Protective effect of ginger (*Zingiber officinale*) against metalaxyl induced hepatotoxicity in albino mice

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Abstract: The present work studied the effect of metalaxyl, an acylalanine fungicide, on the liver of albino mice and the possible role played by the aqueous extract of Ginger (Zingiber officinale) in attenuating the hepatotoxicity of metalaxyl. Metalaxyl treatment induced many histological changes in the liver including congestion of blood vessels, cytoplasmic vacuolization of the hepatocytes, necrosis and fatty degeneration. Metalaxyl caused marked elevation in serum ALT and AST. It also caused an increase in malondialdehyde and depletion of the activity of the antoxidant enzymes, catalase and superoxide dismutase in the liver .Treating animals with metalaxyl and ginger extract led to an improvement in both the histological and biochemical alterations induced by metalaxyl. Moreover, ginger reduced the level of malondialdehyde and increased the activity of antioxidant enzymes, SOD and CAT. These results indicated that ginger have protective effect against liver damage induced by metalaxyl and this may be attributed to its antioxidant and free radicals scavenging properties.

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Keywords: Protective effect; ginger (Zingiber officinale); metalaxyl; hepatotoxicity; albino mice

1.Introduction

The environmental contamination of pesticides as a result of their extensive use has become a serious problem. This stimulated the scientists to study its biological effects. Metalaxyl is a benzenoid fungicide used to control soil-borne fungal diseases on fruits, cotton, soybean, peanuts, ornamental andgrasses (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 only *in vitro* and not *in vivo*. In a long-term feeding study with mice at low levels of exposure, the animals' livers were the primary target for metalaxyl-related effect (Walker and Keith, 1992). Paolini et al. (1996) indicated the cocarcinogenic potential of metalaxyl in Swiss albino mice. Metalaxyl caused dose-dependent bradycardia, and at higher doses (250 and 300 mg/kg body weight) the sustained bradycardia led to cardiac arrest (Naidu and Radhakrishnamurty, 1988). Sakr and Lamfon (2005) reported that metalaxyl induced histological and biochemical alterations in the liver of albino mice. Demsia et al. (2007) found that imidacloprid and metalaxyl separately or in combination induced in vitro micronucleus formation and sister-chromatid exchange induction in human lymphocytes and in vivo micronucleus induction in polychromatic erythrocytes of the rat bone-marrow. Sakr and Abdel-Samie (2008) reported that metalaxyl induced apoptosis and bax expression in hepatocytes of mice. Dasgupta et al. (2011) reported that residues of buprofezin, chlorpyriphos, metalaxyl, and myclobutanil were detected in incurred grape and wine samples.

The potential role of dietary antioxidants to reduce the activity of free radical-induced reaction Has drawn increasing attention. 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). Ginger was found to relief the symptoms of nausea and vomiting associated with motion sickness, surgery and pregnancy (Gilani and Rahman, 2005).The pharmacological effects of ginger and its pungent constituents, fresh and dried rhizome were investigated. Among the effects demonstrated are anti-platlet, antioxidant, anti-tumour , anti- rhinoviral, anti-hepatotoxicity , anti arthritic and anti-diabetic effect (Fisher-Rasmussen et al.1991, Sharma et al.1994, Kamtchoving et al.2002, Islam and Choi, 2008). 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).Ginger extract-pretreated rats attenuated in a dose-dependent manner, CCl4 and acetaminophen-induced increases in the activities of ALT, AST, ALP, LDH and SDH in the blood serum (Yemitan and Izegbu , 2006). The present work was conducted to study the effect of ginger extract on the hepatotoxicity of the metalaxyl in albino mice.

2. Materials and Methods

Animals

Sexually mature male albino mice (*Mus musculus*) weighing 20±5 g was used. 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. Water was available *ad libitum*.

Preparation of ginger aqueous extract

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).

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 LD50 (130mg/kg body weight) three times per week for continuous 4 week (Sakr and Lamfon, 2005).

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 4weeks. This dose of ginger was selected according to Sakr et al., (2011).

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, 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 liver 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 and examined under light microscope.

Biochemical 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 until assayed for the biochemical parameters. Aspartate aminotransferase AST) and alanine aminotransferase (ALT) were measured using a fully automated Hitachi 911 analyzer (Tokyo, Japan). A commercial randox kits (Randox Laboratories, LTD, Ardomre, Crumlin, United Kingdom) were used in these analysis. In hepatic tissue samples, the extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive product (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 H2O2 (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, P.A).

3. Results

i. Histological results

Liver of control animals or animals given ginger extract showed normal structure (Fig.1).Examination of liver of mice treated with metalaxyl displayed many histopathological alterations. After two weeks from the beginning of the administration of the fungicide, the liver tissue revealed disruption of normal cords arrangements of the hepatocytes and the intrahepatic blood vessels were congested (Fig.2). Infiltrations by large mass of leucocytic infilammatory cells were observed (Fig.3) and the hepatocytes displayed cytoplasmic vacuolization (Fig.4). The histopathological changes of the liver were more pronounced after four weeks where the hepatic cells appeared with giant nuclei and their cytoplasms contained fatty droplets (Fig.5). Animals treated with metalaxyl and ginger for two weeks revealed that some hepatocytes showed fat droplets (fig.6).Examination of liver sections after four weeks revealed that liver tissue restored its normal structure and most cells displayed a certain degree of recovery besides the appearance of some binucleated ones

ii. Biochemical results

Change in ALT and AST:

Figure (7) showed the effect of different treatments on serum ALT activity. Non-significant difference in serum ALT activity was recorded in mice treated with ginger extract in comparison with control group. Animals treated with metalaxyl showed a significant increase in serum ALT activity after 2 and 4 weeks of treatment. On the other hand, animals treated with metalaxyl and ginger revealed a significant decrease in ALT activity when compared with metalaxyl group. Figure (8) showed non-significant difference in serum AST activity in animals treated with ginger when compared with control group. Animals treated with metalaxyl showed significant increase in serum AST activity while animals treated with metalaxyl and ginger showed a significant decrease in AST activity when compared with metalaxyl treated group.

Change in MDA, SOD and CAT:

Table (1) showed the effect of different treatments on malondialdehyde (MDA) (index of tissue lipid peroxidation), superoxide dismutase (SOD) and catalase (CAT) in liver of animals examined after 4 weeks. MDA level was increased significantly, whereas the activity of SOD and CAT was found to be decreased in metalaxyl-treated animals when compared to the control group. Treating rats with ginger and metalaxyl increased MDA level and returned SOD and CAT activity to nearly that of the control.

4. Discussion

Results obtained in the present study indicated that metalaxyl induced many histopathological alterations in the liver tissue of mice such as tissue impairment, congestion of intrahepatic blood vessels, cytoplasmic vacuolization of the hepatocytes and fatty degeneration. Similar results were reported by Sakr and Lamfon (2005) and they added that metalaxyl affected liver enzymes (transaminases) in mice. The alterations induced by metalaxyl were also observed in liver of some mammalian animals exposed to various fungicides. When male and female rats were exposed to mancozeb, the liver showed centrilobular necrosis with extramedullary haemopoiesis and the kidney showed tubular dilation, necrosis and congestion of blood vessels (Szepvolgyi *et al.*,1989). Selmanoglu *et al.* (2001) revealed congestion of blood vessels, increase in number of Kupffer cells, cellular infiltration and hydropic degeneration in liver of male rats treated with carbendazim.

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. Hanukoglu *et al.* (1993) reported that lipid peroxidation and reactive oxygen species are produced by electron leakage outside the electron transfer chains and these oxygen radicals can initiate lipid peroxidation, to inactivate P_{450} enzymes. Mathews *et al.* (2000) mentioned that the damage occurred in the cell membrane by hydroxyl radicals induced oxidation of polyunsaturated fatty acids in membrane lipid in a process called lipid peroxidation. Moreover, Banks and Soliman (1997) recorded increase in serum hydroperoxides and decrease in reduced glutathione after benomyl toxicity in rats. The authors added that the *in vivo* toxicity of benomyl may be associated with oxidative stress. 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 et al. (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 Liver toxicity recorded in the present study may be due to the oxidative stress resulted from metalaxyl or its metabolite.

The obtained results showed that treating rats with metalaxyl and ginger improved the histopathological and biochemical changes induced in the liver by metalaxyl. This indicated the effectiveness of ginger in prevention of metalaxyl hepatotoxicity. The effect of ginger on hepatic damage was studied by some investigators. The effect of the ethanol extract of the rhizome of *Zingiber officinale* was tested against carbon tetrachloride and acetaminophen-induced liver toxicities in rats. CCl_4 and acetaminophen induced many histopathological changes and increased the activities of ALT, AST, ALP, LDH and SDH in the blood serum. Ginger extract was found to have a protective effect on CCl_4 and acetaminophen-induced damage as confirmed by histopathological examination of the liver (Yemitan and Izegbu , 2006). Bhandari et al.(2003) studied the effect of an ethanol extract of ginger on country-made liquor (CML)-induced liver injury in rats.

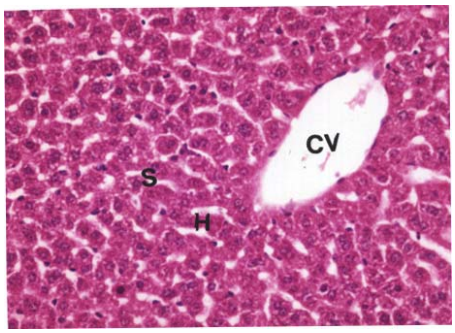

Fig.1. liver section of a control mouse showing hepatic strands (H), hepatic sinusoid (S) and central vein (CV), (X 400).

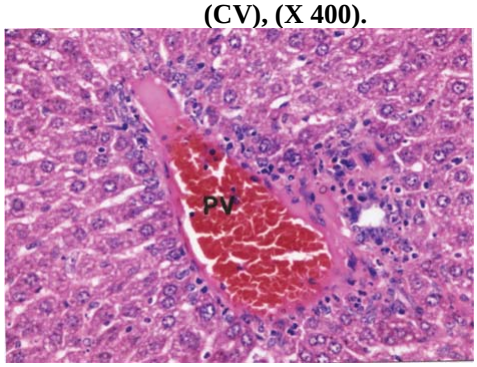

Fig.2. Section of liver of a mouse treated with metalaxyl showing congestion of portal vein with eroded lining (PV), (X 400).

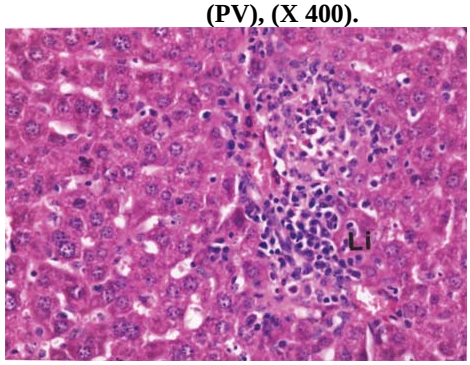

Fig.3 Specimen obtained from mouse treated with metalaxyl showing leucocytic infilteration, (X 400).

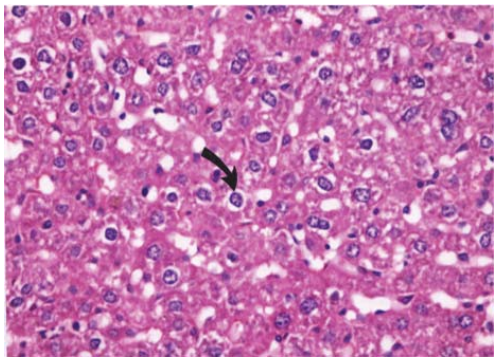

Fig.4. Liver section of a treated mouse showing cytoplasmic vacuolizations of the hepatocytes (arrow), (X 400).

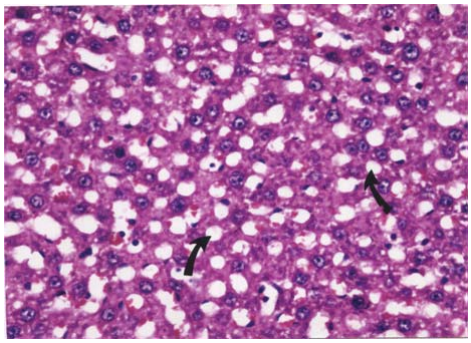

Fig.5. Liver section of a treated mouse showing fatty degeneration (arrows), (X 400).

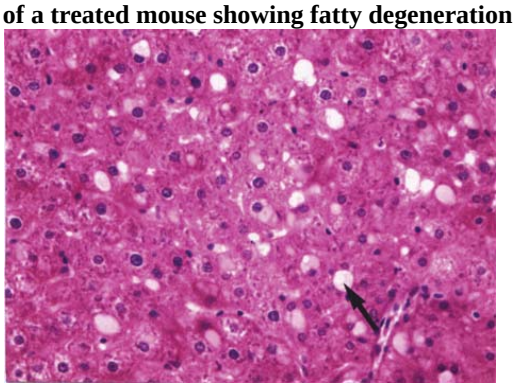

Fig.6. Liver section of a mouse treated with metalaxyl and ginger showing advanced degree of improvement with few number of fat droplets (arrow), (X 400).

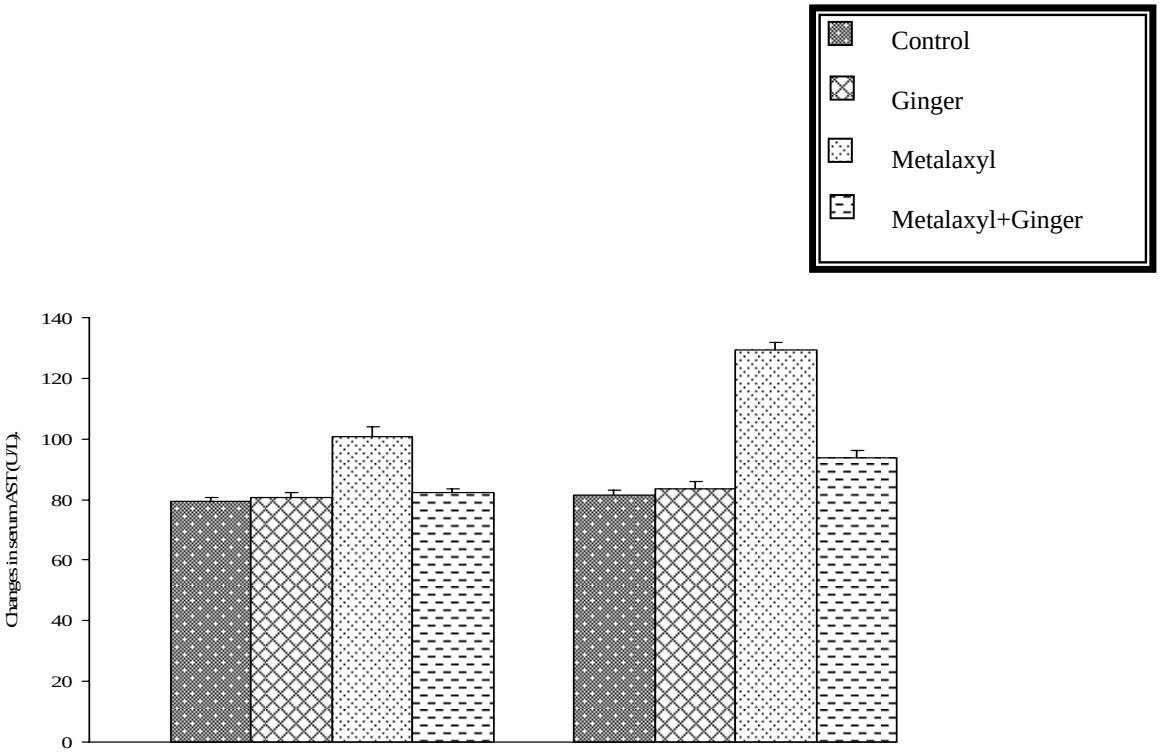

Fig.8. Effect of different treatments on serum ALT.

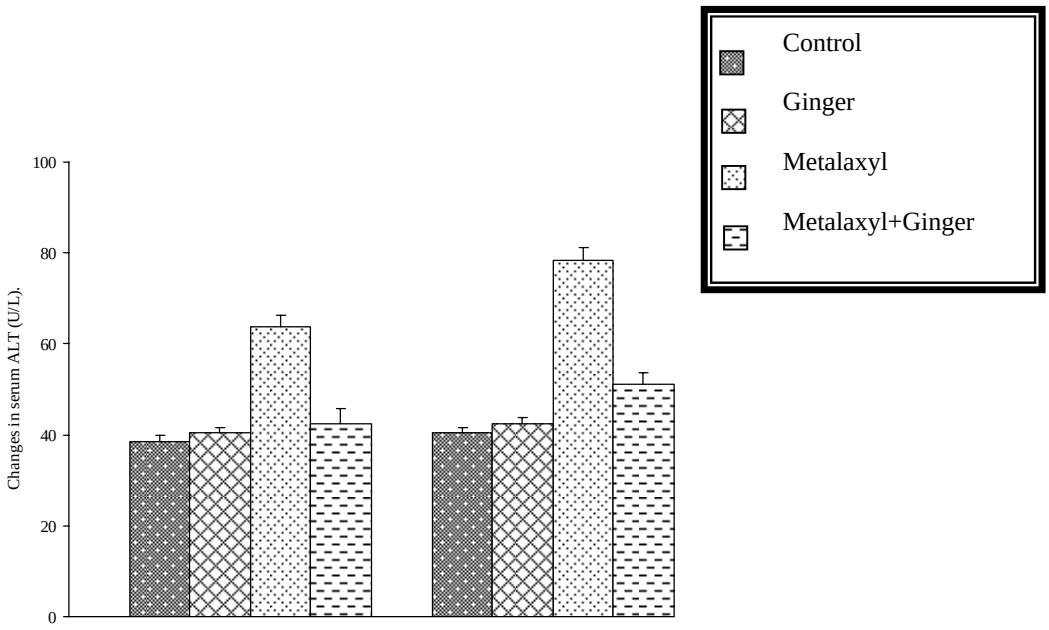

Fig.8. Effect of different treatments on serum ALT.

Table (1). Effect of Metalaxyl and/or ginger extracts on level of MDA, CAT and SOD activities in liver of mice.

Group	MAD (n mol/ml)	CAT (µ mol/ml))	SOD (µ mol/ml)
Control	15.5 ± 1.5	6.5 ± 1.2	140.5 ± 5.5
Ginger	16.2 ± 1.4	6.1 ± 0.9	141.3 ± 4.3
Metalaxyl	30.5 ± 2.8*	2.5 ± 0.6*	106.2 ± 3.5*
Metalaxyl+Ginger	20.5 ± 1.6	4.8 ± 0.2	132.2 ± 2.6

(*)Statistically significant (P<0.05)

Their results showed that administration of ginger ethanolic extract (200 mg/kg) orally from day 15 to day 21 along with CML produced significant ($P < 0.01$) lowering of serum AST, ALT, ALP and tissue lipid peroxide levels. Moreover, ginger reduced the level of serum malondialdhyde acting as lipid peroxidation marker and increased the serum level of antioxidant enzyme, superoxide dismutase. Similarly, 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.officinal* increased the activities of testicular antioxidant enzymes, superoxide dismutase, glutathione and catalse and reduced level of malondialdhyde. Ghasemzadeh et al.(2010) reported that young rhizome of *Z.officinale* had higher content of flavonoids with high antioxidant activity. Sakr et

al.(2011) reported that that ginger has ameliorative effect against kidney damage induced by metalaxyl and reduced lipid peroxidation and increased the serum level of antioxidant enzymes, SOD and CAT.

It is concluded from the present study that, ginger extract have protective effect against metalaxyl-induced hepatotoxicity. This effect may be mediated by free radicals scavenging activity of ginger.

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