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Hepatoprotective potential of *Rumex vesicarius* against malathion hepatotoxicity in adult albino rats

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SUMMARY

The aim of this work was to elucidate a possible protective role of *Rumex vesicarius L. (RV)* against malathion (*MT)*-induced hepatotoxicity in adult male albino rats. Forty-eight adult male albino rats were divided into three groups: Group I (control group), 12 rats, divided into two equal subgroups: subgroup Ia received a single intraperitoneal (ip) dose of ½ ml corn oil. Subgroup Ib received oral *RV* 200 mg/kg daily. Two rats from each subgroup were sacrificed at one day, seven and 28 days. Group II (MT- treated group) 18 rats, each received a single ip dose of 250 mg/kg *MT*. They were further divided into three equal subgroups: IIa sacrificed at one day, IIb at seven days and IIc at 28 days. Group III (MT plus RV-treated group) 18 rats, each received a single ip dose of *MT* plus daily *RV* orally. They were further divided into three equal subgroups: IIIa sacrificed at one day, IIIb at seven days and IIIc at 28 days. Body (BW) and liver weights were recorded and blood samples were collected for Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP). Livers of rats were processed for light and electron microscopic examination.

MT administration induced significant decrease in BW and relative liver weights and significant increase in ALT, AST, and ALP levels in addition to histological alterations. Some hepatocytes showed pyknotic nuclei, rarefied, vacuolated, or dark cytoplasm, fat-like droplets with congestion of blood sinusoids and widened space of Disse, appearance of collagenous bundles, abnormal mitochondria with fusion of cytoplasmic organelles in Electron microscopy. Addition of RV to MT in group III significantly improved BW and relative liver weights, biochemical parameters as well as histological picture. *MT* administration resulted in marked degeneration in the liver that was ameliorated by with concomitant administration of *RV*.

Key words: Malathion – *Rumex vesicarius L.* – Liver – Histology – Electron microscopy

INTRODUCTION

Pesticide use has increased markedly in Saudi Arabia going parallel with the increased development in agriculture. In this regard, the consumption of organophosphorous (OP) pesticides is high and increasing dramatically (Saggu et al., 2016).

The incidence of OP poisoning has increased in recent years in Saudi Arabia. Poisoning has a higher incidence in males both by agricultural contamination and by accidental or suicidal exposures (Al Jumaan et al., 2015). It was found that household OP poisoning can occur due to lack of knowledge, easy access and unsafe storage of these products (Barghash et al., 2016).

Organophosphorous (OP) compunds as malathion, cypermethrin, fenitrothion and thiodan showed neurotoxic effects directly associated with cholinesterase inactivation. They also possessed mutagenic and carcinogenic properties and showed organ-specific toxicity in relation to the heart, kidney and other organs (Cabello et al., 2001; Amer et al., 2002). In addition, Hazarika et al. (2003) suggested that OP insecticides caused oxidase-catalyzed bioactivation to axons that could induce

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cholinergic crisis in mammals.

Malathion (*MT*) is an insecticide of the OP group showing strong insecticidal properties (Ballantyne et al., 2009). It is one of the most frequently used OP insecticides in the world, both in agriculture as well as in residential settings. It has been used in malaria eradication programs in Africa and Central America. It was also used in wide-scale pest control plans including the Mediterranean fruit fly in the United States through aerial applications (Buratti et al., 2005). The reason for such widespread use of *MT* lies in its relatively low toxicity to mammals and high selectivity toward insects, paralleled by its moderate persistence in the environment, when compared with other *OP* insecticides. However, *MT* has a high potential risk for human exposure (Delegado et al., 2006; Abd.Rashid et al., 2008). Shampoos containing small amounts of *MT* are considered safe and effective in the treatment of head lice despite considerable skin absorption (Wananukul et al., 2011).

Many health complications have been reported that were attributed to MT toxicity such as visual loss, kidney damage (Albright et al., 1983), lung damage (Division of Toxicology & Physiology, 1983) and other myriad negative health effects. It has been linked to child leukemia, aplastic anemia and adult leukopenia (Reeves et al., 1981). MT exposure was associated with an increased incidence of breast cancer (Omran and Omer, 2015). It directly disturbs the biosynthesis of thyroid hormone (Xiong et al., 2017). MT has disruptive effects on sperm motility and life span. (Kocabaş et al., 2018). It has toxic effect on the ovaries in female rats. It causes follicular degeneration, vascular congestion, hemorrhage, edema and inflammatory cell infiltration (Ozsoy et al., 2016). Higher concentrations of MT were associated with thinner endometrial thickness, lower retrieval, and fertilization and embryo cleavage rates. High MT concentrations in the follicular fluid can adversely affect the embryological artificial fertilization outcomes (Al- Hussaini et al., 2018).

MT exposure is a proven risk for the development of hyperglycemia. This may be due to the induction of insulin resistance (Mostafalou et al. 2012; Lasram et al. 2015; Ramirez-Vargas et al., 2017) or enhancing hepatic glucose production (Vosough-Ghanbari et al., 2007). The greatest accumulation of MT was found to occur in the liver and kidney (Ballantyne et al., 2009). Several key biological processes have been reported to be involved in MT-induced hepatotoxicity, such as disturbances in the antioxidant defense system, oxidative stress, apoptosis, and mitochondrial and microsomal metabolism disturbance (Karami-Mohajeri et al., 2017).

Inhibition of liver acetylcholinesterase (AChE) activity is normally regarded as a practical parameter of poisoning by OP pesticides. Malathion's toxicity is enhanced by its metabolites and contaminates. It is readily oxidized into its metabolite malaoxon in mammals, insects, and plants, which is the major source of malathion's toxicity and is 40 times more toxic than malathion (Aldridge et al., 1979).

Medicinal plants are believed to be a rich source of antioxidants. Moreover, they are considered as cost effective and potent alternatives with minimal side effects in the management of liver disorders, when compared with the existing conventional therapeutic drugs available in the market (Ortega-Ramirez et al., 2014; Guan and He, 2015).

Rumex vesicarius L. *(RV),* locally known as Al-Humaid, is a wild edible annual plant, eaten fresh or cooked (Mostafa et al., 2011). It grows throughout desert and semi-desert areas of North Africa, Asia, and Australia (Rechinger, 1984). In Saudi Arabia, R. vesicarius *(RV)* is widely used as food, as a medicinal herb, and as an antidote to scorpion stings. The leaves are used as aperient, diuretic, and considered as an antidote against snake and scorpion venom (Ganaei et al., 2015). The antibacterial and antioxidant activities of *RV* have been well known (Panduraju et al., 2009).

The medicinal importance of *RV* is a reflection to its chemical composition, since the plant contains many bioactive substances including flavonoids (vitexin, isovitexin, orientin and isorientin). The plant is also rich in anthraquinones, particularly emodin and chrysophanol in roots. The plant also contains carotenoids, vitamins (especially vitamin C), proteins, lipids and organic acids (Barbosa-Filho et al., 2008). The previously mentioned bioactive phytochemicals (such as polyphenols, flavonoids, carotenoids, tocopherols and ascorbic acid) have a role as antioxidant and detoxifying agents. The intake of dietary antioxidant phytochemicals like carotenoids, phenolic compounds and flavonoids may lead to the protection against non-communicable diseases in humans such as cancer, cardiovascular diseases, and cataract (Matkowski, 2008).

Although the liver is the primary organ affected by the *MT* toxicity, to our knowledge, only few studies have described in detail its effect on the histological and ultrastructure of the liver. Also, no previous study has tested the use of *RV* as a possible protective measure against *MT* hepatotoxicity. Therefore, the aim of the current study was to illustrate clearly the effect of administration of *MT* on the histological structure of the liver and to find out the potential protective role of concomitant administration of *RV* in adult albino rats.

MATERIALS AND METHODS

Drugs and chemicals

MT was obtained from the National Organization for Drug Control and Research, Cairo, Egypt.

RV was collected as the whole plant from Al-Kharj area, Kingdom of Saudi Arabia and was authenticated in the College of Pharmacy, Riyadh Elm University, Riyadh, Saudi Arabia. The plants were cleaned, dried under shade, ground to a coarse powder, and stored in an air-tight container at 25ºC for further use. Then the air-dried and fine powder of *RV* was subjected to extraction with

methanol by using the percolation method, three times, with an interval of 24 hours each. The container with its contents was sealed and kept for a period of 4 days with occasional shaking and stirring. The extract was filtered in the Buchner funnel and the filtrate was concentrated with a rotary evaporator at bath temperature not exceeding 40ºC to have a gummy concentrate of greenish black extract (Ganaei et al., 2015).

All other chemicals were of analytical grade and chemicals required for all biochemical assays were obtained from Sigma- Aldrich Chemicals Company (St. Louis, Mo, USA).

Animals and treatments

This study was carried out on forty-eight male adult albino rats weighing between 180–200g in the animal house of Kasr-Al-Aini, Faculty of Medicine, in Cairo University. All experimental procedures were conformed to the principles laid down by the National Research Council Guide for the Care and Use of Laboratory Animals with CU-IACUC reviewers approval number CUIIIS1418.

The animals were divided into three groups:

Group I (negative control group): twelve rats subdivided into two subgroups:

Subgroup Ia (corn oil-treated group): six rats that received a single dose of ½ ml corn oil intraperitoneally (IP). Two rats were sacrificed at one, seven and 28 days respectively after corn oil administration.

Subgroup Ib (*RV*-treated group): six rats that received methanolic extract of *RV* 200 mg/kg orally by a gastric gavage daily (Ozsoy et al., 2016). Two rats were sacrificed at one, seven and 28 days respectively after *RV* administration.

Group II (*MT*-treated group): eighteen rats that received a single dose of 250 mg/kg MT dissolved in ½ ml corn oil IP (Franco et al., 2009). They were further divided into 3 subgroups (6 rats each) according to the time of sacrifice: subgroup IIa was sacrificed at one day (Banasik et al., 2003), subgroup IIb was sacrificed at seven days (David et al., 2007) and subgroup IIc was sacrificed at 28 days (Franco et al., 2009) after MT administration.

Group III (*MT* plus *RV*-treated group): eighteen rats that received a single dose of 250 mg/kg *MT* dissolved in ½ ml corn oil IP and a daily dose of *RV* 200 mg/kg orally by gastric gavage. They were further divided into 3 subgroups (6 rats each) according to the time of sacrifice: subgroup IIIa was sacrificed at one day, subgroup IIIb was sacrificed at one week & subgroup IIIc was sacrificed at 28 days after *MT* and *RV* administration.

Experimental procedure

On the due date for sacrifice in each group, each rat was weighed and then was sacrificed by decapitation under isoflurane anesthesia after blood collection for biochemical study. Blood samples were collected for the biochemical assays. The anterior abdominal wall was opened by a midline incision. The liver was carefully dissected, weighed and then a small specimen (1x1 mm) was cut for electron microscopy tissue processing, and the rest of the liver was reserved for light microscopy.

Body and liver weights:

Body weight of all rats was recorded at the beginning and before sacrifice. After blood collection, the rats were sacrificed by cervical dislocation; their livers were separated and weighted individually. Then, the relative liver weight was calculated (the relative weight of the organ equals the weight of the organ divided by the weight of whole rat body).

Biochemical assay:

Blood samples of the rats were taken from retro-orbital venous plexus, placed into sterile tubes and centrifuged at 3500 rpm for 20 min to separate the serum. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined according to Reitman and Frankel (1957) and Alkaline phosphatase (ALP) was determined according to Kochmar and Mossa (1976).

Light microscopic study:

The liver tissue was dehydrated and embedded in paraffin. Sections of 5µm-thickness were cut by microtome, subjected to Hematoxylin & Eosin staining technique and examined by light microscopy (Bancroft & Gamble, 2002).

Electron microscopic study:

The specimen selected for electron microscopy was fixed in fresh 3% glutaraldehyde at 4°C for four hours, washed in 0.15M phosphate buffer, pH 7.4, for two hours (two changes), postfixed in 1% osmium tetroxide for one hour at 4°C, dehydrated and embedded in epoxy resin. Serial semithin sections were cut at 1µm-thickness by Seo UMTP-6M ultramicrotome, stained with 1% toluidine blue and examined by light microscope. For electron microscopy, ultrathin sections (0.1 µm thick) were prepared using the same ultramicrotome and stained with uranyl acetate and lead citrate (Hayat, 2000). The sections were examined by Seo transmission electron microscope (TEM).

Statistical Analysis

Body/liver weights and biochemistry data were expressed as mean values ± SD and statistical analyses were performed using SPSS statistical software package for Windows version 22 (SPSS® Inc., USA). The criterion for statistical significance was set at $P < 0.05$.

RESULTS

Body and liver weights

No mortality occurred during the experimental period. Data of final body weights and relative liver weights of rats subjected to different treatments are shown in Table 1. It was observed that MT-treated rats (Group II) presented significant decreases ($P<0.01$) in body weights and relative liver weights compared to control (Group I) and *MT* and

Table 1. Body and relative organs weights of experimental rats.

	Initial BW (g)	Final BW (g)	BW gain (g)	Liver weight (g)	Liver-to-body weight ratio
Group I (sacrificed after 1 day)	183±5.7	185±10.7	2±6.9	4±0.2	0.022±0.002
Group I (sacrificed after 7 days)	187±8.3	204±11.2	10±8.1	4.2±0.1	0.021±0.001
Group I (sacrificed after 28 days)	186±4.4	228±13.6	38±9.3	4.3±0.2	0.019±0.001
Group IIa (sacrificed after 1 day)	185±6.2	180±17.7*	0.3±14.6*	3.8±0.2*	0.021±0.001*
Group IIb (sacrificed after 7 days)	183±7.9	185±22.8*	2±19.5*	3.1±0.1*	0.017±0.002*
Group IIc (sacrificed after 28 days)	189.9±3.8	207±14.3*	7.1±21.7*	2.8±0.1*	0.014±0.001*
Group IIIa (sacrificed after 1 day)	186±5.1	196.5±9.4**	0.5±13.8**	3.9±0.2**	0.020±0.001**
Group IIIb (sacrificed after 7 days)	182±6.6	196±12.8**	6±17.5**	3.6±0.1**	0.018±0.002**
Group IIIc (sacrificed after 28 days)	191±7.2	222±17.1**	31±22.3**	3.5±0.1**	0.016±0.002**

Values are expressed as mean ± S.D. *P < 0.01 relative to Group I; **P < 0.01 relative to Group II. Group I – Control. Group II – MT. Group III - MT+ RV.

RV-treated group (Group III). The decrease in body weights and relative liver weights was significantly less (P<0.01) in the *MT* and *RV*-treated group (Group III) when compared to the MT-treated rats (Group II). Moreover, no significant differences were observed in the body weights and relative liver weights between *MT* and *RV*-treated group (Group III) and those of the control group (Group I) (Table 1).

Biochemical assay

Biochemical parameters showed significant (p<0.05) increase in ALT, AST, and ALP levels in MT group (Group II) when compared with the control group (Group I). Moreover, the increase in the ALT, AST, and ALP levels in the MT plus RV treated group (Group III) was significantly less (P<0.01) when compared to the MT-treated rats (Group II), as shown in Table 2.

Light Microscopic study:

All sections of **group I** (negative control group) revealed the sound histological structure of the liver, with cords and plates of hepatocytes radiating from the central vein and enclosing between them the blood sinusoids. The hepatocytes exhibited acidophilic cytoplasm and vesicular nuclei with one or two nucleoli (Figs. 1A, 1B). Von Kupffer cells were seen with their heterochromatic nuclei and prominent nucleoli (Fig. 1B).

MT administration in **group II** resulted in many alterations in the liver architecture. **Subgroup IIa** presented few apparently normal hepatocytes, while others were degenerated with rarefied or vacuolated cytoplasm. Some hepatocytes had pyknotic nuclei and othersshowed darkly stained cytoplasm. The central vein appeared congested. The sinusoids displayed mild dilatation and congestion. Von Kupffer cells appeared unremarkable (Figs. 1C, 1D).

In **subgroup IIb**, few hepatocytes showed darkly stained cytoplasm and pyknotic nuclei (Fig. 1E). The cytoplasm of most of hepatocytes was markedly vacuolated (Fig. 1F). The branches of the portal vein and some blood sinusoids were moderately dilated and congested. The portal tract showed mild mononuclear cell infiltration (Figs. 1E, 1F). Von Kupffer cells were unremarkable (Fig. 1F).

In **subgroup IIc,** some hepatocytes showed pyknotic nuclei (Fig. 1G). Many hepatocytes were degenerated with vacuolation or rarefaction of their cytoplasm. Moderate mononuclear cell infiltration was observed. The central vein and blood sinus-

Table 2. Serum biochemical parameters (Mean± SE) in control, MT and MT+RV groups.

	AST (U/L)	ALT (U/L)	ALP (U/L)
Group I (sacrificed after 1 day)	32.72±1.7	43.84±2.1	102.23±11.9
Group I (sacrificed after 7 days)	33.68±1.5	41±1.3	105±14.8
Group I (sacrificed after 28 days)	30.96±2.1	44±1.9	103±9.7
Group IIa (sacrificed after 1 day)	59±9.8*	65±19.4*	109±17.3*
Group IIb (sacrificed after 7 days)	67±21.0*	78±13.7*	144±19.6*
Group IIc (sacrificed after 28 days)	71±16.8*	84±12.8*	158±13.7*
Group IIIa (sacrificed after 1 day)	52±8.7**	58±9.9**	111±16.2**
Group IIIb (sacrificed after 7 days)	48±11.0**	53±12.6**	132±11.8**
Group IIIc (sacrificed after 28 days)	46±13.3**	51±17.9**	127±14.9**

Values are expressed as mean ± S.D. *P < 0.01 relative to Group I; **P < 0.01 relative to Group II. Group I – Control. Group II – MT. Group III - MT+ RV.

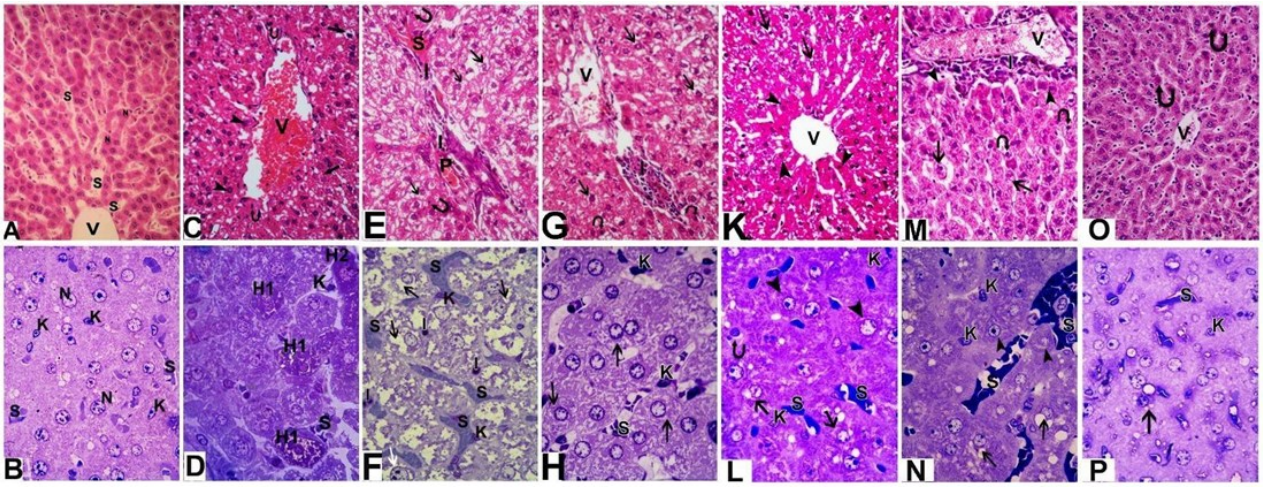

Fig 1. Photomicrographs from light microscopy. (**A**) Negative control rat: presenting the sound structure of the liver with a central vein (V). Cords and plates of hepatocytes radiate from the central vein with sinusoids (S) between them. The hepatocytes appear with acidophilic cytoplasm and vesicular nuclei (N). (H&E, X 400). (B) Negative control rat: liver sinusoids (S). The hepatocytes look with vesicular nuclei (N) which show one or two nucleoli. Von Kupffer cells (K) appear with heterochromatic nuclei and prominent nucleoli. (Toluidine blue, X 1000). (**C**) Rat of subgroup IIa: few apparently normal hepatocytes (arrow head), while others are degenerated with rarefied or vacuolated cytoplasm (arrow) or appear with pyknotic nuclei (curved arrow). The central vein (V) appears congested. (H&E, X 400). (**D**) Rat of subgroup IIa: displaying some degenerated hepatocytes. The hepatocytes appear with vacuolated cytoplasm and pyknotic nuclei (H1) or with darkly stained cytoplasm (H2). The sinusoids (S) show mild dilatation and congestion with normal Von Kupffer cells (K). (Toluidine blue, X 1000). (**E**) Rat of subgroup IIb: few hepatocytes show darkly stained cytoplasm and pyknotic nuclei (curved arrow). Most of the hepatocytes are degenerated with marked vacuolation of their cytoplasm (arrow). A portal tract appears with mild mononuclear cell infiltration (I). Moderately congested blood sinusoid (S) and a branch of the portal vein (P) can be observed. (H&E, X 400). (**F**) Rat of subgroup IIb: degenerated hepatocytes with markedly vacuolated cytoplasm (arrow). The blood sinusoids (S) are moderately dilated and congested. Mononuclear cell infiltration (I) can be observed. Von Kupffer cells (K) appear normal. (Toluidine blue, X 1000). (**G**) Rat of subgroup IIc: few hepatocytes with pyknotic nuclei (curved arrow). Many hepatocytes are degenerated with vacuolation of their cytoplasm (arrow). The central vein (V) appears mildly congested. Moderate mononuclear cell infiltration (I) is observed. (H&E, X 400). (**H**) Rat of subgroup IIc: rarefaction of the cytoplasm of most of hepatocytes (arrow). The blood sinusoids (S) are dilated and mildly congested. Von Kupffer cells (K) appear normal. (Toluidine blue, X 1000). (K) Rat of subgroup IIIa: most hepatocytes are with sound structure (arrow head) and few are degenerated (arrow) with rarefied or vacuolated cytoplasm. The central vein (V) appears normal. (H&E, X 400). (L) Rat of subgroup IIIa: some hepatocytes with vacuolated cytoplasm (arrow). A hepatocyte appears with pyknotic nucleus (curved arrow). Other hepatocytes appear with sound structure (arrow head). The blood sinusoids (S) show minimal congestion and dilatation. Von Kupffer cells (K) appear normal. (Toluidine blue, X 1000). (M) Rat of subgroup IIIb: most of hepatocytes appear with vacuolated cytoplasm (arrow). Few of them show pyknotic nuclei (curved arrow). Some hepatocytes appear normal (arrow head). The central vein (V) appears congested surrounded by mononuclear cell infiltration (I). (H&E, X 400). (N) Rat of subgroup IIIb: some hepatocytes exhibited vacuolated cytoplasm (arrow). Other hepatocytes appear normal (arrow head). The blood sinusoids (S) are moderately dilated and congested. Von Kupffer cells (K) appear normal. (Toluidine blue, X 1000). (O) Rat of subgroup IIIc: most of the hepatocytes appear normal. Few hepatocytes have pyknotic nuclei (curved arrow). The central vein (V) appears normal. (H&E, X 400). (P) Rat of subgroup IIIc: most hepatocytes are normal. Few hepatocytes appear with vacuolated cytoplasm (arrow). The blood sinusoids (S) show minimal congestion. Von Kupffer cells (K) appear normal. (Toluidine blue, X 1000).

oids showed mild congestion and dilatation (Figs. 1G, 1H). Von Kupffer cells had a normal appearance (Fig. 1H).

Little histological alterations were detected in **group IIIa** in most hepatocytes, with only few hepatocytes occasionally presenting vacuolated or rarefied cytoplasm (Figs. 1K, 1L). Von Kupffer cells appeared normal and the blood sinusoids were minimally congested (Fig. 1L). Most hepatocytes of **group IIIb** had vacuolated cytoplasm and only few cells looked normal (Figs. 1M, 1N). Few hepatocytes showed pyknotic nuclei. The central vein appeared congested and surrounded by mononuclear cellular infiltration (Fig. 1M). The blood sinusoids were moderately congested. Von Kupffer cells seemed normally appearing (Fig. 1N). No abnormal histological changes were observed in **group IIIc** apart from few hepatocytes displaying pyknotic nuclei (Fig. 1O) or vacuolated cytoplasm (Fig. 1P). The blood sinusoids appeared minimally congested (Fig. 1P).

Electron microscopic study

Electron microscopic examination of liver tissues of **group I** revealed the normal ultrastructure of the liver. The hepatocytes nuclei were euchromatic with prominent nucleoli (Fig. 2A). The cells showed many normally looking mitochondria and rough endoplasmic reticulum (Figs. 2A, 2B). The space of Disse was identified between the hepatocytes and blood sinusoids. The blood sinusoids contained red blood corpuscles (Fig. 2B).

Many ultrastructural alterations in the liver were observed after MT administration **(group II). Subgroup IIa** showed rarefaction of the cytoplasm of the hepatocytes, apparent mild congestion of

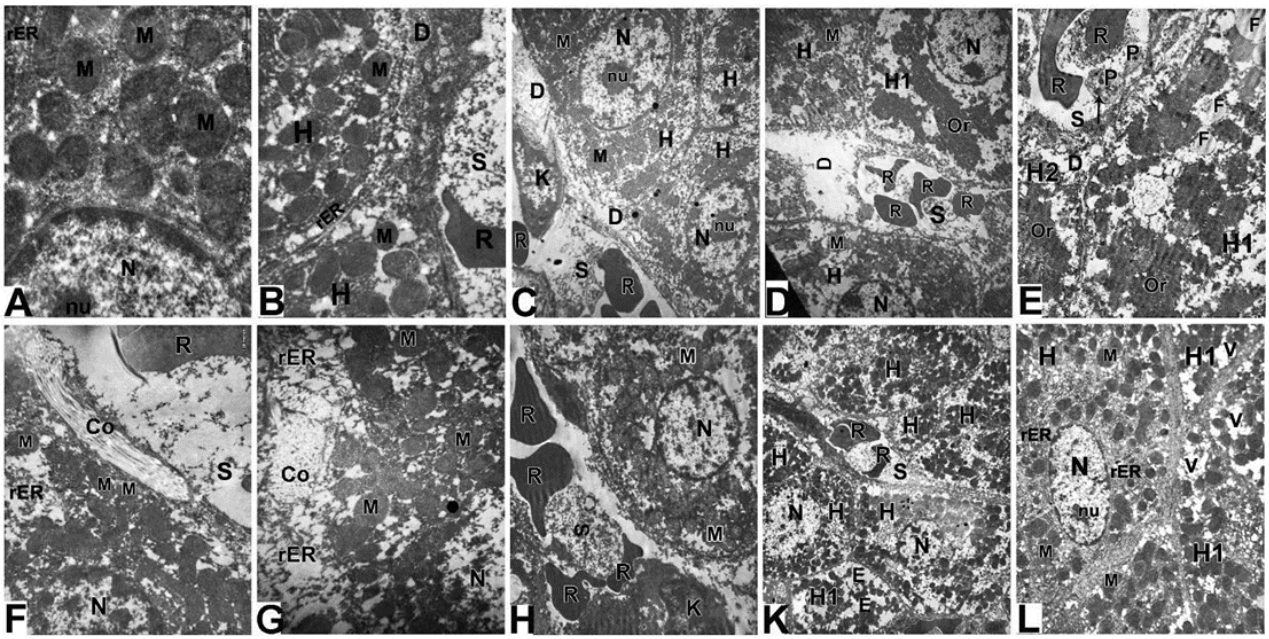

Fig 2. Images from transmission electron microscopy. (A) Negative control rat: a part of a hepatocyte with euchromatic nucleus (N) with prominent nucleolus (nu). Many preserved mitochondria (M) and rough endoplasmic reticulum (rER) are observed (X 15,000). (B) Negative control rat: part of two apparently normal hepatocytes (H) with many preserved mitochondria (M) and rough endoplasmic reticulum (rER). The space of Disse (D) is observed between the hepatocytes and the blood sinusoid (S) which contains a red blood corpuscle (R) (X 8000). (C) Rat of subgroup IIa: parts of three hepatocytes (H) appear with rarefied cytoplasm. Two of them show euchromatic nuclei (N) with prominent nucleoli (nu). A Von Kupffer cell (K) is observed lining a mildly congested blood sinusoid (S) with red blood corpuscles (R) in its lumen. The space of Disse (D) is widened (X 3000). (D) Rat of subgroup IIb: Two hepatocytes (H) show mitochondria (M) and rarefied cytoplasm. One hepatocyte (H1) shows marked rarefaction of the cytoplasm with marked degeneration and fusion of organelles (Or). Nuclei (N) of two hepatocytes are observed. A congested blood sinusoid (S) appears with red blood corpuscles (R) in its lumen. The space of Disse (D) is widened (X 2000). (E) Rat of subgroup IIb: parts of two hepatocytes (H1 & H2) with rarefied cytoplasm and degenerated fused organelles (Or). One hepatocyte (H1) contains fat like droplets (F). The blood sinusoid (S) is congested and contains red blood corpuscles (R). The processes (P) of Von Kupffer cells, containing many lysosomes (arrow), are observed protruding into the lumen. The space of Disse (D) is widened (X 4000). (F) Rat of subgroup IIc: a part of hepatocyte with heterochromatic (condensed) nucleus (N) and rarefied cytoplasm. The cytoplasm contains rough endoplasmic reticulum (rER) and many mitochondria (M) which appear nearly normal. The blood sinusoid (S) is mildly congested and contains a red blood corpuscle (R). A perisinusoidal longitudinal bundle of collagenous fibers (Co) is observed (X 6000). (G) Rat of subgroup IIc: a part of hepatocyte appears with euchromatic nucleus (N) and rarefied cytoplasm. The cytoplasm contains rough endoplasmic reticulum (rER) and many mitochondria (M) which appear nearly normal. A transversely cut bundle of collagenous fibers (Co) is observed (X 8000). (H) Rat of subgroup IIIa: parts of some hepatocytes (H) appear with rarefied cytoplasm. Two of them show euchromatic nuclei (N). One hepatocyte (H1) shows marked rarefaction of cytoplasm. A mildly congested blood sinusoid (S), with red blood corpuscles (R), is observed. The space of Disse (D) appears normal. A part of Von Kupffer cell (K) can be observed (X 2000). (K) Rat of subgroup IIIb: a part of hepatocyte with euchromatic nucleus (N) and rarefied cytoplasm. The cytoplasm contains many mitochondria (M). The blood sinusoid (S) appears congested and contains many red blood corpuscles (R) (X 3000). (L) Rat of subgroup IIIc: parts of three hepatocytes are seen. One of them (H) appears nearly normal with euchromatic nucleus (N) with prominent nucleolus (nu), many mitochondria (M) and rough endoplasmic reticulum (rER). The other two hepatocytes (H1) show vacuolations (V) in their cytoplasm (X 4000).

the blood sinusoids enclosing red blood corpuscles and widening of the space of Disse (Fig. 2C). In **subgroup IIb**, most hepatocytes showed rarefied cytoplasm. Some hepatocytes appeared degenerated with fusion of their organelles. The blood sinusoids appeared congested with red blood corpuscles. The space of Disse was widened in some areas (Figs. 2D, 2E). Additionally, some hepatocytes contained fat-like droplets.

Von Kupffer cells containing many lysosomes were observed and their processes were seen protruding into the sinusoidal lumen (Fig. 2E). In **subgroup IIc**, some hepatocytes appeared with heterochromatic condensed nuclei. The blood sinusoids appeared mildly congested with red blood corpuscles (Fig. 2F). Many hepatocytes showed rarefied cytoplasm with many nearly normally appearing mitochondria and rough endoplasmic reticulum. Collagenous bundles were observed in the interstitial tissue between the hepatocytes and around the blood sinusoids (Figs. 2F, 2G).

S**ubgroup IIIa** showed rarefied hepatocytes cytoplasm which was marked in few of them. The blood sinusoids appeared mildly congested with red blood corpuscles. The space of Disse had a normal appearance (Fig. 2H). In **subgroup IIIb,** some hepatocytes exhibited rarefied cytoplasm with many mitochondria. The blood sinusoids appeared moderately congested and contained red blood corpuscles (Fig. 2K). The ultrastructure of

the liver of **subgroup IIIc** appeared nearly normal apart from few hepatocytes having vacuolated cytoplasm (Fig. 2L).

DISCUSSION

Malathion *(MT)* is an organophosphorus (OP) insecticide used in agriculture, commercial extermination, fumigation, veterinary practices, domestic and other public health purposes (Brocardo et al., 2007). Because of its relatively low mammalian toxicity, *MT* has become one of the most commonly used OP compounds in the world; and hence became one of the major sources of occupational exposure to pesticides (Gwinn et al., 2005, Bonner et al., 2007).

The liver was chosen to be the study organ in this work as it has been reported that the greatest pesticides accumulation, after exposure, was in the liver and kidney (Ballantyne et al., 2009).

In toxicological studies, organ and relative organ weights are important criteria for evaluation of toxicity (Crissman et al., 2004). In the current study, the body weight and relative liver weights of rats treated with MT were significantly lower than those of the control group. This may be attributed to anorexia leading to decreased food intake due to treatment related toxicity. Furthermore, MT may induce oxidative stress leading to generation of free radicals and alterations in antioxidant status or ROS which can cause metabolic disorder and weight loss. For this reason, treatment with remedies rich in antioxidants and free radical scavengers can decrease the oxidative stress and improve the metabolic processes in MT exposed rats, hence improving food intake and consequently their BW and liver/body weight ratio. In the present study, supplementation with RV in MT-treated rats significantly improved BW and relative liver weights as compared to the rats of the MT-treated group.

It has been shown that OP compounds can elevate the enzymatic activities of AST, ALT, and ALP (Ogutcu et al., 2008; Farghaly and El-Maghraby, 2009). AST and ALT are two hepatic enzymes released into the blood in the event of hepatic cellular destruction proportionate to the intensity of the cellular injury (Vinogradova et al., 1989). Consequently, these serum enzymes are deemed markers of liver damage (Eissa and Zidan, 2010). In this study, the results of AST ALT and ALP might mirror the degenerative changes and disturbed liver function as the effect of MT was hepatocytes damage and release of their intracellular enzymes to the blood. The same explanation was reported by Ncibi et al. (2008).

In the present study, *MT* administration resulted in many histological alterations in the liver. After one day, many hepatocytes were degenerated with rarefied or vacuolated cytoplasm. Some of them appeared with pyknotic and heterochromatic nuclei or with darkly stained cytoplasm. Those degenerative changes were marked and affected most of the hepatocytes after one week. Those changes became less intense but affecting many hepatocytes four weeks after administration. These findings were in correspondence with those of Abdel-Salam et al. (2017), who found that a single acute *MT* intoxication resulted in severe damage of liver structure along with disarrangement of hepatic lobules with formation of fibrotic strands. The hepatic cells showed degenerative features in form of vacuolar ballooning cytoplasm with pyknotic nuclei, inflammatory infiltrations especially around hepatic vessels which appeared mostly dilated and congested with bile ductal epithelium hyperplasia. Similar results were reported by Rezg et al. (2007), who found significant decrease in attic proteins and lipid contents in the hepatocytes that altered their functions 32 days after *MT* exposure. Also, Saadi et al. (2008) described disarrangement and degeneration of hepatocytes 40 days after *MT* administration.

Moreover, Velmurugan et al. (2009) found degeneration and cloudy swelling of the hepatocytes after one week of cypermethrin administration. Comparative results were obtained by Afshar et al. (2008), who described parenchymatous degeneration of the hepatocytes and mild necrosis four weeks after fenitrothion administration. On the other hand, Tós-Luty et al. (2003) reported that parenchymatous degeneration affected only 10% of the hepatocytes after *MT* administration. However, their route of administration was dermal. More severe results of *MT* administration were mentioned by Mamun et al. (2015). They described severe damage in hepatic tissue including prominent enlargement of sinusoids, mononuclear cell infiltration, vacuole formation, hemorrhage and necrosis. However they administered *MT* daily for 15 days in their experiment.

In the present work, fat-like droplets were detected in the cytoplasm of the hepatocytes one week after *MT* administration. Similar findings were observed by Tós-Luty et al. (2000, 2003), where they described lipid droplets four weeks after daily *MT* administration. Moreover, Velmurugan et al. (2009) reported almost the same findings one week after cypermethrin administration. This could be related to be the process of fatty degeneration development in the hepatocytes.

In the current work, *MT* induced congestion in the central vein and sinusoids. This congestion was maximal 7 days after *MT* administration. These findings were in correspondence with those of Baiomy et al. (2015), who found congestion of the central vein after *MT* administration. They proved that the *MT* induced hepatotoxicity could be ameliorated by the addition of ginger and ZnCl2 mixture concomitant with the exposure to the insecticide.

In the present study, it was observed that most mitochondria of the examined hepatocytes were degenerated with fusion of organelles, 7 days after *MT* administration, while they appeared normal one day and 28 days after *MT* administration. On the other hand, Tós-Luty et al. (2003) found swollen mitochondria and residues of cell organelles in

focal areas four weeks after *MT* administration. However, they administered the *MT* daily in their study.

The current study revealed congestion in some central and portal veins as well as in the blood sinusoids. This congestion was moderate one week, but milder one day and four weeks after *MT* administration. These findings were coinciding with the findings of Saadi et al. (2008), who described dilated sinusoidal spaces 40 days after *MT* administration. Similar findings were observed by Manna et al. (2004) and Afshar et al. (2008) four weeks after cypermethrin and fenitrothion administration respectively. Also, the space of Disse appeared widened at one day and one week after *MT* administration suggesting the presence of edema as a part of the inflammatory process.

In the present work, one week after *MT* administration, Von Kupffer cells appeared filled with numerous lysosomes and their processes were seen protruding into the sinusoidal lumen. Nowak and Kingsford (2003) described prominent lysosomes four weeks after thiodan administration.

In this study, perivascular mononuclear cellular infiltration was observed one and 28 days after *MT* administration. Such findings were previously reported four weeks after *MT* administration by Tós-Luty et al. (2000, 2003) and four weeks after of fenitrothion administration by Afshar et al. (2008). Also, the present study revealed the presence of collagenous bundles in the interstitial tissue between the hepatocytes and around the blood sinusoids four weeks after *MT* administration. This, together with cellular infiltration, raised the assumption that *MT* induced hepatic interstitial inflammation that ultimately ended by interstitial fibrosis.

Moreover, the findings of the present work are supported by those of David et al. (2007) who described degenerative effects in hepatocytes, evidenced by biochemical assays, through the first 15 days after *MT* administration which returned near normal after 25 days from administration.

The mechanism of cytotoxicity of *MT* on the hepatocytes was the concern of many authors. Masten et al. (2001) found that *MT* affected the gap junction intercellular communications between the hepatocytes which led to their affection. Abdel-Salam et al. (2017) attributed *MT* toxicity to a significant increase in lipid peroxidation in the liver of rats. They found that *MT* administration caused elevation of liver aspartate aminotransferase, alanine aminotransferase. It also caused significant decrease in liver glutathione and paraoxonase-1 activity and total antioxidant capacity, suggesting that the mechanism of liver cell damage by *MT* involved the excessive generation of free radicals. The same findings were reported by Ince et al. (2017) who demonstrated that taurine alleviated MT induced lipid peroxidation, oxidative stress, and proinflammatory cytokine gene expressions in rats. Additionally, Coban et al. (2015) showed that Boron attenuated *MT*-induced oxidative stress and acetylcholinesterase inhibition in rats.

Delegado et al. (2006) reported that the *MT* induced excessive generation of reactive oxidative species (ROS) due to mitochondrial respiratory chain dysfunction which led to the development of oxidative stress. However, Mişe et al. (2017) attributed the oxidative stress to the inhibition of the antioxidant defenses as glutathione reductase, glutathione peroxidase, glutathione S-transferase, catalase and superoxide dismutase. In addition, Naraharisetti et al. (2008) reported that oxidative stress of *MT* affected cytochrome P450, cytochrome b5 and microsomal and cytosolic proteins of the hepatocytes.

The resulting oxidative stress would lead to lipid peroxidation as evidenced by Akhgari et al. (2003) and Fortunato et al. (2006). This lipid peroxidation led to the degradation of membrane lipids, resulting in deterioration of the cellular integrity (Rezg et al., 2008).

In contrast, Ramirez-Vargas et al. (2017) suggested that alteration of glucose homeostasis was a possible mechanism for the hepatotoxicity associated with *MT* exposure.

Furthermore, Amer et al. (2002) postulated that the degenerative effects of *MT* were attributed to its genotoxicity. Giri et al. (2002) also considered *MT* to be a potent genotoxic agent that can be regarded as potential mutagenic agent. This was supported by Muniz et al. (2008) and Réus et al. (2008), who referred the cytotoxic effect of *MT* to be due DNA damage caused by oxidative stress. Réus et al. (2008) added that DNA damage may lead to cell mutation and cancer development. Moreover, Gao et al. (2018) reported that *MT* disturbed gut microbiome development and the quorum- sensing system.

It was evident in the current study that the degenerative effects of *MT* were attenuated by concomitant administration of *RV*. After one day, most hepatocytes had a normal appearance with only few hepatocytes displaying rarefied or vacuolated cytoplasm. After one week, some hepatocytes looked normal, but the rest appeared mildly degenerated with vacuolated cytoplasm and pyknotic nuclei. Four weeks after *MT* and *RV* administration, the normal histological picture of the liver was restored apart from few hepatocytes still having rarefied cytoplasm and pyknotic nuclei. The congestion detected in some central and portal veins as well as in the blood sinusoids was marked at seven days but milder at one and 28 days after *MT* and *RV* administration. However, perivascular mononuclear cellular infiltration was observed only at seven days after *MT* and *RV* administration.

The protective role of *RV* may be attributable to its antioxidant effect as it contains high levels of phenolic compounds and omega 3-fatty acids (Abou Elfotoh et al., 2013). The antioxidant activity was reported by (Tukappa et al., 2015) who investigated cytotoxicity and hepatoprotective attributes of methanolic extract of *RV* against CCl4- induced hepatotoxicity. Moreover, Ganaie et al. (2015) stated that treatment with *RV* reversed the changes produced by CCl4 on the liver. They added that

RV significantly decreased hepatic lipid peroxidation levels and increased the levels of hepatic biochemical markers to near normal levels. Pretreatment with *RV* was also able to raise the levels of glutathione. Hence, the hepatoprotective effect of *RV* may be the result of preservation of the glutathione levels in the tissues. Furthermore, Shahat et al. (2015) reported that *RV* protected against hepatocellular carcinoma. They found that *RV* extract reversed the significant increase in liver enzymes activity, CEA, AFP, AFU, glypican 3, golgi 73 and VEGF levels in serum as compared to the untreated hepatocellular carcinoma counterparts. In addition, the favorable impact of RV treatment was evidenced by the marked improvement in the histopathological features of the liver of the RV treated groups. They owed this protective effect to both its potent antiangiogenic activity as well as its effective antiproliferative capacity.

In this study, it could be concluded that the marked liver degeneration induced by *MT* administration was minimized by concomitant *RV* treatment. This might be due to the *RV* antioxidant and anti-inflammatory properties, thus precluding any lipid peroxidation or DNA damage in the hepatocytes. So, it is recommended to avoid the excessive use of *MT* in agriculture and for insect control whenever possible. It is also advisable for agricultural workers and insecticides sprayers exposed to OP compounds to wear protective clothes and face masks and to be supplemented with *RV.*

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