

Full Length Research Paper

## Could grape seed extract modulate nephritic damage induced by methomex in male rats?

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**Methomex (Metho)** is classified as a carbamate insecticide. The present study was designed to examine the influence of grape seed oil (GSO) on the histopathological changes in methomex-induced kidney damage in male rats. Rats were divided into 6 groups, the first of which was considered as the control. The 2nd group was treated with 4 ml/kg GSO. Rats from 3<sup>rd</sup> and 4<sup>th</sup> groups were treated with Metho at dose level of 2.4 and 4.8 mg/kg, respectively. Rats from 5<sup>th</sup> and 6<sup>th</sup> groups pre-administered with GSO were treated with 2.4 and 4.8 mg/kg Metho, respectively. Metho administration caused destruction of the normal pattern of the renal tissue. These damages were encountered by the presence of some glomeruli appeared atrophy with distension of Bowman's space and degeneration of their parietal epithelial cells. The lumina of distal and proximal convoluted tubules contain hyaline casts of dead cells. The renal medulla showed dilated collecting tubules stuffed with red blood cells (RBCs). Pre-administration of GSO to Metho-induced rats revealed apparent normal renal parenchyma. The proximal convoluted tubules and collecting tubules appeared near to normal with their narrow lumen. Pre-administration with GSO exhibited that it had a protective effect against methomex-induced toxic effects in the kidney. The present study advocated using GSO in the daily diets.

**Key words:** Grape seed oil, methomex, kidney, histopathology.

### INTRODUCTION

Carbamate insecticides are widely used in industry, agriculture and for public health purposes. Numerous incidents of acute carbamate poisoning have resulted from inhalation of sprays or contamination of crops or food (Mahgoub and El-Medany, 2001). This is due to the misuse of pesticides by concerned individuals and the absent or weak national controlling methods regarding the safe use of these chemicals (Ibitayo, 2006). Methomex (Metho) is classified by the Environmental Protection Agency (EPA) as a restricted-use pesticide (RUP) or class IB (highly hazardous) (Farré et al., 2002). Methomex, a derivative of carbamic acid, has been widely

marketed since 1967 as a broad-spectrum insecticide to control ticks and spiders (WHO, 1996). Its application as an insecticide is highly effective against a wide variety of pests, particularly those that are resistant to organophosphorus. It induced significant toxicity against the treated rats (El-Fakharany et al., 2011) by exerting its toxic effect via peroxidative damage to the hepatic, renal and splenic cell membranes and induces DNA damage in these organs (El-Khawaga, 2005).

Grape seed (*Vitis vinifera*) extracts are known to have high antioxidant activity and contain numerous polyphenols. The polyphenols have been shown to have positive

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**Abbreviations:** RUP, Restricted-use pesticide; GSO, grape seed oil; SOD, superoxide dismutase; GST, glutathione S-transferase; LPO, lipid peroxidation; HSCs, haematopoietic stem cells.

effects on vascular injury; it is also known to have free radicals scavenging and antimutagenic activity (Çetin et al., 2008). Grape seeds are rich sources of monomeric phenolic compounds such as catechin, epicatechin and dimeric, trimeric and tetrameric proanthocyanidins (Shin et al., 2010). These molecules possess a structure that confers on them an antioxidant property, which has been demonstrated to exert a novel spectrum of biological, pharmacological, therapeutic, and chemoprotective effects against oxygen free radicals and oxidative stress, which can be used as herbal remedies especially for controlling oxidative damages (El-Ashmawy et al., 2007; Dulundu et al., 2007). Several studies have indicated that extracts obtained from grape seed inhibit enzyme systems that are responsible for the production of free radicals, and that they are antimutagenic and anticarcinogenic (Pineiro et al., 2010). For this reason, grape seed extract is widely consumed as a dietary supplement in addition to the chemotherapeutic agents in cancer treatment (Çetin et al., 2008). Grape seed extracts have been reported to possess a broad spectrum of pharmacological and therapeutic effects including anti-inflammatory activity and reduced apoptotic cell death (Ashtiyani et al., 2013). The present study, therefore, investigated the protective effect of grape seed extract against nephritic damage induced by methomex in male rats.

## MATERIALS AND METHODS

### Reagents and doses

Methomex was obtained from Agriculture Pesticides Laboratory, Agriculture, Research Center, Giza, Egypt and used in the present study (methomex LD<sub>50</sub> = 48 mg/kg. body weight orally) according to the study of Thomson (1992). Grape seed oil (GSO) was obtained from the Unit of Squeeze and extraction of National Oils in National Research Center, Dokki, Cairo, Egypt. Grape seed oil was administered to rats at a dose level (4 ml/kg b.w.) according to the study of Maheswari and Rao (2005).

### Experimental animals

Adult male albino rats of the *Rattus rattus* strain weighing 100±10 g were obtained from the Egyptian Organization for Vaccine and Biological Preparations at Helwan, Egypt. They were housed in a controlled environmental room, with a 12 h light/dark cycle. The animals were classified into six groups (7 rats each).

### Experimental design

The duration of the present study was eight weeks. Rats were divided into 6 groups. The 1<sup>st</sup> group served as the control; these received a daily oral administration of saline by gastric tube. The 2<sup>nd</sup> group was orally administered GSO (4 ml/kg). The 3<sup>rd</sup> and 4<sup>th</sup> groups were given daily oral dose of 1/10 and 1/20 of LD<sub>50</sub>/day of methomex, respectively. Rats belonging to the 5<sup>th</sup> and 6<sup>th</sup> groups were pre-administered with GSO, then after 2 h, rats received oral dose of 1/10 and 1/20 of LD<sub>50</sub>/day of methomex, respectively. At the end of treatment period, rats were anaesthetized by ether and renal tissue samples were collected for histological and histochemical investigations.

## Histopathological studies

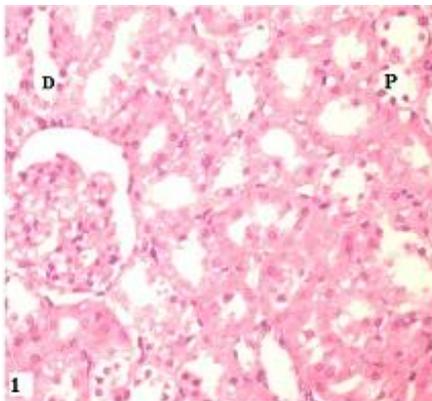
For light microscopic investigations, renal tissue specimens were fixed in 10% neutral buffered formalin, dehydrated in alcohol series, clearing in xylol and embedding in paraffin. Paraffin sections (5 µm) were stained with hematoxylin and eosin according to the method of Humason (1979) and examined under a photomicroscope.

## RESULTS

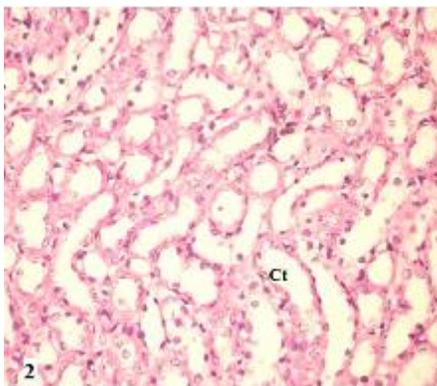
Sections of kidney from control rats illustrated that preserved architecture of the renal tissue appeared in the preserved renal parenchyma, rounded glomeruli, distal convoluted tubules with wide lumen and proximal convoluted tubules with narrow lumen lined by cuboidal epithelium in the renal cortex (Figure 1) and the renal medulla showed normal collecting tubules (Figure 2). Sections from kidney tissue of rats treated with 4 ml/kg GSO showed no histopathological changes when compared with control animals.

The effects of methomex (Metho) with both doses on nephritis damage were evaluated by histopathologic examination of the kidney sections by H&E staining. In contrast to the normal group of rats, the administration of Metho caused extensive disruption of tissue architecture. These disturbances were more obvious in the higher dose. Oral administration of Metho (1/20 LD<sub>50</sub>) caused destruction of the normal pattern of the renal tissue. These damages were encountered by the presence of hypertrophy of glomerular tuft, thickening of parietal layer of Bowman's capsule as well as focal interstitial nephritis, focal area of mononuclear cellular infiltration is clearly seen, the lumina of renal tubules containing hyaline cast and cellular debris (Figure 3). Lymphocytic infiltrations, tubulointerstitial nephritis which means necrosis of tubular cells and necrosis in the interstitial cells, also the renal tubules appeared with pyknotic nuclei (Figure 4). Kidney sections from rats administered with Metho (1/10 LD<sub>50</sub>) showed necrosis of renal tubules, atrophy of glomerular tuft, distension of Bowman's space and destruction of the renal tubules (Figure 5). Congestion of blood vessel, necrobiosis of renal tubular epithelium with pyknosis of their nuclei and swelling with multivacuolations of the cytoplasm with obliteration of their lumina and focal area of severe haemorrhage was observed (Figure 6). Shrunken renal corpuscle and widened Bowman's space, degeneration of the parietal epithelial cells of Bowman's capsule was also observed. The lumina of distal and proximal convoluted tubules contain hyaline casts of dead cells and congested blood vessel can be also detected (Figure 7). The renal medulla showed dilated collecting tubules stuffed with R.B.Cs. (Figure 8).

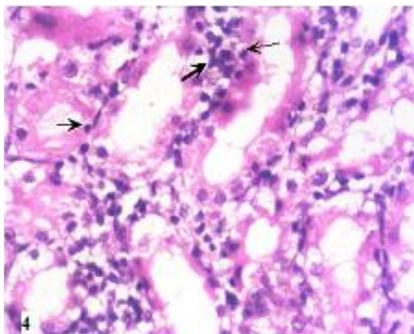
The effects of grape seed oil (GSO) on methomex-induced kidney damage were evaluated by histopathologic examination of the kidney sections by H&E staining. The treatment of Metho caused extensive necrosis, vacuolar degeneration and disruption of renal tissue architecture.



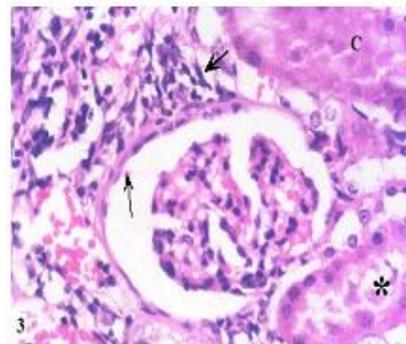
**Figure 1.** Photomicrograph of the renal cortex from control rat, showing the normal histological structure of renal parenchyma, rounded glomeruli, distal convoluted tubules with wide lumen (D) and proximal (P) convoluted tubules with narrow lumen lined by cuboidal epithelium. (H-E, X 400).



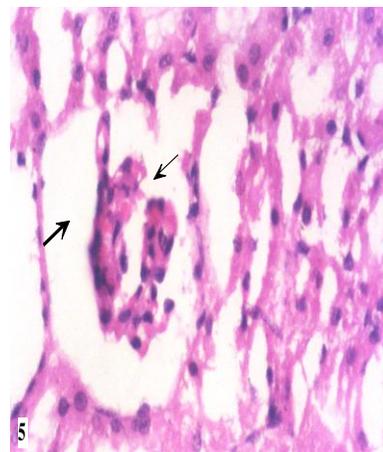
**Figure 2.** Photomicrograph of the renal medulla from control rat showing normal collecting (Ct) tubules. (H-E, X 400).



**Figure 4.** Photomicrograph of the kidney section from rat administered with 1/20 LD<sub>50</sub> Metho, showing tubulointerstitial nephritis and the renal tubules appeared with pyknotic nuclei (thin arrows), beside the presence of lymphocytic infiltrations (thick arrow). (H-E, X 400).

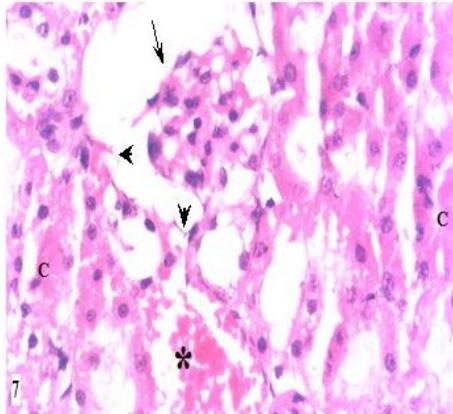


**Figure 3.** Photomicrograph of the renal cortex, from rat treated with 1/20LD<sub>50</sub> Metho showing hypertrophy of glomerular tuft, thickening of parietal layer of Bowman's capsule (thin arrow) as well as focal interstitial nephritis, focal area of mononuclear cellular infiltration is clearly seen (thick arrow) notice: the lumina of renal tubules containing hyaline cast (C) and cellular debris (\*). (H-E, X400).

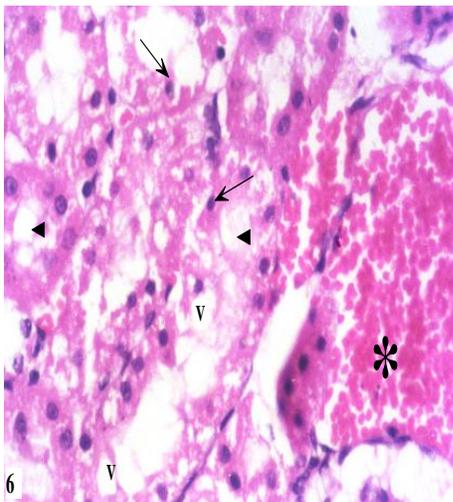


**Figure 5.** Photomicrograph of the renal cortex, from rat administered with 1/10 LD<sub>50</sub> Metho, showing necrosis of renal tubules, atrophy of glomerular tuft (thin arrow), distension of Bowman's space (thick arrow) and destruction of the renal tubules. (H-E, X 400).

rats. In this study, pre-administration of GSO protected the Renal tissue from injuries and improved the renal lesions where encountered in the renal tissue as a result of Metho administration. Pre-administration of GSO to Metho-induced rats 1/20 LD<sub>50</sub> (mg/kg), kidney sections revealed slight hypertrophy and vacuolation of glomerular tuft with slight distension of Bowman's capsule, also the lumen of the proximal convoluted tubules appeared filled with debris (Figure 9). The renal medulla, showed the normal structure of the collecting tubules (Figure10). Kidney sections from rats administered with GSO + 1/10

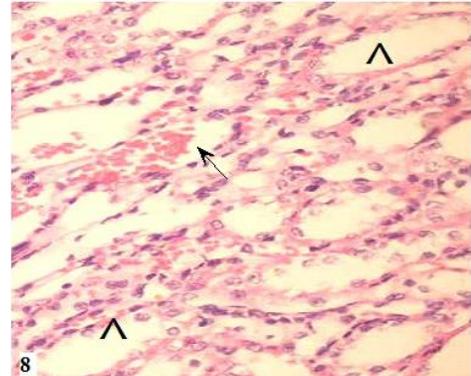


**Figure 7.** Photomicrograph of the renal cortex from rat treated with 1/10  $LD_{50}$  Metho showing shrunken renal corpuscle (arrow) with widening of the urinary space, degeneration of the parietal epithelial cells (head arrow) of Bowman's capsule. The lumina of distal and proximal convoluted tubules contain hyaline casts (C) of dead cells. Congested blood vessel can be also detected (\*). (H-E, X 400)

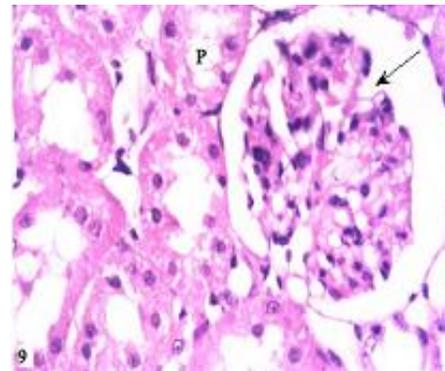


**Figure 6.** Photomicrograph of the renal cortex, from rat treated with 1/10  $LD_{50}$  Metho showing congestion of blood vessel, necrobiosis of renal tubular epithelium with pyknosis (thin arrow) of their nuclei and swelling with multiple vacuolations of the cytoplasm (V) with obliteration of their lumina (head arrows) and focal area of severe haemorrhage is observed (\*). (H-E, X 400).

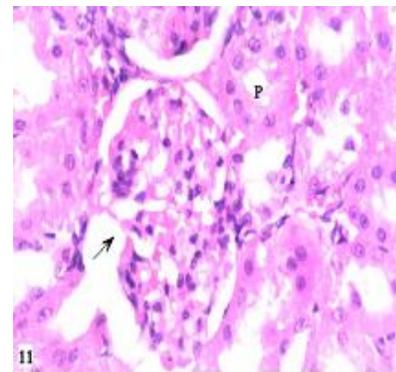
$LD_{50}$  mg/kg Metho revealed apparent normal renal parenchyma, but still glomeruli appeared with slight hypertrophy. The proximal convoluted tubules appeared near to normal with their narrow lumen (Figure 11). The renal medulla delineated near to normal appearance of the collecting tubules as appeared in Figure 12.



**Figure 8.** Photomicrograph of the renal medulla from rat treated with 1/10  $LD_{50}$  Metho, showing dilated (^) collecting tubules stuffed with R.B.Cs. (arrow) (H-E, X400).



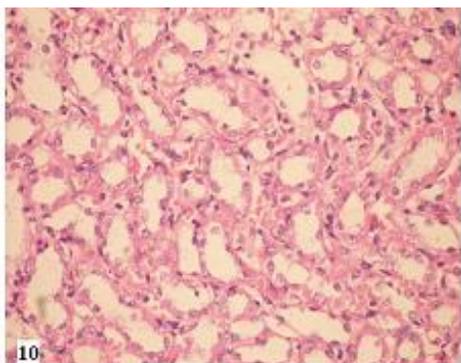
**Figure 9.** Photomicrograph of the renal cortex, from rat treated with GSO+1/10  $LD_{50}$  Metho showing slight hypertrophy and vacuolation of glomerular tuft with slight distension of Bowman's capsule, note the lumen of the proximal convoluted tubules appeared filled with debris (P). (H-E, X400).



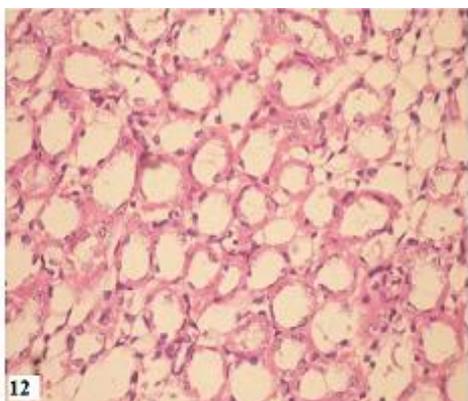
**Figure 11.** Photomicrograph of the renal cortex, from rat treated with GSO+1/20  $LD_{50}$  Metho showing apparent normal renal parenchyma, but still glomeruli appeared with slight hypertrophy. The proximal convoluted tubules appeared near to normal with their narrow lumen (P) (H-E, X 400).

## DISCUSSION

Methomex (Metho) application as an insecticide is highly effective against a wide variety of pests, particularly those that are resistant to organophosphorus (Farré et al., 2002). Metho exerts its toxic effect via peroxidative damage to the hepatic, renal and splenic cell membranes and induces DNA damage in these organs (El-Khawaga, 2005). Kidneys are responsible for the elimination of metabolic waste and the control of the amount and composition of the body fluids. Nephrotoxicity can result in systemic toxicity causing decreased ability to excrete



**Figure 10.** Photomicrograph of the renal medulla, from kidney rats administered with GSO+1/10  $LD_{50}$  Metho showing the normal structure of the collecting tubules (H-E, X 400).



**Figure 12.** Photomicrograph of the renal medulla, of kidney section from rat administered with GSO+1/10  $LD_{50}$  Metho showing near to normal appearance of the collecting tubules. (H-E, X 400).

body wastes, inability to maintain body fluid and electrolyte balance and decreased synthesis of essential hormones (For example, erythropoietin) (Finn 1977; Laurent et al., 1988).

The histological disturbances in the renal tissue in this study come in accordance with the results obtained by Radad et al. (2009). The authors reported that exposure of rats to Metho (2 mg/kg) markedly affected glomeruli, tubules and interstitium. Glomeruli appeared swollen Bowman's spaces. Glomerular swelling was primarily caused by congestion of glomerular capillaries and thickening of glomerular basement membranes. There were also mild proliferation of the glomerular epithelial cells and thickening of the Bowman's capsule and periglomerular fibrosis. Renal tubular epithelium appeared swollen and sometimes showed hyaline droplet degeneration and certain degree of necrobiotic changes. In some cases, renal tubules showed dysplastic changes characterized by abnormal mitotic figures and nuclear pleomorphism. There was also fibroblastic proliferation in the interstitium in some cases. Metho treated rats showed histopathologic changes in the kidney, and spleen of male and female rats. Similarly, enzymatic alterations of acetyl cholinesterase and liver glucose-6 phosphate dehydrogenase were also observed (Fayez and Bahig, 1992).

In the kidney, Metho treatment damaged the glomeruli, the tubules and the interstitium. Similarly, Nariman et al. (1995) and Selmanoglu et al. (2001) observed proliferation and swelling of glomerular endothelial cells and tubular degeneration, mononuclear cell infiltration and fibrosis in thiodicarb and carbendazim treated rats, respectively. Dysplastic changes seen in the tubules of some methomex-treated rats are of great concern. Together with increased frequency of normal mitosis in the liver, they might suggest that Metho is a potentially carcinogenic substance. However, there was no evidence of carcinogenicity in both rats and mice fed Metho for 2 years (EPA, 1987). Methomex was found to be potentially toxic to liver, kidney, lungs, spleen and testicles when applied repeatedly at a dose of 2 mg/kg. The observed renal damages could predispose to hepatic insufficiency and renal failure in exposed individuals (Radad et al., 2009).

The nephritic damage may appear due to the oxidative damages as a result of Metho administration. El-Khawaga (2005) and Mansour et al. (2009) showed that Metho decreased superoxide dismutase (SOD) and glutathione S-transferase (GST) activities and increased the level of lipid peroxidation (LPO) as well as the percentage of haemolysis. The response occurred in a concentration-dependent manner. The study suggested that methomyl has the capability to induce oxidative damage as evidenced by increasing LPO and perturbations in various antioxidant enzymes.

Pre-administration of GSO with Metho exhibited that GSO had a protective effect in preserving the architecture of the renal tissue. GSO is an extract by-product obtained from the grape seed and it contains a variety of biologically active species used for protection against oxidative stress induced by free radicals and ROS (Baiges et al., 2010; Ashtiyani et al., 2013). In relation to their polyph-

nol compounds, GSO contains mainly flavonoids, all involved in ameliorating the oxidative stress *in vitro* and *in vivo* through their ability to balance the oxidant-antioxidant status (Sehirli et al., 2008). The damaged renal are potent sources of reactive oxygen intermediates and these compounds exert paracrine stimulation of stellate cells. Therefore, the hepatoprotective effects of GSO may decrease paracrine stimuli, which lead to hepatic fibrosis via activated haematopoietic stem cells (HSCs) (Shin et al., 2010).

Grape seed extract has a protective effect on oxidant-induced production and deposition of extracellular matrix components, which results in hepatic fibrosis (Dulundu et al., 2007). Furthermore, GSO treatment reversed all the injury parameters and the levels of inflammatory mediators while protecting the liver tissue against reperfusion-induced oxidative injury (Sehirli et al., 2008). In the same line, El-Ashmawy et al. (2007) concluded that grape seed procyanidin extract are useful herbal remedies, especially for controlling oxidative damages, by enhancing the expression profile of copper/zinc-superoxide dismutase (Cu/Zn-SOD), an enzyme that defends against oxidative stress (Puigràs et al., 2009). The GSO along with MTX-administration (used to treat cancer and some inflammatory diseases) significantly reversed these parameters toward to near normal. These results indicated that GSO could reduce hepatic and nephritic damage induced by MTX-treatment in young rats therefore having free radical scavenging (Pinheiro et al., 2010).

## Conclusion

Methomex administration caused extensive destruction to the renal tissue. This damage was more pronounced in the higher dose which may cause renal failure. However, co-administration of the extracts of protective plants resulted in minimizing the deleterious effects of methomex toxicity on male renal tissues. It may be concluded that grape seed oil is useful as a herbal remedy, especially for controlling oxidative damages.

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