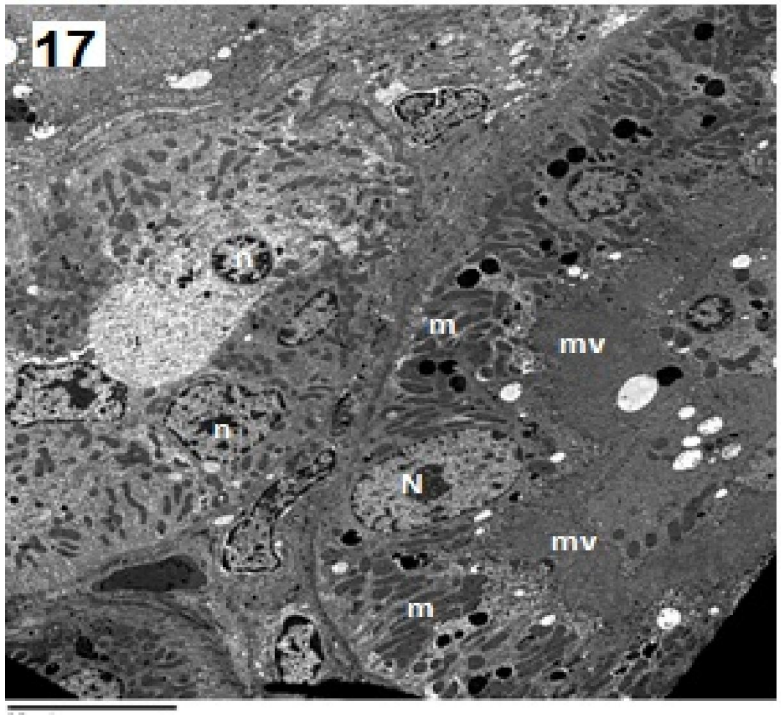


Fig. (17): Electron micrograph of ultrathin section of Malathion plus Roselle shows part of (PCT) with normal nucleus **(N),** microvilli **(mv)** and mitochondria (to the right side of the picture). Also part of (DCT) with normal nucleus **(n)** and clear lumen (to the left side of the picture). **(TEM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

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 ±SD	P value		
			VS control	Vs ROS-treated	Vs Malathion - treated
Absolute Kidney weights (g)	Control	19.85±0.86			
	ROS-treated	20.17±0.45	P>0.05-		
	Malathion–treated	22.05±0.54	P<0.000	P<0.000*	
	Malathion+ROS treated	20.67±0.66	P>0.05-	P<0.000*	P<0.001*
Urea (mg/dl)	Control	38.3±0.675			
	ROS-treated	37.9±0.876	P>0.05-		
	Malathion–treated	44.2±1.751	P<0.001*	P<0.001*	
	Malathion+ROS treated	37.9±0.994	P>0.05-	P>0.05-	P<0.001*
Creatinine (mg/dl)	Control	1.29±0.057			
	ROS-treated	1.28±0.034	P>0.05-		
	Malathion–treated	1.43±0.048	P<0.001*	P<0.00*	
	Malathion+ROS treated	1.28±0.063	P>0.05-	P>0.05-	P<0.001*
Tissue MDA (nm/g of tissue)	Control	1.81±0.025			
	ROS-treated	1.61±.152	P<0.001*		
	Malathion–treated	2.13±0.131	P<0.001*	P<0.001*	
	Malathion+ROS treated	1.60±0.063	P<0.001*	P>0.05-	P<0.001*
Tissue Catalase (mmol/ g of tissue)	Control	0.53±0.008			
	ROS-treated	0.53±0.067	P>0.05-		
	Malathion–treated	0.26±0.014	P<0.001*	P<0.001*	
	Malathion+ROS treated	0.50±0.063	P>0.05-	P>0.05-	P<0.001*
Tissue SOD (U/ g of tissue)	Control	28.62±0.261			
	ROS-treated	29.23±0.281	P<.015*		
	Malathion–treated	19.83±0.117	P<0.001*	P<0.001*	
	Malathion+ROS treated	23.96±0.996	P<0.001*	P<0.001*	P<0.001*
Tissue GSH (U/ g of tissue)	Control	4.28±0.071			
	ROS-treated	4.17±0.307	P>0.05-		
	Malathion–treated	1.54±0.092	P<0.001*	P<0.001*	
	Malathion+ROS treated	3.85±0.128	P<0.001*	P<0.001*	P<0.001*

* The mean difference is significant at the 0.05 level.

4- Evaluation of Biochemical Results:

1-Results of the oxidative stress markers:

The activity of CAT in the Malathion-treated group was significantly (P<0.001) 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<0.001). Renal SOD activity was decreased significantly (p<0.001) in the Malathion treated-animals compared to control group. Treatment with the extract of *Roselle* significantly elevated (P<0.001) the SOD levels as compared to the malathion-treated animals. The GSH level reduced significantly (P<0.001) along with increased in MDA concentration in the Malathion treated group as compared to the control group (p<0.001). However on treatment with *Roselle* extract, the GSH level was found to be enhanced significantly (p<0.001) and the MDA contents were reduced in (P<0.001) 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<0.001 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<0.001 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).**

4. 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 golmerular 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 proteinurea in kidney after administration of adriamiycin 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 in 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 ingnificantly 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 roslle 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.

Corresponding author
Olfat A. Abd-El Aty
Department of Anatomy, Faculty of Medicine, Al-Azhar –University (girls)
olfat_fair@yahoo.com

References:

Abbas, M; Shirin, M; Patricia, K and Mohammad, GK (2011): The Effect of Hibiscus Sabdariffa on Lipid Profil Creatinine, and Serum Electrolytes: A Randomized Clinical Trial. *Gastroenterology*, 2011: 4.

Abdel Rahman, MF and Zaki, ZT (1992): Histopathological and histochemical effects of sevin on the renal and hepatic tissues of mice. *Journal of the Egyptian German Society of Zoology,* 8C:115-126.

Abdollahi, M; Mostafalou, S; Pournour mohammadi, S; Shadnia, S. (2004): Oxidative stress and cholinesterase inhibition in saliva and plasma of rats following subchronic exposure to malathion. *Comp Biochem Physiol C Toxicol Pharmacol., 137:29-34.*

Abdul, G (1990): Introduction to Pharmacognosy. University press, 1st Edn., pp: 175-200.

Afshar, S; Farshid, AA; Heidari, R and Ilkhanipour, M (2008): Histopathological changes in the liver and kidney tissues of Wistar albino rat exposed to fenitrothion. *Toxicology and Industrial Health,* 24(9): 581–586.

Ahmed, RS; Seth, V; Pasha, ST and Banerjee, BD (2000): Influence of dietary ginger (*Zingiber officinales* Rosc) on oxidative stress induced by Malathion in rats," *Food and Chemical Toxicology*, 38: 5, 443–450.

Akhtar, N; Kayani, S; Ahmad, M and Shahab, M (1996): Insecticide-induced changes in secretory activity of the thyroid gland in rats. *J. Appl. Toxicol.,* 16:5:397-400.

Al-Attar, AM (2010): Physiological and Histopathological Investigations on The Effects of α-Lipoic Acid in Rats Exposed Malathion. *Journal of Biomedicine and Biotechnology,* 2010: 1-8.

Ali, BH, Mousa, HM and Mougy, ES (2003): The effect of water extract and anthocayanins of *Hibicus sabdariffa L.* on paracetamol induced hepatoxicity in rats. *Phyto. Res.*, 17 (1): 56-59.

Ali, BH; Al Wabel, N and Blunden, G (2005): Phytochemical, pharmacological and toxicological aspects of Hibiscus sabdariffa L.: a review. *Phytother Res.,* 19(5): 369-375.

Alli, MB and Salih, M (1991): Investigation of the antispasmodic potential of Hibiscus sabdariffa calyces. *J. Ethnopharmacol.,* 31: 249-257.

Amin, A and Hamza, AA (2005): Hepatoprotective effects of Hibiscus, Rosmarinus and Salvia on azathioprine-induced toxicity in rats. Life Sci., 77: 266–78.

Amin, A and Hamza, AA (2006): Effects of ginger and roselle on cisplatin induced reproductive toxicity in rats. Asian J Androl., 8: 607–12.

Amr, A; Alaaeldin, AH; Amr, K and Sayel, D (2008): Herbal extracts counteract cisplatin-mediated cell death in rat testis. *Asian Journal of Andrology*, 10: 291–297.

Antunes Madeira MC and Madeira, VMC (1987): Partition of Malathion in synthetic and native membranes .*Biochem Biophys Acta;* 901: 61–67.

Bagchi, D; Bagchi, M; Hassoun, EA and Stohs, SJ (1995): *In vitro* and *in vivo* generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. *Toxicology,* 104-129-136.

Bako, IG; Mabrouk, MA; Maje, IM; Buraimoh, AA and Abubakar, MS (2010): Hypotensive Effect of Aqueous Seed Extract of Hibiscus sabdariffa linn (Malvaceae) on Normotensive Cat. *Int. J. Anim. Sci. Veter.* Adv., 2(1): 5-8.

Bancroft, JD and Steven, SA (1996): Theory and practice of histological techniques. 4thed. Churchill Livingston, *Edinburgh, London*, 184-193.

Bergmeyer, HU, Gowehn, K and Grassel, H (1974): Methods of enzymatic analysis. *Weinheim Verlag Chemine.*, 438-439.

Bertani, T; Poggi, A; Pozzani, R; Delaini, F; Sacchi, G; Thova, Y; Mecca, G; Remuzzi, G and Donati, MB (1982): Adriamycin-induced ephritic syndrome in rats: sequence of pathologic events. Lab,. 707.

Cabello, G; Valenzuela, M; Vilaxa A (2001): A rat mammary tumor model induced by the organophosphorous pesticides parathion and malathion, possibly through acetylcholinesterase inhibition. Environ Health Perspect., 109:471-9.

Carlberg, I and Mannervik, B (1975): Glutathione reductase levels in rat brain. J. Biol. Chem., 250: 5475-5479.

Datta, C; Dasgupta, JG and Sengupta, D (1994): Interaction of organophosphorous insecticides phosphamidon and Malathion on lipid profile and Acetylcholinesterase activity in human erythrocyte membrane. *Indian J. Med. Res.,* 100: 87–94.

Durak, D; Uzun, FG; Kalender, S; Ogutcu, A; Uzunhisarcikli, M and Kalender, Y (2009): Malathion-induced oxidative stress in human erythrocytes and the protective effect of vitamins C and E in vitro, *Environ. Toxicol.,* 24(3): 235–242.

Enan, EE; El Sabaawi, EA and Mashali R (1986): Enzymatic and histological studies of profenofos on white male rats. *The Egyptian Journal of Histology,* 9 (1):182.

Farombi, EO and Fakoya, A (2005): Free radical scavenging and antigenotoxic activities of natural phenolic compounds in dried flowers of Hibiscus sabdariffa L. *Mol Nutr Food Res.,*49(12):1120-1128.

Fatma Gökçe ,U and Yusuf, K (2011): Protective Effect of Vitamins C and E on Malathion- Induced Nephrotoxicity in Male Rats. *GU J Sci*., 24(2):193-201.

Franco, JL; Posser, T; Mattos, JJ (2009): Zinc reverses Malathion-induced impairment in antioxidant defences. *Toxicology Letters*, 187 (3):137–143.

Frankowski, BL (2004): American Academy of Pediatrics guidelines for the prevention and treatment of head lice infestation. Am J Manag Care;10 suppl:S269-72.

Gallo, M J and Lawryk, NJ (1991): Organic phosphorus pesticides. Handbook of Pesticide Toxicology; Hayes Jr., W. J.; Laws Jr., E. R.,Eds.; Academic Press, Inc.: San Diego,917-1123.

Galloway, T and Handy R, (2003): Immunotoxicity of organophosphorous pesticides. *Ecotoxicology*, 12:345-63.

Gervais, JA; Luukinen, B; Buhl, K and Stone, D (2009): Malathion Technical Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services.

Giri, S; Prasad, SB; Giri, A and Sharma, GD(2002): Genotoxic effects of malathion an organophosphorous insecticide, using three mammalian bioassays in vivo. Mutat.Res., 514:223 - 231.

Hazarika, A; Sarkar, SN; Hajare, S; Kataria, M and Malik, JK (2003): Influence of Malathion pretreatment on the toxicity anilofos in male rats: a biochemical interaction study. *Toxicology*, 185: 1-8.

Herrera-Arellano A; Flores-Romero, S; Chávez-Soto, MA and Tortoriello, J (2004): Effectiveness and tolerability of a standardized extract from Hibiscus sabdariffa in patients with mild to moderate hypertension: a controlled and randomized clinical trial. Phytomedicine; 11: 375–82.

Husain, K; Whitworth, C and Rybak, LP (2004): Time response of carboplatin-induced nephrotoxicity in rats. *Pharmacol. Res.,* 50: 291-300.

IARC, C (1983): Monograph on the evaluation of carcinogenic risk of chemicals to man .Miscellaneous pesticides. *International Agency for research on cancer,*30. Lyon France.

John, S; Kale M; Rathore, N (2001): Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. *J Nutr Biochem*., 12:500-4.

Jollow, DJ; Mitchell, JR; Zampaglione, N; Gillete, JR (1974): Bromobenzene induced liver necrosis: Protective role of glutathione and evidence for 3,4 bromobenzene oxide as the hepatotoxic intermediate. Pharmacology, 11: 151–169.

Josiah, SJ; Omotuyi O; Oluyemi, KA; Ezea, UI; Uhunmwangho, ES (2010): Protective role of aqueous extract of Hibiscussabdariffa (calyx) against potassium bromate induced tissue damage in wistar rats. *African Journal of Biotechnology,* 9 (21): 3218-3222.

Kalender, S; Uzun, FG; Durak, D; Demir, Fand Kalender, Y (2010): Malathion-induced hepatotoxicity in rats: The effects of vitamins C and E. Food Chem .*Toxicol*., 48: 633–63.

Kalyan, SB; Christeana, AJ; Sayma, BS; Selvakumar, S and Sundara SK (2009): Antilithiatic activity of of Hibiscus Sabdariffa on ethylene glycol-induced lithiasis in rat. *Natural Prodect Radiance,* 8(1): 43-47.

Kamrin, MA (1997): Pesticide Profiles: Toxicity, Environmental Impact, and Fate; Lewis Publishers: New York: 191-195.

Kerem, M; Bedirli, N; Gürbüz, N; Bedirli, A; Akkaya, T; Ekinci, Ö; Sakrak, Ö and Pasaoglu, H (2007): Effects of acute fenthion toxicity on liver and kidney function and histology in rats. *Turkish Journal of Medical Sciences.*, 37(5):281–288.

Lasram, MM; Annabi, AB; Rezg, R; Elj, N; Slimen, S; Kamoun, A; El-Fazza, S and Gharbi, N (2008): Effect of short-time malathion administration on glucose homeostasis in Wistar rat. *Pestic Biochem. Phys.,* 92: 114-119.

Lin, HH; Chen, JH; Kuo, WH and Wang, CJ (2007): Chemo preventive properties of Hibiscus sabdariffa L. on human gastric carcinoma cells through apoptosis induction and JNK/p38 MAPK signaling activation. *Chem Biol Interact.,* 165 (1):59-75.

Liu, C; Wang, J; Chu, C; Cheng, M and Tseng, T (2002): In vivo protective effect of protocatechuic acid on tert-butyl hydroperoxide-induced rat hepatotoxicity. *Food Chem Toxicol.,* 40: 635–41.

Lo , CW; Huang, HP; Lin, HM; Chien, CT and Wang, CJ (2007): Effect of Hibiscus anthocyanins-rich extract induces apoptosis of proliferating smooth muscle cell via activation of P38 MAPK and p53 pathway. *Mol Nutr Food Res.,* 51(12):1452-1460.

Lowry, OH; Rosebrough, NJ; Farr, AL and Randal, RJ (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem*., 193: 265-275.

Mansour, SA and Mossa, AH (2010): Oxidative damage biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. *Pestic. Biochem. Phys.,* 96: 14-23 .

Massoulie, J and Bon, S (1982): The molecular forms of cholinesterase and acetylcholinesterase in vertebrates. *Annu. Rev. Neurosci*., 5: 57-106.

Mc Cord, JM and Fridovich, I (1978): The biology and pathology of oxygen radicals. *Ann. Intern. Med.*, 82: 122-127.

Mohandas, J; Marshall, JJ; Duggin, GG; Horvath, JS and Tiller, D (1984). Differential distribution of glutathione and glutathione related enzymes in rabbit kidney: possible interactions in analgesic neuropathy. *Cancer Re.*, 44: 5086-5091.

Mora, LO; Antunes, LM; Francescato, HD; Bianchi, ML (2003): The effects of oralglutamine on cisplatin-induced nephrotoxicity in rats. *Pharmacological Research,* 47 (6): 517-522.

Morton, JF (1998): Roselle. In: fruits of warm climate. Dowling, C. F. (Ed). *Media Inc. Greensboro, NCP*: 281-286.

Okasha MM; Abubakar, MS and Bako, IG (2008): Study of the effect of Aqueous *Hibiscus sabdariffa s*eeds extract on serum prolactin level of lactating female albino rat**.** *European Journal of scientific research*, 22(4):575-583.

Olvera-García, V; Castaño-Tostado, E; Rezendiz-Lopez, RI et al., (2008): Hibiscus sabdariffa L. extracts inhibit the mutagenicity in microsuspension assay and the proliferation of HeLa cells. *J Food Sci*., 73(5):75-81.

Poovala, VS; Huang, H and Salahudeen, AK (1999): Role of reactive oxygen metabolites in organophosphate-bidrin-induced renal tubular cytotoxicity. *J. Am. Soc. Nephrol*., 10:1746-1752.

Possamaia, FP; Fortunatob, JJ; Feierb, G ; Agostinhob, FR ; Quevedob, J; Wilhelm Filhoc, D and Dal-Pizzola F (2007): Oxidative stress after acute and sub-chronic malathion intoxication in Wistar rats Environmental Toxicology and Pharmacology, 23 (2): 198-204.

Prenesti, E; Berto, S; Daniele, PG and Toso, S (2007): Antioxidant power quantification of decoction and cold infusions of Hibiscus sabdariffa flowers. Food Chem., 100: 433–8.

Rai, S., Wahile, A., Mukherjee, K., Saha, B.P. and Mukherjee, P.K. (2006): Antioxidant activity of Nelumbo nucifera [sacred lotus] seeds. *J. Ethnopharmacol*., 104: 322-27.

Reigart, JR and Roberts, JR (1999): Organophosphate Insecticides. Recognition and Management of Pesticide Poisonings, 5th ed.; U.S Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC, pp 34-47.

Resendiz-Lopez, I; Loara-pina, B and Castano-Tostado, E (1998): Antimutagenicity of natural phenolics compounds in dried flowers from Hibiscus sabdarafa L. against1-nitropyrene. *On line:File://A:\Hib5.htm*

Rossi, A; Serraino, I and Dugo, P (2003): Protective effects of anthocyanins from blackberry in rat model of acute lung inflammation. *Free Radical Res.* ,37(8): 891-900.

Saadi, L; Lebaili, N and Benyoussi, M(2008): Exploration of cytotoxic effect of malathion on some rat organs structure. Communications in Agricultural and Applied Biological Sci-nces, 73(4): 875–881.

Tebekeme, O and Ibiba, FO (2008): The effect of Hibiscus sabdariffa calyx extract on cisplatin-induced tissue damage in rats .*Biokemistri*, 20 (2): 47-52.

Tos-luty, S; Obuchowska-Przebirowska, D; Latuszynsxa, J; Tokarska-Rodak, M and Haratym Maj, A (2003): Dermal and oral toxicity of malathion in Rat. *Ann Agric Environ. Med*., 10: 101-106.

Tsai PJ and Huang HP (2004): Effect of polymerization on the antioxidant capacity of anthocyanins in Roselle. *Food Res Intern*., 37: 313–8.

Tsai, PJ; McIntosh, J; Pearce, P; Camden, B and Jordan BR (2002): Anthocyanin and antioxidant capacity in Roselle (Hibiscus sabdariffa L.) extract. *Food Res. Intern.,* 35: 351–6.

Tseng, T; Kao, ES; Chu, CY; Chou, FP; LinWu, HW and Wang CJ (1997 a): Hisbiscus protocatechuis acid protects against oxidative damage induced by tert-butyl hydroperoxide in rat primary hepatocytes. *Chem. Biol. Int.,* 101: 137-148.

Tseng, T; Kao, ES; Chu, CY; Chou, FP; LinWu, HW; Wang CJ (1997 b): Protective effects of dried flower extracts of Hibiscus sabdariffa L. against oxidative stress in rat primary hepatocytes. *Food Chem Toxicol.,* 35: 1159–64.

Tseng, TH; Hsu, JD; Lo, MH; Chu, CY; Chou, FP and Huang, CJ (2005): Inhibitory effect of Hibiscus Protocatechuic acid on tumor promotion in mouse skin. *Cancer Lett.,* 126(2): 199.

Uzun, FG; Kalender, S; Durak, D; Demir, F and Kalender, Y (2009): Malathion-induced testicular toxicity in male rats and the protective effect of vitamins C and E", *Food. Chem. Toxicol.,* 47: 1903-1908.

Verma, SR; Gupta, AK; Bansal, SK and Dalela, RC (1978): In vitro disruption of ATP dependent active transport following treatment with aldrin and its epoxyanalog dieldrin in a fresh water teleost. *Labeo rohita. Toxicolgy* ,11: 193-201.

Wang, CJ; Wang, JM; Lin, WL; Chu, CY; Chou, FP; Tseng, TH (2000):Protective effect of Hisbiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. *Food Chem Toxicol.;*38: 411–6.

Yang, H; Lauv, S and Sinclair, DA (2006): Nampt/PBEF/Visfatin: a regulator of mammalian health and longevity. *Exper. Geront.,* 41:718-726.

Young, DS (2000): Effects of drugs on clinical laboratory tests . 5th edition . AACC Press .

Zeid, MM ; El-Barouty, G ; Adbdel-Reheim, E; Blancato, J; Dary, C; El-Sebae, AH and Saleh, M(1993): Malathion's disposition in dermally and orally treated rats and its impact on the blood serum acetylcholine esterase and protein profile. *J. Environ.Sci. Health, Part B*, 28 (4): 413-430.

Zhang, XZ (1992): Crop Physiology Research Methods. *China Agricultural Press Beijing*, 131:207.

11/2/2011