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Effect of Ginger (*Zingiber Officinale*) on Mancozeb Fungicide Induced Testicular Damage in Albino Rats

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Abstract: The effect of mancozeb fungicide on testicular activity in albino rats was studied. Treating rats with mancozeb at a dose level of 1/10 LD_{50} (313.6 mg/kg body weight) 3 times per week for 6 weeks affected the serum level of testosterone and leuteinizing hormone (LH). A significant decrease in testosterone ($P<0.05$) and insignificant decrease in LH level was recorded in mancozeb-treated animals compared with control. The diameters of the seminiferous tubules and heights of their germinal epithelium were significantly reduced. In addition ,testes of treated rats showed inhibition of speramatogenesis as indicated by the decrease of the number of different spermatogenic cells. Histological observations revealed degeneration of spermatogenic cells, congestion of blood vessels and destruction of Leydig cells. These results indicated that mancozeb affected testicular function . Treating animals with mancozeb and ginger led to an improvement in the histological structure of the testis with an increase in the number of spermatogenic cells. Moreover, there was an increase in testosterone and LH level. It is speculated that the curative effect of *Z.officinale* against testicular damage induced by mancozeb may be due to its antioxidant properties.

Key words: Ginger; Fungicide; testis; rat; hormones

INTRODUCTION

Fungicides are used for controlling the fungi of different agricultural crops. 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). Despite all good beneficial properties of mancozeb it 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 bengin skin tumours.

The simplest dream for many ordinary people allover the world , is living in safety far from diseases .They tend to use nearly every natural things in their life .The modern medicine come to achieve this dream from few recent years by the so-called Complementary and Alternative Medicine. Some plants, herbs and spices act as alternative medicine. 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).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). Many studies were carried out on ginger and its pungent conistituents, fresh and dried rhizome. Among the pharmacological effects demonstrated are anti-platlet, 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). The present work was conducted to study the effect of ginger on mancozeb fungicide-induced testicular damage in albino rats.

MATERIALS AND METHODS

Adult male rats (*Rattus norvegicue*) weighing 120 ± 5 g were used. Animals were kept in the laboratory under constant temperature (24±2 ^{o}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 groups. Group1: animals of this group (20 rats) were given orally the fungicide mancozeb dissolved in water at a dose

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level of 1/10 LD_{50} (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. (Kamatchoving *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 in different groups and their controls were sacrificed by decapitation. The blood samples were obtained from the animals by heart puncture. The circulating level of testosterone and leutienizing hormone (LH) were determined using radioimmunoassay kits supplied by diagnostic Co. Los Angeles according to the method of Maruyama (1987). For histological examination, the testes were removed and fixed in Bouin's fluid. They were subsequently embedded in paraffin wax, sectioned at 5 microns and stained with haematoxylin and eosin. Seminiferous tubules diameter and germinal epithelial height were measured from the normal spermatogenetic cells on the inner surface of the basement membrane through the most advanced cell types lining the lumen of the tubules. For statistical analysis of the data, the Student's t-test was used.

RESULTS AND DISCUSSION

l. Morphometric results:

Data exist in figures (1&2) represented the morphometric changes in the diameter of the seminiferous tubules and their epithelial heights .Treating rats with ginger for 6 weeks caused insignificant increase in the diameter of the seminiferous tubules compared with control group. Rats treated with mancozeb showed a significant decrease ($P < 0.05$) in tubules diameter and their epithelial heights after 4 and 6 weeks in comparison with control . Animals treated with mancozeb followed by ginger showed a significant increase in the diameter of seminiferous tubules and their epithelial heights in comparison with mancozeb group .

II. Hormonal results:

-Testosterone Hormone:

Data in figure (3) revealed that there was no significant difference in total testosterone hormone in serum of control animals and those treated with ginger. Treating animals with mancozeb induced a significant reduction ($P<0.05$) in serum total testosterone after all periods of treatment. On the other hand treatment with mancozeb followed by ginger caused significant increase in serum total testosterone in comparison with rats given mancozeb only.

- Leutinizing Hormone:

Data in figure (4) showed that there was no significant difference in serum Leutinizing hormone (LH) in control groups and animals treated with ginger . Animals treated with mancozeb showed insignificant decrease in the activity of this hormone. An insignificant increase in LH was recorded in animals treated with mancozeb followed by ginger in all treatment periods.

lII. Histopathological results:

Figure (5) showed testis of control rat. Animals treated with ginger showed the same histological structure of testis as in those served as controls. Testes of rats administered with mancozeb exihibited a distinct histological difference compared to control. After 2 weeks of treatment with mancozeb, the seminiferous tubule showed a reduction in the number of spermatogenic cells and their normal arrangement were lost. The number of the sperm were reduced and the intertubular blood vessels were dilated and congested with blood. Examination of testis of rats after 4 weeks revealed sloughing off of germinal epithelium at many points. There was a marked reduction in the number of spermatogenic cells and sperm bundles in the lumen of the seminiferous tubules. The leydig cells were degenerated and the intertubular blood vessels were congested Fig.6). These histopathological alternations were exaggerated in rats examined at 6 weeks post-treatment with mancozeb. In these specimens, the intertubular blood vessels were congested, there was severe decrease in the number of spermatogenic cells and exfoliated degenerated cells appeared in the lumen of the tubules. The sperm bundles were degenerated in some of tubules and completely absent in the others (Fig .7).

Examination of testis sections from rats treated with mancozeb followed by ginger for 2 and 4 weeks revealed that the histopathological changes were less prominent when compared with the same periods of mancozeb group only .The seminiferous tubules showed normal arrangement of the spermatogenic cells and the intertubular blood vessels appeared with few congestion . An improvement of most of the histopathological alternation induced by mancozeb were observed after 6 weeks of treatment (Fig.8).

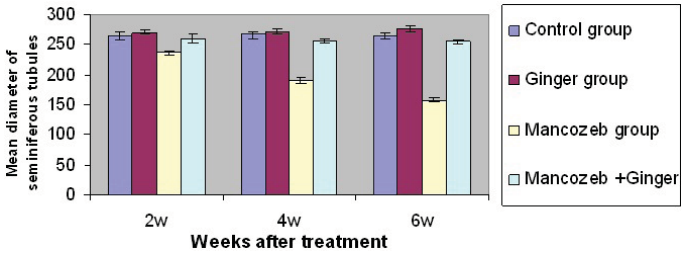

Fig. 1: Change in diameter of seminiferous tubules (mean±SD)

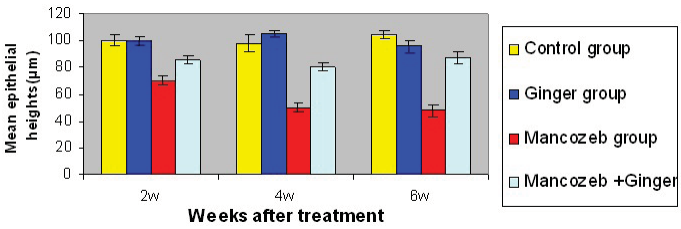

Fig. 2: Change in germinal epithelial heights (mean±SD)

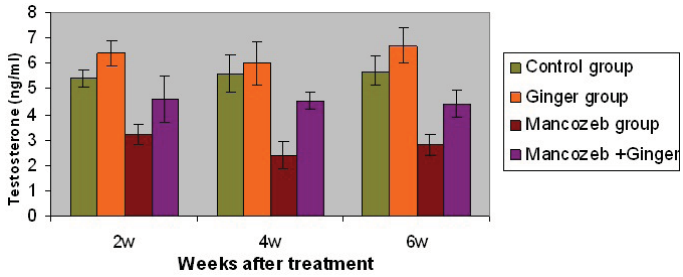

Fig. 3: Change in testosterone hormone (ng/ml) (mean±SD)

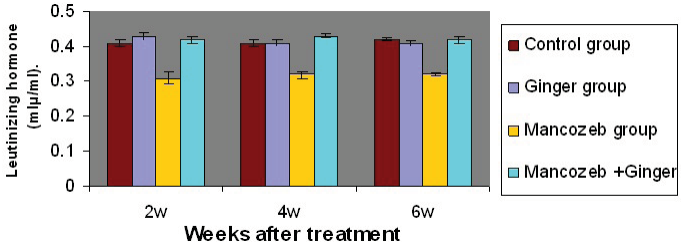

Fig. 4: Change in leutinizing hormone (mlµ/ml) (mean±SD)

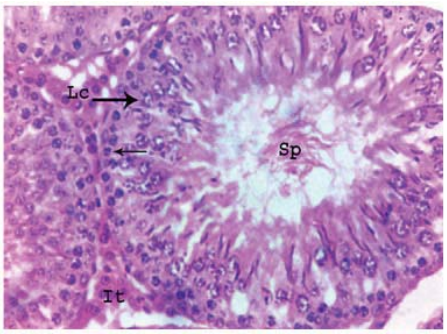

Fig. 5: Section in the testis of a control rat showing seminiferous tubule with active spermatogenesis, sp:sperm, sg:spermatogonia, It: interstitial tissue, X 400.

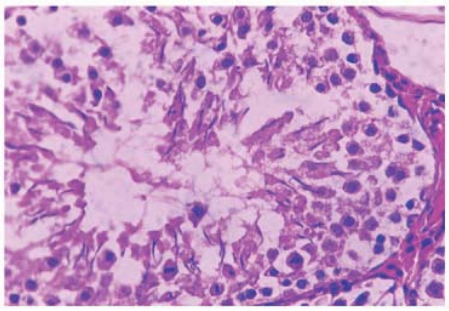

Fig. 6: seminiferous tubule of a rat treated with mancozeb for 4 weeks showing reduction of spermatogenic cells, X400.

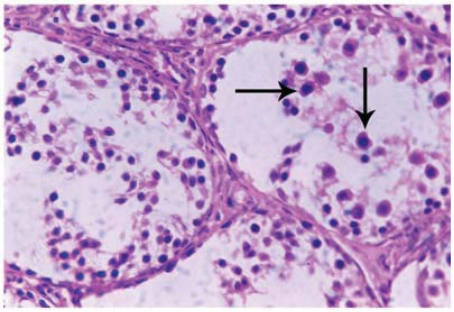

Fig. 7: seminiferous tubule of a rat treated with mancozeb for 6 weeks showing inhibition of spermatogenesis and exfoliated spermatogenic cells (arrows), X400.

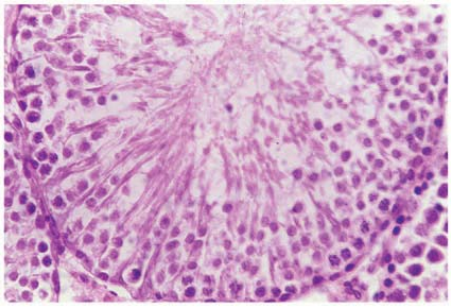

Fig. 8: seminiferous tubule of a rat treated with mancozeb and ginger for 6 weeks showing restoration of spermatogenesis X400.

Discussion:

The present study revealed that mancozeb induced morphometrical as well as histopathological alterations in the testes of albino rats. Similarly, Khan and Sinha(1994) studied the effect of mancozeb in Swiss mice , they demonstrated that there was a decrease in sperm count and a higher frequency of sperm with aberrant head morphology. Kackar *et al*., (1997) also reported that mancozeb caused histopathological changes in gonads of male rats after chronic exposure. These changes include a significant increase in testes and decrease in epididymis weights, degeneration in seminiferous and epithelial tubules with loss of sperm. Sakr and Okdah (2004) studied the effect of benomyle fungicide on the testis of albino mice. Their results showed a degeneration of the spermatogenic cells, absence of sperm bundles and a significant reduction in the diameter of the seminiferous tubules and the height of the germinal epithelium.

Treating animals with mancozeb decreased the level of testosterone and lutenizing hormone . Hormonal abnormalities due to exposure to pesticides were reported by many investigators. Blystone *et al*., (2007) reported that the fungicide prochloraz (PCZ) induced malformations in androgen-dependent tissues in male rats when administered during sex differentiation. They added that progesterone and 17alpha-hydroxyprogesterone production levels were increased significantly whereas testosterone levels were significantly decreased. On the contrary, Goetz *et al*., (2007) studied the effect of 3 triazole fungicides on male rats. Their results showed that

treatment with myclobutanil, triadimefon and propiconazole induced an increase in testis weight and serum testosterone Rehnberg *et al* (1989) found that carbendazim treatment resulted in severe seminiferous tubular atrophy and affected the functional capacity of Leydig cells to secrete testosterone. Vinclozolin fungicide was found to have anti –androgenic effects on spermatogenesis in male rat testis (Kubota *et al*.2003) .Sakr *et al*. (2007) reported that mancozeb induced a significant decrease in the serum antioxidant superoxide dismutase and an increase in malondialdehyde which is lipid peroxidation marker in albino rats. Calviello, *et al* .(2006) confirmed the oxidative effect of mancozeb which caused post-apoptotic and necrotic alteration in cell membrane integrity. Therefore, it is suggested that testicular injury induced by mancozeb is mediated by depletion of antioxidants and elevation of lipid peroxidation.

Administration of ginger improved testicular damage induced by mancozeb as shown by increase of spermatogenic cells together with increase of L.H and testosterone. These results are in agreement with Qureshi *et al* (1989) who reported that ginger (Zingiber officinale) significantly increased the sperm motality and sperm contents in the epididymis and vas deference without producing any spermatotoxic effect. Kamtchoving *et al* (2002) investigated the androgenic activity of aqueous extract of *Z.officinal* in male rats. They reported that *Z. officinal* significantly increased weight of testes, the serum testosterone level and epididymal α – glucosidase activity. Also Amin and Hamza (2006) studied the protective effect of ethanol extract of *Z. officinal* against cisplatin – induced reproductive toxicity in rats .They demonstrated that *Z. officinal* extract reduced the extent of cisplatin – induce sperm abnormality, enhanced sperm motility and testicular damage by increase the activities of testicular antioxidants .

Accumulating evidence showed that the antioxidant activity of ginger (Zingiber officinale) could be attributed to its major ingredients namely Zingerone, gingerdiol, Zingiberene ,gingerols and shogoals (Zancan *et al*. 2000). Siddaraju and Dharmesh (2007) elucidated that ginger - free phenolic (GRFP) and ginger hydrolysed phenolic (GRHP) fractions of ginger exhibited free radical scavenging, inhibited lipid peroxidation, DNA protection and reduced power abilities indicating strong antioxidant properties. Ansari *et al* .(2006) showed that the ethanolic extract of *Z.officinale* in isoproternol- treated rats induced myocardial necrosis in rats, enhanced the antioxidant defense (catalase, superoxide dismutase and tissue glutathione) and exhibited cardioprotection property. Thus, the curative effect of *Z.officinale* against testicular damage induced by mancozeb may be due to its antioxidant properties.

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