

Ameliorative Effect of Ginger (*Zingiber officinale*) on Mancozeb Fungicide Induced Liver Injury in Albino Rats

Saber A. Sakr

Zoology Dept., Faculty of Science, Menoufia University, Shebin El-kom, Egypt.

Abstract: Mancozeb is an ethylenebisdithiocarbamate fungicide used against a wide range of fungal diseases of field crops and fruits. Treating albino rats with mancozeb induced various histological changes in the liver. These changes include congestion of blood vessels, leucocytic infiltration, cytoplasmic vacuolization of the hepatocytes and pyknosis. Mancozeb also caused significant elevation in serum ALT and AST enzymes. The result of the oxidative stress and antioxidant enzymes in this work revealed that there was a significant increase in malondialdehyde which is lipid peroxidation marker and a significant decrease in the level of serum antioxidant enzyme, superoxide dismutase activity. Treating animals with mancozeb and ginger led to an improvement in the histological liver picture together with significant decrease in ALT and AST activity. Moreover, ginger reduced the level of serum malondialdehyde acting as lipid peroxidation marker and increased the serum level of antioxidant enzyme, superoxide dismutase. The results of the present work indicated that the ameliorative effect of *Z. officinale* against liver damage induced by mancozeb is mediated by its potent antioxidant activities..

Keywords: Mancozeb, Ginger, Liver, Histology, Antioxidants

INTRODUCTION

Mancozeb (Diathan-M) is an ethylene-bis-dithiocarbamate, fungicide used against a wide range of fungal diseases of field crops, fruits and ornamentals (Worthing, 1991). On the other hand, mancozeb was found to have toxic effects in a variety of experimental animals. O'Hara and DiDonto (1985) reported that mancozeb induced histopathological changes in the liver and adrenal gland of mice. Szepvolgyi *et al.*, 1989 reported that kidney of animals exposed to mancozeb showed tubular dilation, necrosis and congestion of blood vessels. Hagan *et al.* (1986) demonstrated that mancozeb induced multifocal inflammatory cell infiltration, focal or multifocal necrosis in the respiratory tract of rats. Mancozeb was found to produce chromosomal aberrations in Wister rats (Georgian *et al.*, 1983). Shukla *et al.* (1990) studied the tumour incidence in albino mice dermally exposed to mancozeb. They found that after 48 weeks, animals had benign skin tumours.

Today many botanicals natural products are used in therapy of different diseases. Ginger (*Zingiber officinale* Roscoe) is example of botanicals which is gaining popularity amongst modern physicians and its underground rhizomes are the medicinally and wlinary useful part (Mascolo *et al.* 1989). Many studies were carried out on ginger and its pungent constituents, fresh and dried rhizome. Among the pharmacological effects demonstrated are anti-platelet, antioxidant, anti-tumour, anti-rhinoviral, anti-hepatotoxicity and anti arthritic effect (Fisher-Rasmussen *et al.* 1991, Sharma *et al.* 1994, Kamtchoving *et al.* 2002). Ginger was found to have hypocholesterolaemic effects and cause decrease in body weight, blood glucose, serum total cholesterol and serum alkaline phosphatase in adult male rats (Gujral *et al.* 1978). One of the most popular use of ginger is to relief the symptoms of nausea and vomiting associated with motion sickness, surgery and pregnancy (Gilani and Rahman, 2005). The present work was conducted to study the effect of ginger on mancozeb fungicide-induced liver injury in albino rats.

MATERIALS AND METHODS

Adult male rats (*Rattus norvegicus*) weighing 120 ± 5 g were used. Animals were kept in the laboratory under constant temperature (24 ± 2 °C) for at least one week before and throughout the experimental work. They were maintained on a standard diet and water was available *ad libitum*. Animals were divided into 4

Corresponding Author: Saber A. Sakr, Zoology Dept., Faculty of Science, Menoufia University, Shebin El-kom, Egypt.
E.mail: sabsak@yahoo.com

groups. Group1: animals of this group (20 rats) were given orally the fungicide mancozeb dissolved in water at a dose level of 1/10 LD₅₀ (313.6 mg/kg body weight) (Sakr *et al.* 2005) 3 times per week for 6 weeks. Group 2: animals in this group (20 rats) were given the same dose of mancozeb given to animals of group 1 followed by 1 ml of final equous extract of ginger (24 mg / ml)3 times weekly for 6 weeks. The rhizomes of *Z. officinale* were shade dried at room temperature and were crushed to powder. 125 g of the powder were macerated in 1000 ml of distilled water for 12 h. at room temperature and were then filtered to obtain the final aqueous extract. The concentration of the extract is 24 mg/ml equal to 120 mg/kg. In this study each animal was orally given 1 ml of the final aqueous extract. (Kamatchouing *et al.*, 2002). Animals in the third group (20 rats) were given ginger only and those in the fourth group(10 animals) were given water. The treated animals and their controls were killed by cervical dislocation, quickly dissected and small pieces of liver were fixed in Bouin's fluid, dehydrated, embedded in wax and 5 micrometers thick sections were stained with haematoxylin and counterstained with eosin. For enzymes determination, sera were obtained by centrifugation of the blood sample and stored at -20C° until assayed for the biochemical parameters. Serum alanine aminotransferase (ALT) and asparate aminotransferase(AST) were determined on the basis of Gella *et al.*, (1985). Serum malandialdehyde (MDA) (Ohkawa *et al.*, 1979) and superoxide dismutase activity (Rest and Spitznagel 1977) were determined. The results were analyzed statistically using Student's " t " test.

RESULTS AND DISCUSSIONS

Histological Results:

The control animals which orally given water or Ginger showed normal liver structure. Examination of liver sections obtained from rats treated with mancozeb for 2 weeks showed that the normal structural organization of the hepatic lobules was impaired and the characteristic cord-like arrangement of the normal liver cells was lost. The central and portal veins were congested (Fig.1). Liver sections prepared from rats 4 weeks post-treatment with mancozeb revealed that a considerable number of hepatic cells were damaged and lost their characteristic appearance while others showed marked cytoplasmic vacuolization which was so extensive in some cells to the extent that only slight remnants of the cytoplasmic mass cells - frequently forming a narrow peripheral rim was left (Fig.2). The nuclei of these cells were pyknotic. In addition, congestion of the intrahepatic blood vessels and inflammatory leucocytic infiltrations (Fig.3) were observed. The histopathological changes of the liver were more increased after 6 weeks and the liver cells were degenerated and suffered from fatty infiltrations (Fig. 4). Animals treated with mancozeb and ginger for 2 and 4 weeks revealed that the majority of these histopathological changes were diminished but some of the intrahepatic vessels were still congested and some hepatocytes appeared with vacuolized cytoplasm and Kupffer cells were activated (Fig. 5).After 6 weeks of the same treatment,the liver tissue restored most of its normal structure (Fig.6).

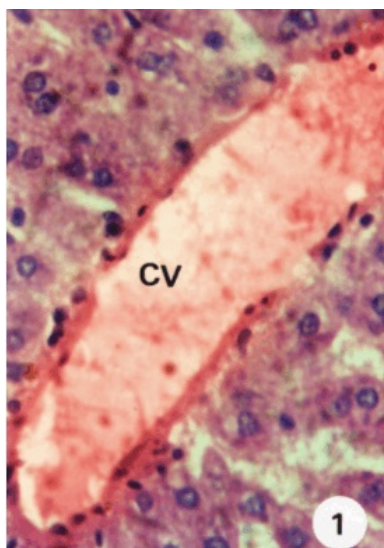


Fig. 1: Section of liver from a rat treated with mancozeb and examined 2 weeks post-treatment showing congestion of central vein (CV), X 400.

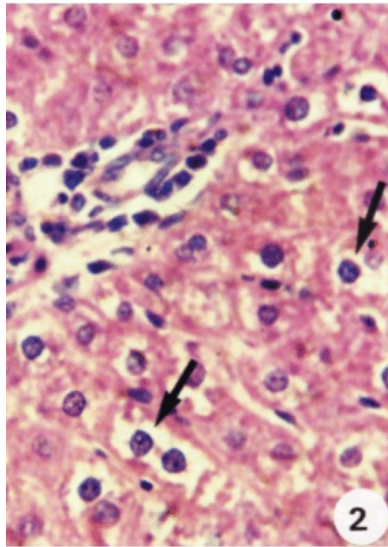


Fig. 2: Specimen obtained 4 weeks after treatment with mancozeb showing cytoplasmic vacuolization of the hepatocytes (arrows), X 400.

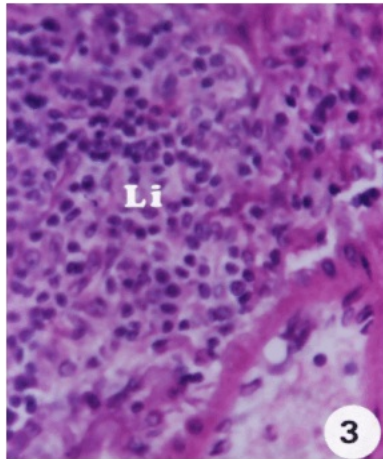


Fig. 3: Liver section of a rat examined after 4 weeks of treatment with mancozeb showing large mass of inflammatory leucocytic infiltration (Li) , X 400.

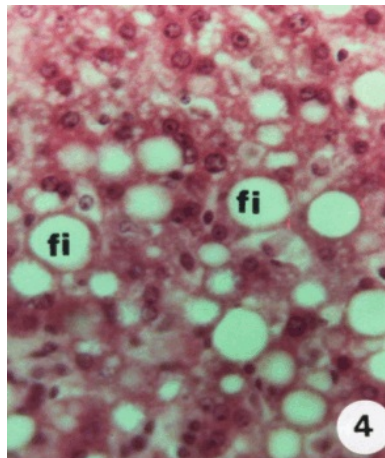


Fig. 4: Liver section obtained after 6 weeks post-treatment with mancozeb showing fatty infiltrations (fi), X400.

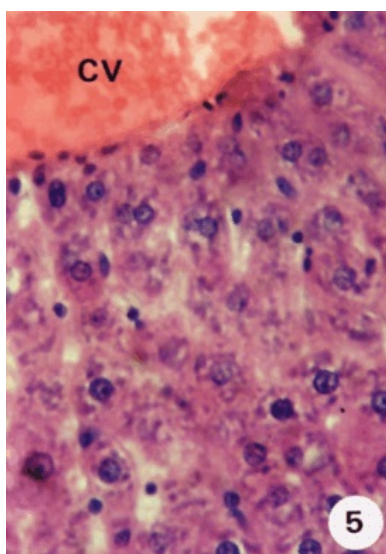


Fig. 5: Liver section obtained from a rat treated with mancozeb followed with ginger and examined after 2 weeks showing restoration of the normal arrangement of the hepatocytes but the central vein appeared congested (CV), X 400.

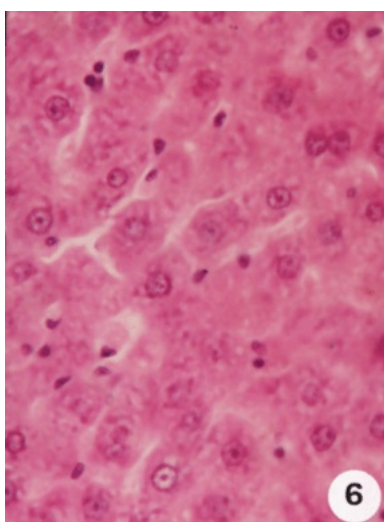


Fig. 6: Liver section of a rat after 6 weeks of treatment with mancozeb and ginger showing improvement of hepatic tissue , X 400.

Liver Function Enzymes:

Data in table 1 shows that there was an insignificant elevation in AST in the sera of animals treated with mancozeb for 2 weeks followed by a significant increase ($P < 0.05$) after 4 and 6 weeks of treatment. Similarly, ALT exhibited an insignificant increase after the second week followed by a significant increase after 4 and 6 weeks. On the other hand, animals treated with Mancozeb and ginger showed insignificant increase in these parameters in comparison with control. There is no significant differences in the activity of ALT and AST in the sera of control group or animals group given ginger (table 1).

Antioxidante Enzymes:

As showed in table (2), the level of superoxide dismutase (SOD) have a significant decrease in animals treated with mancozeb in comparison with control, while animals treated with mancozeb followed by ginger showed significant increase in SOD level in serum in all periods of treatment. Concerning serum level of malondialdehyde (MDA), animals treated with mancozeb revealed a significant increase ($P < 0.05$) in all periods of treatment compared with control. On the other hand, rats treated with mancozeb followed by ginger showed a decrease of the serum level of MDA.

Table 1: Effect of mancozeb alone or in combination with ginger on serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Enzyme	Period of treatments (weeks)	Group			
		Control	Ginger	Mancozeb	Mancozeb +Ginger
AST (u/ ml)	2	123.3± 5.5	128.9±2.4	128.2 ± 4.6	132.0 ± 3.6
	4	119. 6±4.3	130.3± 5.5	146.0 ± 2.2*	137.0 ± 2.3
	6	122. 6±6.2	138.5±4.2	188.0 ± 2.5*	128.3 ± 1.8
ALT (u/ ml)	2	56.3± 2.5	63.2±1.3	59.4 ± 3.5	60.6 ± 2.2
	4	58.5 ±5.4	67.5±5.1	87.5 ± 2.2*	64.2 ± 4.2
	6	60.5±1.4	70.4±2.2	102.0 ± 2.7*	72.3 ± 2.6

Each value represent mean ± standard deviation. * significant at P<0.05.

Table 2: Changes in serum antioxidantes in the experimental groups.

Parameter	Period of treatments (weeks)	Group			
		Control	Ginger	Mancozeb	Mancozeb +Ginger
Superoxidase dismutase (u/ml)	2	61±5.5	77±2.3	35±2.5*	58 ± 4.5
	4	63±3.5	82±4.2	28±3.4*	56 ± 3.2
	6	71±2.2	93±5.2	21±2.4*	62 ± 2.5
Malondialdehyde (n mol/ml)	2	19± 2.5	20± 1.1	36 ± 2.5*	24 ± 1.3
	4	22 ±1.2	18 ±2.3	45 ± 2.2*	30 ± 5.2
	6	20±1.4	19±2.4	63 ± 2.7*	27 ± 2.5

Each value represent mean ± standard deviation. * significant at P<0.05.

Discussion:

The present study showed that mancozeb induced many histopathological changes in the liver of rats. Similarly, Sakr *et al.* (2005) reported that mancozeb induced marked histopathological and ultrastructural changes in the liver of mice. Leucocytic inflammatory infiltrations were observed in liver of mancozeb-treated rats. Hagan *et al.* (1986) mentioned that mancozeb induced multifocal inflammatory cell infiltrations in the respiratory tract of rats. These leucocytic infiltrations were considered as a prominent response of the body tissue facing any injurious impacts (Sakr, 1999). The intrahepatic blood vessels, central and portal veins, were congested in the treated rats. Such lesions were previously observed in the liver of animals exposed to different types of fungicides including mancozeb (Deveci *et al.*, 1997 and Kackar *et al.*, 1999). Liver function enzymes, ALT and AST elevated in the sera of mancozeb-treated rats. This is in agreement with the result of Sakr *et al.* (2005) who found that these enzymes increased in sera of mice administered with mancozeb. Kackar *et al.* (1999) also reported that oral administration of mancozeb to male rats induced changes in the activities of ALT, AST, alkaline phosphatase, lactate dehydrogenase and acetylcholinesterase throughout the period of the study in a dose-dependent manner. Furthermore, Lavric *et al.* (1990) reported that the fungicide bithionol sulfoxide at high doses caused hepatotoxicity including an increase in serum AST.

The result of the oxidative stress and antioxidant enzymes in this work revealed that there was a significant increase in the oxidative stress, malondialdehyde which is lipid peroxidation marker and a significant decrease in the level of serum antioxidant enzyme, superoxide dismutase activity in mancozeb-treated rats. Malondialdehyde is a product formed during peroxidation process. Antioxidant is a substance that delays or inhibits oxidative damage to target molecules (Halliwell, 1996). In this respect, Calviello *et al.* (2006) confirmed the oxidative effect of mancozeb which caused post-apoptotic and necrotic alteration in cell membrane integrity. Similarly Fabra *et al.* (2004) demonstrated that mancozeb produced biochemical alteration in membrane composition, polysaccharides and polyamines.

The obtained results showed that treating rats with mancozeb and ginger improved the histopathological and biochemical changes induced in the liver by mancozeb. This indicated the effectiveness of ginger in prevention of mancozeb 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. CCl₄ 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 CCl₄ and acetaminophen-induced damage as confirmed by histopathological examination of the liver (Yemitan and Izegebu, 2006). Bhandari *et al.* (2003) studied the effect of an ethanol extract of ginger on country-made liquor (CML)-induced liver injury in rats. 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.

The results showed that ginger was scavenging free radical by its potent antioxidant. This results were cleared by the data in which ginger reduced the level of serum malondialdehyde 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 properties. Ansari *et al.* (2006) showed that the ethanolic *Z.officinale* extract pretreatment for 20 days in isoproterenol treated rats induced oxidative myocardial necrosis in rats, enhances the antioxidant defense (catalase, superoxide dismutase and tissue glutathione) and exhibits cardioprotection property. Ajith *et al.*, (2007) reported that ginger ameliorated cisplatin- induced nephrotoxicity and this protection is mediated either by preventing the cisplatin – 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 catalase and reduced level of malondialdehyde.

The results of the present work indicated that the ameliorative effect of *Z.officinale* against liver damage induced by mancozeb is mediated by its potent antioxidant activities.

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