International Scholars Journals

International Journal of Pharmacy and Pharmacology ISSN: 2326-7267 Vol. 7 (4), pp. 001-009, April, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Ginger (*Zingiber officinale*) extract ameliorates metalaxyl fungicide induced nephrotoxicity in albino mice

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Accepted 16 February, 2018

The present work studied the effect of metalaxyl on the structure and function of the kidney of albino mice. The work extends to study the possible role played by the aqueous extract of Ginger (*Zingiber officinale*) in minimizing the toxicity of metalaxyl. Animals were divided into 4 groups. Group 1: given metalaxyl at a dose level of 1/10 LD_{50} for 4 weeks, Group 2: given metalaxyl and ginger, Group 3: given ginger and Group 4: controls. Kidney cortex of metalaxyl-treated mice showed many histopathological alterations. The renal tubules lost their characteristic appearance and their lining epithelial cells appeared with cytoplasmic vacuolation. The glomeruli were degenerated and the renal blood vessels were congested. The intertubular spaces were infiltrated by inflammatory leucocytic cells. Metalaxyl caused marked elevation in serum creatinine and blood urea nitrogen. It also leads to significant increase in malondialdehyde and decreased superoxide dismutase and catalase activities. Treating animals with metalaxyl and ginger led to an improvement in the histological structure of the kidney together with significant decrease in urea and creatinine. Moreover, ginger reduced the level of serum malondialdhyde (lipid peroxidation marker) and increased the serum activity of antioxidant enzymes, SOD and CAT. The present results indicate that ginger has ameliorative effect against kidney damage induced by metalaxyl and this may be mediated by the antioxidant activity of ginger.

Key words: Metalaxyl, nephrotoxicity, ginger, histopathology, antioxidant.

INTRODUCTION

In recent years, environmental contamination with pesticides represents one of the problems of the region as well as world-wide importance. The presence of these toxic chemicals was recorded in water, air, house dust and in the tissues of non-occupationally exposed people, particularly in the adipose tissue, blood and urine (Reisinger et al., 2006). Metalaxyl is a benzenoid fungicide used to control soil-borne fungal diseases on fruits, cotton, soybean, peanuts, ornamental and grasses (Sukul and Spiteller, 2000). On the other hand, metalaxyl showed hazardous effects in mammalian animals. Hrelia et al. (1996) reported that metalaxyl has cytogenetic effects on human and animal chromosomes *in vitro*. Experimental studies in mice demonstrate that liver is the primary target for metalaxyl-treated animals (Walker and Keith, 1992). Sakr and Lamfon (2005) reported that metalaxyl induced histological and biochemical alterations in the liver of albino mice. Paolini et al. (1996) indicated the cocarcinogenic potential of metalaxyl in Swiss albino mice. Sakr and Abdel-Samie (2008) reported that metalaxyl induced apoptosis and bax expression in hepatocytes of mice.

Medicinal plants play an important role in pharmacology and medicine for many years. Today, it is estimated that about 80% of the world population relies on botanical preparations as medicine to meet their health needs (Ogbera et al., 2010). Ginger (*Zingiber officinale* Roscoe) is example of botanicals which is gaining popularity amongst modern physicians and its underground rhizomes are the medicinally useful part (Mascolo et al., 1989). Many studies were carried out on ginger and its pungent constituents, fresh and dried rhizome. One of

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the most popular uses of ginger is to relief the symptoms of nausea and vomiting associated with motion sickness, surgery and pregnancy (Gilani and Rahman, 2005). Among the pharmacological effects demonstrated are anti-platelet, anti-oxidant, anti-tumour, anti-rhinoviral, anti-hepatotoxicity and anti-arthritic effect (Fisher et al., 1991; Sharma et al., 1994; Kamtchouing et al., 2002).Ginger was found to have hypocholesterolaemic effects and cause decrease in body weight, glucose in blood, serum total cholesterol and serum alkaline phosphatase in adult male rats (Bhandari et al., 2005). It was, therefore, of interest to examine the effect of metalaxyl on the structure and function of the kidney of albino mice. Also studies have been made to clarify the possible role played by the aqueous extract of ginger (*Z. officinale*) in minimize the toxicity of metalaxyl.

MATERIALS AND METHODS

Animals

Sexually mature male albino mice (*Mus musculus*) weighing 20±5 g were purchased from the breeding center of experimental animals at Helwan University, Helwan, Egypt. The animals were housed in plastic cages (40×30×16 cm) and kept in the laboratory under constant temperature (22±1°C) for at least one week before and along the period of the experimental work. They were maintained on a standard rodent diet composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminzed starch (Egyptian Company of Oils and Soap Kafr-Elzayat Egypt). Water was available *ad libitum*.

Experimental design

All the experiments were done in compliance with the guide for the care and use of laboratory animals (National Research Council, 1985). Animals were divided into 4 groups:

Group 1: Animals of this group (20 mice) were orally given metalaxyl by gastric intubation at a dose level of 1/10 LD_{50} (130 mg/kg body weight) three times per week for continuous 4 weeks (Sakr and Lamfon, 2005). Metalaxyl was supplied from Central Agricultural Pesticides Laboratory, ARC, Egypt.
Group 2: Animals in this group (20 mice) were given the same dose of metalaxyl given to animals of group 1 followed by 1 ml of final aqueous extract of ginger (24 mg/ml) three times weekly for 4 weeks. This dose of ginger was selected according to Sakr (2007). Ginger (*Z. officinale* Roscoe) rhizome was purchased from the local market at Shebin El-kom, Egypt. One kilogram fresh ginger rhizome was cleaned, washed under running tap water, cut into small pieces, air dried and powdered. 125 g of this powder were macerated in 1000 ml of distilled water for 12 h at room temperature and were then filtered. The concentration of the extract is 24 mg/ml. Each animal in the present study was orally given 1 ml of the final aqueous extract (Kamtchouing et al., 2002).
Group 3: Animals of this group (20 mice) were orally given ginger at the same dose level of group 2. Group 4: This group is a control one, in which animals (20 mice) were orally given water. 10 animals were selected randomly after 2 and 4 weeks of treatment and were sacrificed.

Histopathological examination

The treated animals and their controls were killed by cervical dislocation, quickly dissected and kidney was removed, fixed in Bouin's fluid. After 24 h, tissues were rinsed three times in 70% ethanol, dehydrated using a graded ethanol series and then embedded in paraffin wax. Paraffin sections were cut into 5 micrometers thick slices and stained with haematoxylin and eosin for light microscope examination. The severity of structural renal changes was estimated quantitatively in 10 visual fields in 5 sections of 10 animals in each treatment. The sections were viewed and photographed.

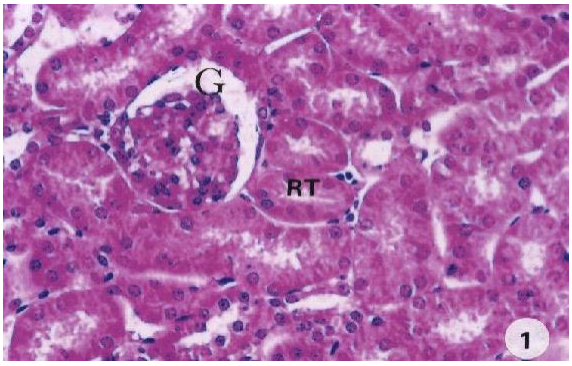


Figure 1. Section of kidney of a control mouse showing the glomerulus (G) and renal tubules (RT) X 400.

Enzyme assays

For enzymes determination, blood samples were collected from animals after 4 weeks of treatment. Sera were obtained by centrifugation of the blood sample and stored at -20°C untill assayed for the biochemical parameters. Creatinine was estimated spectrophotometrically using the method of Henry (1974) in which creatinine in alkaline solution reacts with picrate to form a coloured complex. Urea was assayed by colorimetric method of Berthelot (Patton and Crouch, 1977) in which urea converted to ammonia by urease and in alkaline medium, ammonium ions reacts with salicylate and hypochlorite to form a green coloured indophenol measured at 580 nm. The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive products (malondialdhyde) according to (Ohkawa et al., 1979). Superoxide dismutase activity was measured using the methods of Rest and Spitznagel (1977). The principal of this method depends on the ability of SOD to inhibit the power of phenazine methosulphate-mediated to reduce the nitroblue tetrazolium. Catalase activity was determined from the rate of decomposition of H_2O_2 (Aebi et al., 1974).

Statistical analysis

The results were expressed as mean ± SD of different groups. The differences between the mean values were evaluated by ANOVA followed by Student's "t" test using Minitab 12 computer program (Minitab Inc., State Collage, PA).

RESULTS

Histological results

Histological examination of the kidney of control mice revealed entirely normal structures of the renal cortex

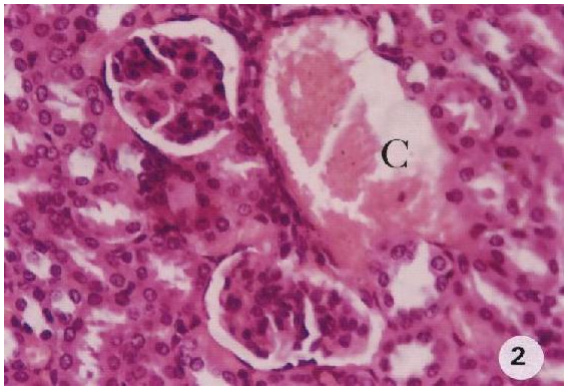


Figure 2. After treatment with metalaxyl for 2 weeks showing congested blood vessel (C) ×400.

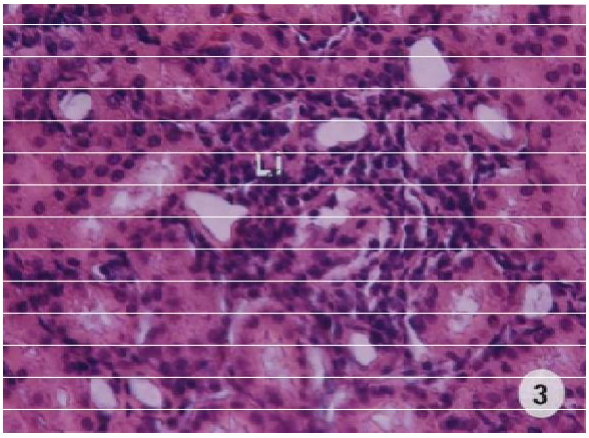


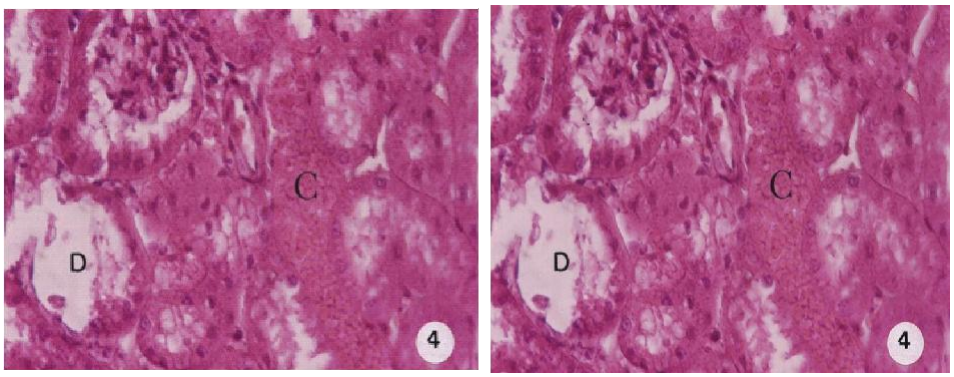
Figure 3. Leucocytic infiltration (Li) ×400.

Table 1. Quantitative assessment of renal structural changes in animals after 4 weeks of treatment.

	Tubular degeneration	Tubular cast	Interstitial leukocytic infiltrations	Glomeruli atrophy
Control	-	-	-	-
Ginger	-	-	-	-
Metalaxyl	+++	+++	+++	+++
Ginger + metalaxyl	+	+	++	+

+ Mild, ++ Moderate, +++ Severe.

which comprised renal corpuscles, proximal and distal convoluted tubules (Figure 1). No histological alterations were observed in animals treated with ginger. Examination of the kidney sections of mice treated with metalaxyl for 2 weeks showed enlarged and congested renal veins (Figure 2). Leucocytic infiltrations were observed in the interstitium (Figure 3). Comparison of changes in renal structures of different groups after 4 weeks are summarized in Table 1. In these specimens, the Malpighian corpuscles lost their characteristic configuration and the renal tubules appeared with wide lumen and their epithelial cells were degenerated (Figure 4). Some glomeruli seemed to have lost their attachments and mesangial stroma and others were atrophied with dilatation in the subcapsular space (Figure 5). The tubular epithelia were exfoliated from their underlying basement membrane and their lining cells exhibited cytoplasmic vacuolation and pyknotic nuclei



Figures 4. Sections of kidneys after 4 weeks of treatment with metalaxyl showing congested vein (C), damaged tubule (D) x400.

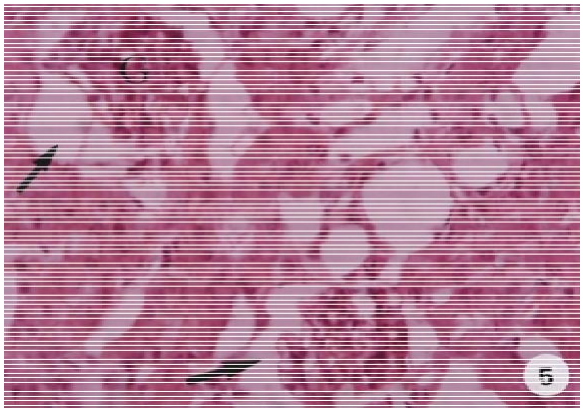


Figure 5. Glomeruli with dilatation of subcapsular space (arrows), exfoliation of epithelial cells of some renal tubules (thick arrows) x400.

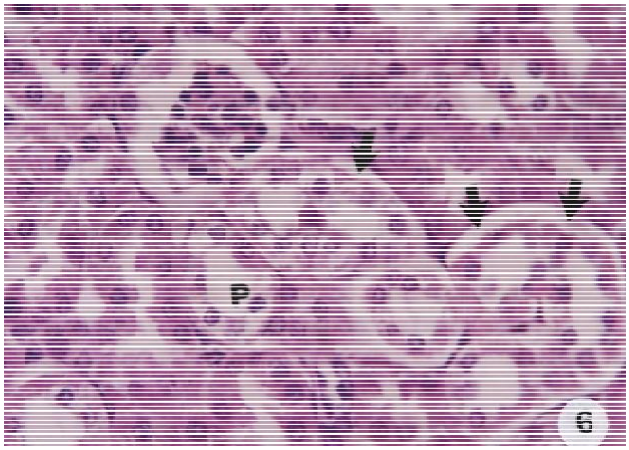


Figure 6. Vacuolated cells with pyknotic nuclei (P) x400.

(Figure 6). The lumen of the convoluted and collected tubules were filled with proteinaceous casts (Figure 7).

Animals treated with metalaxyl and ginger for 2 weeks revealed congestion of renal blood vessels and some

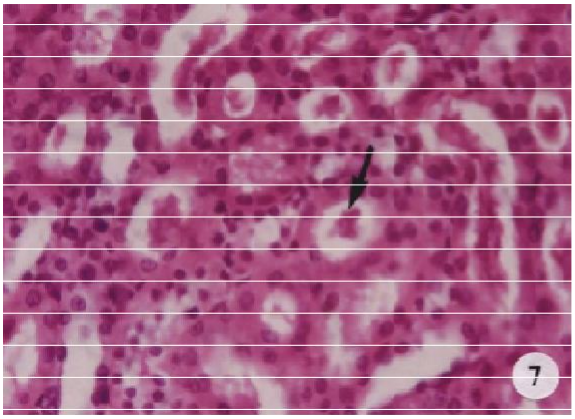


Figure 7. Proteinaceous casts in the lumen of the renal tubules (arrow), x400.

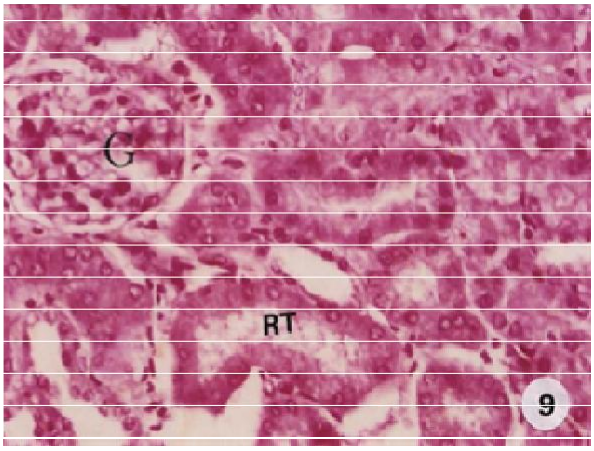


Figure 9. After treatment with metalaxyl and ginger for 4 weeks showing normal glomerulus (G) and renal tubules (RT), x400.

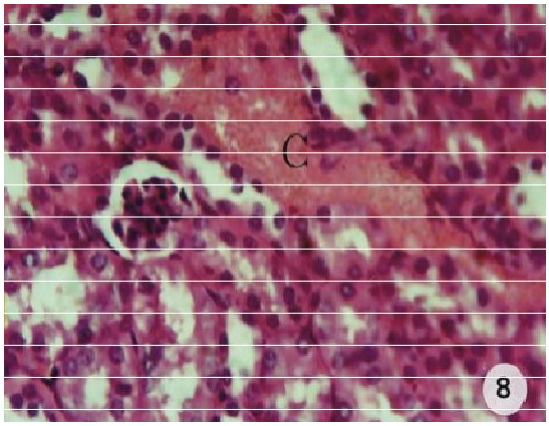


Figure 8. Section of kidney of a mouse treated with metalaxyl and ginger for 2 weeks showing congested vein (C) x400.

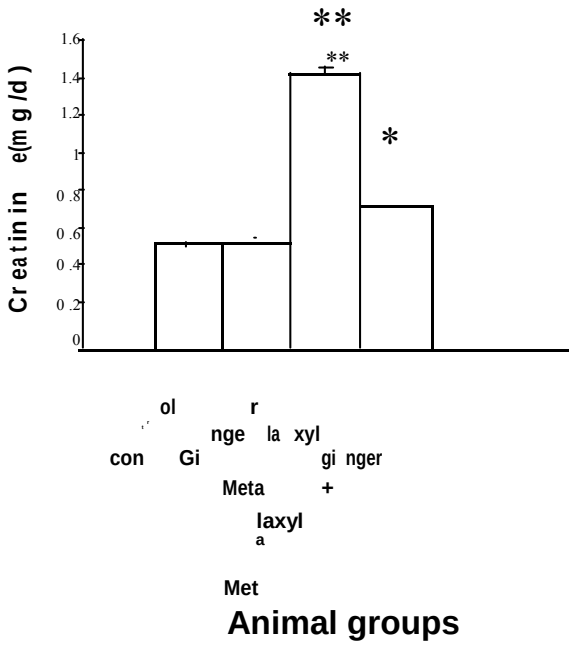


Figure 10a. Effect of metalaxyl and/or ginger on serum creatinine. (*): significant at <0.001 against control,(**): significant at P<0.05 in comparison with metalaxyl group.

tubules still degenerated (Figure 8). Administration of metalaxyl with ginger for 4 weeks attenuate the histological lesions observed in kidney of mice treated with metalaxyl alone and most of the renal tubules appeared normal (Figure 9).

Biochemical results

Effect of treatments on creatinine and urea

Figure (10a) showed that there was an elevation in creatinine in the sera of mice treated with metalaxyl in comparison with control. The mean values were 0.52±0.01 and 1.42± 0.02 mg/dl in controls and metalaxyl group, respectively. This increase was significant ($p<0.05$) after 4 weeks of treatment. Treating animals with metalaxyl and ginger induced significant decrease in creatinine (0.73±0.04) when compared with animals in metalaxyl group. Blood urea exhibited a significant increase with mean value 48.7±2.2 after 4 weeks of treatment with metalaxyl. When animals treated with metalaxyl and ginger, urea became significantly decrease (37.5±2.4 mg/dl) in comparison with group of animals given metalaxyl (Figure 10b). No significant change was recorded in values of creatinine and urea between

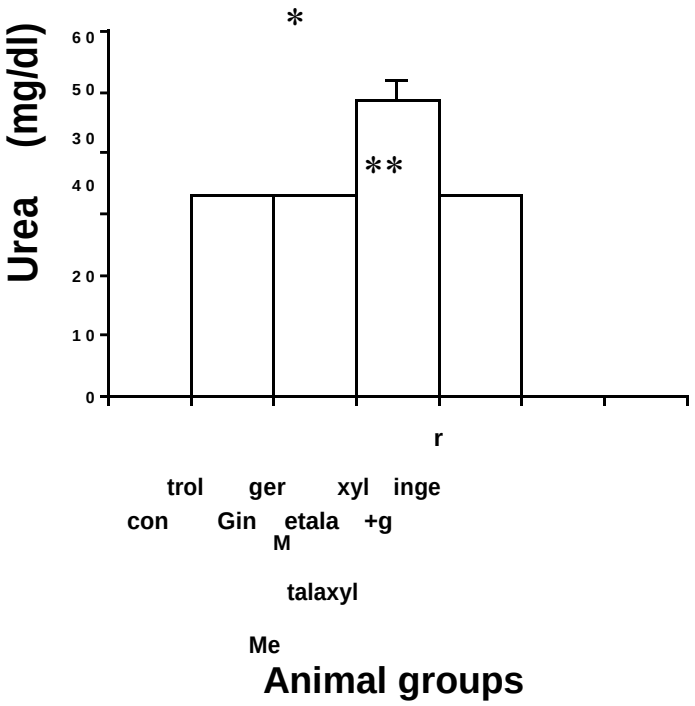


Figure 10b. Effect of metalaxyl and /or ginger on blood urea**.** (*): significant at P<0.05 against control, (**); significant at P<0.05 in comparison with metalaxyl group.

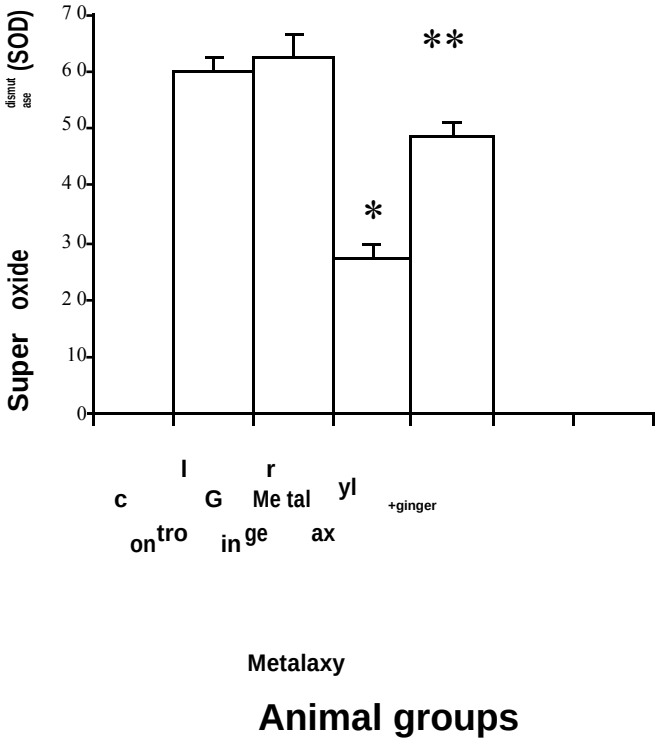


Figure 10d. Effect of metalaxyl and /or ginger on activity of superoxide dismutase. (*):significant at P<0.001 against control,(**):significant at P<0.05 in comparison with metalaxyl group.

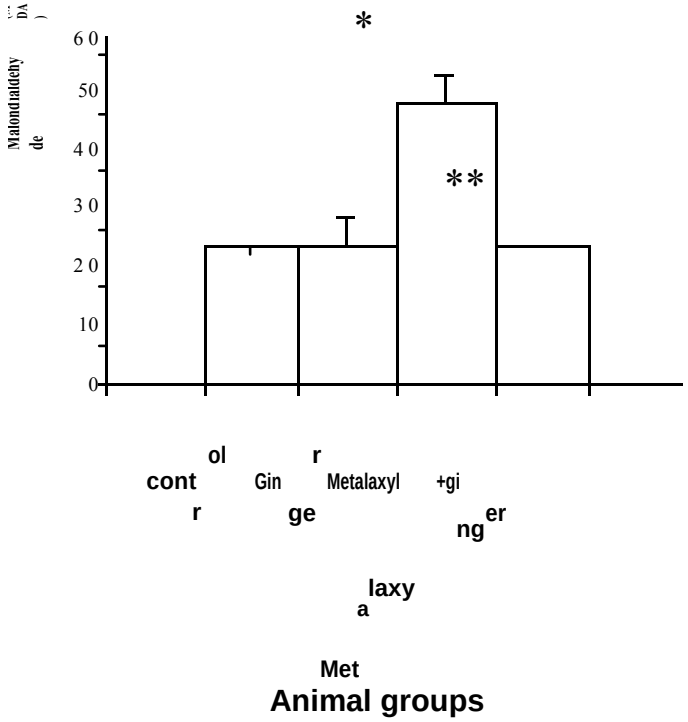


Figure 10c. Effect of metalaxyl and /or ginger on level of malondialdehyde. (*): significant at P<0.001 against control,(**):significant at P<0.001 in comparison with metalaxyl group.

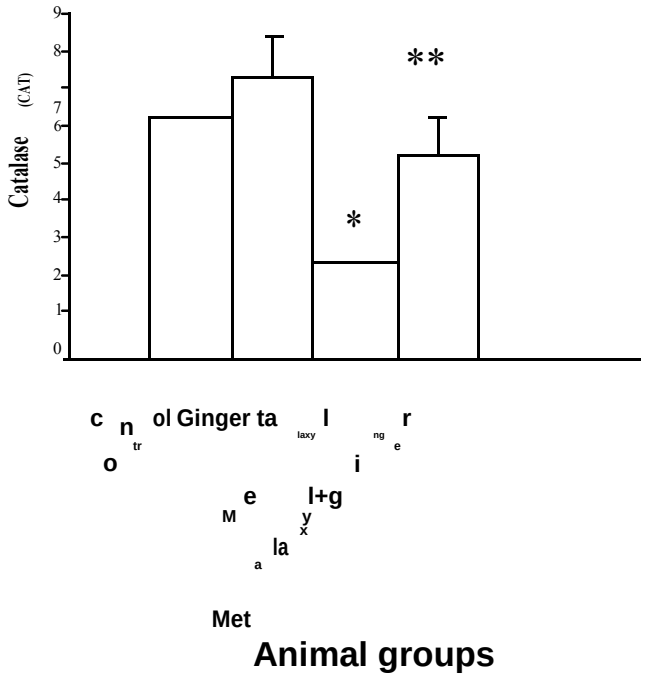


Figure 10e. Effect of metalaxyl and /or ginger on catalase activity. (*):significant at P<0.001 against control,(**):significant at P<0.001 in comparison with metalaxyl group.

animals given and their controls.

Effect of treatments oxidative and antioxidant enzymes

Malondialdhyde (MAD)

Data exist in Figure (10c) revealed that there was significant (P<0.05) increase in the level of MAD in seraof animals treated with metalaxyl compared with control group. Animals treated with both metalaxyl and ginger showed decrease of the MAD level compared with metalaxyl group. The mean values were 25.6±1.1, 49.2±2.1 and 24.14±1.5 nmol/ml in control, MAD and MAD+ ginger groups, respectively. No significant change was recorded between control and ginger treated group.

The antioxidant enzymes (SOD, CAT)

Animals treated with metalaxyl showed decrease in the level of SOD and CAT enzymes compared with control group. Animals given metalaxyl and ginger showed significant (P<0.05) increase in the level of these enzymes compared with metalaxyl group. The mean values of CAT activity were 6.2±0.5, 2.3±0.2 and 5.2±1.2 u/ml in control, metalaxyl and metalaxyl + ginger groups, respectively. The mean values of SOD were 60.3±3.2, 27.28±3.5 and 48.7±2.2 u/ml in control, metalaxyl and metalaxyl + ginger groups, respectively. No significant change was recorded in SOD or CAT levels in mice treated with ginger for 4 weeks (Figures 10d and 10e).

DISCUSSION

The results of the present investigation demonstrate the adverse effect of metalaxyl on kidney of mice. Treating mice with metalaxyl induced many histopathological changes in the kidney. The renal tubules as well as the glomeruli were affected. These alterations seemed to follow almost the same pattern as that previously enumerated by some investigators under the effect of different fungicides. Szepvolgyi et al. (1989) reported that when male and female rats were exposed to mancozeb fungicide, the kidney showed tubular dilation, necrosis and congestion of blood vessels and the liver showed centrilobular necrosis with extramedulary haemopoiesis. Cellular infiltrate of leucocytes observed in the interstitial kidney tissue of metalaxyl-treated animals and it might be considered as a prominent response of body tissues facing the injurious impacts. In agreement with this result, Hagan et al. (1986) mentioned that mancozeb induce multifocal inflammatory cell infiltrations in the respiratory tract of rats and considered it as sign of toxicity and consequent activation of defensive mechanism. Exposing mice to maneb and zineb fungicides caused blood congestion and mononuclear inflammatory cell infiltrations in the liver and kidney tissues (Ozbay et al., 1991). Carbendazim fungicide was found to cause congestion, mononuclear cell infiltration and tubular degeneration in kidney of rats (Selmanoglu et al., 2001). Dithiocarbamates (DTCs) fungicides have toxic effects on liver, kidney, testis and placenta, excessive exposure to the DTCs maneb and zineb caused acute renal failure and nephrotic syndrome in agricultural workers and led to kidney damage and reduced body weights in the offspring of exposed pregnant rats (Odermatt, 2004).

Significant elevation in creatinine and blood urea was recorded in metalaxyl-treated animals. These pathophysiological changes are a consequence of decreased glomerular filtration which, in the present work, could have developed due to atrophy of glomeruli or decrease of renal tubule reabsorption due to degeneration of tubular epithelial cells and their des-desquamation with the appearance of proteinaceous debris in the lumen tubules. Rankin et al. (1989) studied the nephrotoxic potential of the three fungicides N-(3,5-dichlorophenyl) succinimide (NDPS), vinclozolin (VCLZ) and iprodione (IPDO) in Male Fischer 344 rats. They found that NDPS induced renal effects characterized by marked diuresis, increased proteinuria, elevated blood urea nitrogen (BUN) concentration and kidney weights, decreased organic ion accumulation by renal cortical slices and proximal tubular necrosis. In contrast, IPDO and VCLZ administration resulted in only minor or no alterations in the renal function parameters studied and renal morphology. Increased blood urea nitrogen and creatinine levels were recorded in rats fed on thiophanate-methyl fungicide (Takaori, 1993). Significant rise in creatinine level was observed from the 4th week of exposure to thimet fungicide in serum of male Swiss albino mouse (Mohssen, 2000).

Oxidative stress due to abnormal production of reactive oxygen molecules (ROM) is believed to be involved in the etiology of toxicities of many xenobiotics. Evidences suggested that ROM is involved in the nephrotoxicity of widely used pesticides. Treating animals with metalaxyl induced a significant increase in the oxidative stress, malondialdhyde which is lipid peroxidation marker and a significant decrease in the level of serum antioxidant enzymes, superoxide dismutase and catalase. Malondialdhyde is a product formed during peroxidation process. Antioxidant is a substance that delays or inhibits oxidative damage to target molecules (Halliwell, 1996). According to Calviello, et al. (2006) fungicides-induced damage is closely associated with increase in lipid peroxidation and the decrease in the antioxidant enzymes. Sakr (2007) found that mancozeb fungicide induced a significant decrease in the serum antioxidant superoxide dismutase and an increase in malondialdehyde which is lipid peroxidation marker in albino rats. The precise mechanism of metalaxyl-induced

toxicity is not known. Metalaxyl may be metabolized to a reactive metabolite which may initiate a chain reaction with respect to lipid peroxidation and other tissue damaging effects Therefore, it is suggested that kidney injury induced by metalaxyl is mediated by depletion of antioxidants and elevation of lipid peroxidation.

The obtained results showed that treating animals with metalaxyl and ginger improved the histopathological and biochemical changes induced in the kidney by metalaxyl. The effect of ginger on kidney damage was studied by some investigators. Ajith et al. (2008) studied the effect of the ethanol extract of *Z. officinale* on doxorubicin-induced nephrotoxicity in rats. Doxorubicin caused many histopathological changes and increased the level of creatinine and urea in the serum. Ginger extract was found to have a protective effect on doxorubicin -induced damage as confirmed by decrease of creatinine and urea level and improving of the kidney structure. Uz et al. (2009) reported that renal damage resulted from ischemia/perfusion injury in the kidney of rats was improved after administration of ginger. The nephroprotective effects of ethanol extract of *Z. officinale* alone and in combination with vitamin E (α-tocopherol) were evaluated using cisplatin induced acute renal damage in mice. The results indicated that *Z. officinale* significantly and dose dependently protected the nephrotoxicity induced by cisplatin (Ajith et al., 2007).

The results showed that ginger reduced the level of serum malondialdhyde acting as lipid peroxidation marker and increased the serum level of antioxidant enzymes, superoxide dismutase and catalase. Similarly, Shanmugam et al. (2010) reported that superoxide dismutase, ascorbic acid, glutathione and uric acid levels were decreased and xanthine oxidase, glutathione-s-transferase activities were increased in alcohol treated rats. Treatment with ethanolic extract of ginger attenuated the parameters to normalcy showing the antioxidant effect of ginger. Siddaraju and Dharmesh (2007) reported that ginger - free phenolic and ginger hydrolysed phenolic fractions exhibited free radical scavenging, inhibition of lipid peroxidation, DNA protection and reducing power abilities indicating strong antioxidant properities. Ansari et al. (2006) showed that the ethanolic *Z. officinale* extract pretreatment for 20 days in isoproternol treated rats induced oxidative myocardial necrosis in rats, enhances the antioxidant defense (catalase, superoxide dismutase and tissue glutathione) and exhibites cardioprotection property. Ajith et al. (2007) reported that ginger ameliorated cisplatin- induced nerphrotoxicity and this protection is mediated either by preventing the cispaltin–induced decline of renal antioxidant defense system or by their direct free radical scavenging activity. Amin and Hamza (2006) demonstrated that *Z. officinale* increased the activities of

testicular antioxidant enzymes, superoxide dismutase, glutathione and catalse and reduced level of malondialdhyde. Accumulating evidence showed that the antioxidant activity of *Z. officinale* could be attributed to its major ingredients namely: Zingerone, gingerdiol, zingiberene, gingerols and shogoals (Zancan et al., 2000). Ghasemzadeh et al. (2010) reported that young rhizome of *Z. officinale* had higher content of flavonoids with high antioxidant activity. Results of the present study revealed that, ginger extract ameliorated metalaxyl-induced nephrotoxicity. This effect is mediated by either preventing metalaxyl-induced decline of renal antioxidant defense system or by its direct free radicals scavenging activity.

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