RESEARCH ARTICLE

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GINGER (ZINGIBER OFFICINALE) AMELIORATES MANCOZEB-INDUCED HISTOLOGICAL AND HISTOCHEMICAL ALTERATIONS IN THE ADRENAL CORTEX OF THE ALBINO RAT

ABSTRACT:
Mancozeb is an ethylenebisdithiocarbamate fungicide used against a wide range of fungal diseases of field crops and fruits. Ginger (Zingiber officinale) is a monocotyledonous plant with many pharmacological properties. Treating albino rats with mancozeb at a dose level of (1/10 LD₅₀) 3 times/week for 2, 4 and 6 weeks induced a significant decrease in their body weight in all such periods of treatment in comparison with control. Animals treated with mancozeb and ginger water extract showed a significant increase in their body weight compared with those of mancozeb group. Adrenal cortex of animals administered with mancozeb exhibited obvious histological and histochemical alterations. The histological changes include atrophy of zona fasciculata and zona reticularis, thickening of adrenal capsule and vacuolation of zona glomerulosa. The blood sinusoids in zona reticulaires was dilated and congested. Histochemical results revealed a marked depletion of polysaccharides in zona glomerulosa and zona fasciculata and lacking of such polysaccharides in zona reticularis. Total proteins content showed a marked decrease in zona glomerulosa and zona fasciculata, while the cells of zona reticularis appeared with a moderate amount of total proteins. Animals treated with mancozeb followed by ginger showed that the architecture of the three cortical zones was nearly returned to normal picture but the connective tissue capsule appeared thicker than in the control. Moreover, ginger increased polysaccharides and total protein contents in the cortical zones. It is speculated that the ameliorative effect of Z. officinale against adrenal damage induced by mancozeb is mediated by its potent antioxidant activities.

KEY WORDS:
Mancozeb, zinger, adrenal cortex, rat, histology.

INTRODUCTION:
Plants have been used as food and also for medicinal purposes for centuries. Plant medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. Today many botanicals natural products are used in therapy of different diseases. Ginger (Zingiber officinale Roscoe) is an example of botanicals which gained popularity among modern physicians; its underground rhizomes are the medicinally and winery 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-arthritis 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).

Fungicides are a group of pesticides used to control fungi. Although they are used in a wide range, they may cause different side effects either to animals or human. Mancozeb (Diathan-M) is an ethylene-bisdithiocarbamate, 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. Treating animals with mancozeb caused tubular dilation, necrosis and congestion of blood vessels in kidney (Szepvolgyi et al., 1989). Hagan et al. (1986) demonstrated that mancozeb induced multifocal inflammatory
cell infiltration, focal or multifocalf necrosis in the respiratory tract of rats. Recently, Sakr et al. (2009) reported that mancozeb affected testis structure and function in mice. Mancozeb was found to produce chromosomal aberrations in Wister rats (Georgian et al., 1983). Benign skin tumours were observed in albino mice dermally exposed to the fungicide mancozeb for 48 weeks (Shukla et al., 1990). The present work was conducted to study the effect of ginger on mancozeb-induced histological and histochemical changes in albino rats.

MATERIAL AND METHODS:

Adult male rats (Rattus norvegicus) weighing 120 ± 5g were used. Animals were kept in the laboratory under almost 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 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, according to 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 aqueous 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 hr. 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). The animals in the third group (20 rats) were given ginger only and those in the fourth group (10 animals) were given water. The treated and control animals were sacrificed after 2, 4 & 6 weeks after treatment and their adrenal glands were removed and fixed either in alcholic Bouin’s (for histological study and histochemical demonstration of polysaccharides) or 10% formalin for histochemical demonstration of total proteins. Fixed materials were embedded in parafine wax and sections of 5μm thickness were cut. Slides were stained with haematoxylin and counterstained with eosin for histopathological examination. For histochemical purposes, polysaccharides were demonstrated using periodic acid Schiff’s (PAS) technique (Hotchkiss, 1948). Total proteins were demonstrated using mercury bromophenol blue method (Mazia et al., 1953). The changes in thickness of zona fasciculata, zona reticularia and zona granulosa were measured in haematoxylin and eosin-stained section, by using calibrated ocular scale grid. The data was expressed in micrometers and the mean values were calculated for each group. The significances of differences between the means were calculated according to the Students “t” test.

RESULTS:

1. Change in body weight:

Data in table 1 show that treatment with ginger for 6 weeks caused insignificant increase in body weight of rats in comparison with controls. On the other hand, there was a reduction in body weight of animals treated with mancozeb in comparison with control rats. This reduction became significant (P < 0.05) after 4 weeks and 6 weeks of treatment (Table 1). Treatment with mancozeb followed by ginger caused significant increase in body weight of rats in comparison with the mancozeb group.

Table 1. Changes in body weight (g) of animals treated with mancozeb followed by Ginger.

<table>
<thead>
<tr>
<th>Period of treatment</th>
<th>Control group</th>
<th>Ginger group</th>
<th>Mancozeb group</th>
<th>Mancozeb + Ginger group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>2 weeks</td>
<td>197.0 ± 9.1</td>
<td>202.4 ± 8.4</td>
<td>149.0 ± 13</td>
<td>183.0 ± 7.1</td>
</tr>
<tr>
<td>4 weeks</td>
<td>242.4 ± 5.1</td>
<td>254.0 ± 5.9</td>
<td>156.4 ± 7*</td>
<td>216.4 ± 4.3**</td>
</tr>
<tr>
<td>6 weeks</td>
<td>285.2 ± 7.1</td>
<td>286.4 ± 5.5</td>
<td>157.2 ± 4.9*</td>
<td>270.6 ± 8.4**</td>
</tr>
</tbody>
</table>

(*) significant decrease (P < 0.05) in comparison with the control group.

(**) significant increase (P < 0.05) in comparison with the mancozeb group.

2. Morphometrical results:

Table 2 shows that treating rats with mancozeb for 6 weeks induced significant decrease in the thickness of the three cortical zones: zona glomerulosa, zona fasciculata and zona reticularis in comparison with control group. Treating animals with mancozeb and ginger improved the thickness of the three cortical zones.

Table 2. Effect of different treatments on the thickness of the three cortical zones.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Thickness of the three zones in μm. (Mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z.G.</td>
</tr>
<tr>
<td>Control</td>
<td>63.0±3.3</td>
</tr>
<tr>
<td>Ginger</td>
<td>64.0±2.4</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>38.5±2.2</td>
</tr>
<tr>
<td>Mancozeb + Ginger</td>
<td>58.5±4.2</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05.

Histological results:

The adrenal gland of the control rats is covered with a thin capsule of collagenous connective tissues capsule. It consists of two...
layers: a peripheral layer, the adrenal cortex; and a central layer, the adrenal medulla (Fig. 1).

The adrenal cortex is composed of three distinct zones, zona glomerulosa, zona fasciculate and zona reticularis. The zona glomerulosa is the layer immediately beneath the connective tissue capsule, in which the columnar or pyramidal cells are arranged in closely packed, rounded, or arched clusters, surrounded by capillaries. The middle layer is zona fasciculate; its cells are polyhedral or rectangular with a great number of lipid droplets in their cytoplasm. As a result of the dissolution of lipids during tissue preparation, the cytoplasm of faciculata cells appeared vacuolated. Zona reticularies is the inner most layer of the adrenal cortex; it lies between the zona fasciculate and medulla. It is composed of cells arranged in an anastomosing network of clumps columns with a capillary network closely apposed to the cell membranes. These cells are smaller than those of the other layers and they have deeply stained nuclei (Figs 2&3).

The adrenal medulla is the centre of the adrenal gland. The cells of this layer are polyhedral parenchymal cells arranged in cords, clumps or columns and surrounded by a rich network capillary. The cells have a large, commonly pale staining nuclei and their cytoplasm is usually finely granular (Fig. 4). Examination of the adrenal gland of rats treated with ginger showed the same histological structure as those served as controls.

Adrenal gland of rats administered with mancozeb exhibited an obvious histological difference compared to control. The histological changes recorded after 2 and 4 weeks include thickness of the capsule, irregular arrangement in cells of zona glomerulosa and some of these cells showed vacuolated cytoplasm and pyknotic nuclei. There was interference with both zona
glomerulosa and zona fasciculata or zona fasciculata with zona reticularis without differentiation of their cells from each others (Fig. 5). Blood sinusoids in zona reticularis were dilated and congested (Fig. 6). All these histopathological features were exaggerated after six weeks of treatment (Fig. 7). Animals treated with mancozeb followed by ginger after 2, 4, and 6 weeks showed that the architecture of the three cortical zones was almost normal although there was a slight histopathological features such as few congested blood sinusoids (Fig. 8).

**Histochemical results:**

1- Polysaccharides

Section of control rats stained with PAS reaction showed polysaccharide content and distribution in the form of deeply stained reddish granules in the cytoplasm of the cortical cells. The capsule and the separating connective tissue limits were heavily stained and the three cortical zones showed a moderate reaction with PAS method (Fig. 9). The adrenal gland of rats treated with ginger showed the same PAS stain-ability as those served as control.

Animals treated with mancozeb and examined after 2 and 4 weeks showed that the capsule exhibited a strong reaction with PAS but the cytoplasm of the cortical zones showed a reduction in polysaccharide content (Fig. 10). After 6 weeks of treatment the adrenal gland exhibited a moderate to weak capsule reactivity and a marked depletion of the polysaccharide content was observed in the cells of cortical zones (Fig. 11). Examination of adrenal gland of rats treated with mancozeb followed by ginger showed a gradual increase of the polysaccharide content in the cytoplasm of cortical cells with marked improvement in comparison with mancozeb group in all periods of treatment (Fig. 12).
Sakr et al., Ginger (Zingiber Officinale) Ameliorates Mancozeb-Induced Alterations in the Adrenal Cortex of the Albino Rat

Fig. 10. Section of adrenal cortex of a rat treated with mancozeb for 4 weeks showing a depletion of polysaccharides in the cells of ZG and ZF, PAS × 400.

Fig. 11. Section of adrenal cortex of a rat treated with mancozeb for 6 weeks showing a marked depletion of polysaccharides in capsule and the cells of ZG and ZF, PAS × 400.

Fig. 12. Section of adrenal cortex of a rat treated with mancozeb and ginger showing increase of polysaccharides in capsule and cells of ZG and ZF, PAS × 400.

2- Total protein:

Total proteins appeared in the cortical cells of control rats in the form of small bluish granular bodies in the cytoplasm. The cell membrane and nuclear membrane exhibited strong reaction with mercury bromophenol blue method. The adrenal capsule as well as zona reticularis showed a strong reaction but the zona glomerulosa and zona fasciculata showed a moderate stain-ability (Fig. 13). Adrenal cortex of rats treated with ginger showed the same results as those served as control.

Animals treated with mancozeb and examined after 2 and 4 weeks showed that total proteins decreased in the capsule and the cells of zona glomerulosa and zona fasciculata while the zona reticularis cells showed moderate reactivity (Fig. 14). After treatment with mancozeb for 6 weeks there was a marked decrease in the protenic content in the capsule and the cells of zona glomerulosa and zona fasciculata which exhibited a low affinity towards the stain (Fig. 15). Treating rats with mancozeb followed by ginger showed a gradual increase in the protein content in the capsule and cortical zones (Fig. 16).

Fig. 13. Section of adrenal cortex of control rat showing moderate amount of total protein content in ZG & ZF, while ZR displayed a strong stain-ability, Bromophenol blue × 100.

Fig. 14. Section of adrenal cortex of treated rat with mancozeb for 4 weeks showing a reduction of the total proteins in ZG & ZF, while ZR showed moderate amount of total proteins, Bromophenol blue × 400.

Fig. 15. Section of adrenal cortex of treated rat with mancozeb for 6 weeks showing a marked reduction of the total proteins in the cortical zones, Bromophenol blue × 400.
DISCUSSION:

In the present study there was a significant decrease in the body weight after exposure to mancozeb. This result was obtained by some authors. Lu and Kennedy (1986) reported that mancozeb showed teratogenic effect in rats and caused severe weight loss. Kackar et al. (1997) studied the gonadal toxicity of male rats after chronic exposure to mancozeb. Signs of toxicity, mortality pattern and loss in body weight were observed in dose-dependent manner.

Treating animals with mancozeb induced many histopathological changes in the adrenal gland. It caused an increase in thickness of capsule, irregular arrangement in cells of zona glomerulosa, vacuolated cytoplasm and pyknotic nuclei in cells of zona glomerulosa, dilatation and congestion of blood sinusoids in both zona reticularis and medulla. These results were confirmed by some investigators. Bisson and Hontela (2002) indicated that mancozeb disrupted the sites downstream of the generating steps of the cortisol synthetic pathway in vitro; leading to adrenal toxicity. Hypertrophy of cells in zona glomerulosa was seen at dose level 1000 ppm of mancozeb in the adrenal gland of rats (WHO, 1993). O’Hara and Didnto (1985) reported that treatment with mancozeb at 1000, and 10,000 ppm increases deposits of brownish pigments in the zona reticularis of the adrenal cortex in both sexes of mice.

Adrenal cortex of animals treated with mancozeb exhibited a marked decrease in the polysaccharide content in cortical cells in time-dependent manner. At the same time, adrenal gland of the same group showed a marked decrease in its proteinic content. These histochemical results are similar to those reported in different organs under the effect of mancozeb. Mehadevaswami et al. (2001) reported that mancozeb at a dose level of 600, 700, and 800 mg / kg day induced a significant decrease in the level of glycogen in the liver albino rats. Sakr and Sarhan (2009) reported a decrease in the liver content of protein and glycogen in albino mice treated with mancozeb. A significant decrease in protein, glycogen and total lipids was observed in ovary of Wistar rats given mancozeb (Baligar and Kaliwal, 2001). The decrease in proteins may be due to change in enzymes related to protein degradation. In this concern, Igbedioh and Akinwale (1992) recorded a significant increase in alanine and aspartate transaminases activities (concerned with protein degradation) in rats treated with benomyl. Sakr et al. (2004) also observed reduction of total proteins in liver of benomyl-treated rats. The authors suggested that the reduction may be due to either arrested metabolism or to use it to build up new cells or enzymes to reduce the stress. In a paradoxical study on male Wistar rats fed mancozeb in the diet for 90 days, the protein content of the adrenal and thyroid gland increased (Nicolaou, 1982).

Animals treated with mancozeb and ginger in the present work exhibited a marked degree of improvement in the histological and histochemical alterations induced by mancozeb; this reflects the ameliorative effects of ginger on biological system. The effect of ginger on tissue 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 Izejebu, 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 orally administration of ginger ethanolic extract (200 mg/kg) 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 of Sakr et al. (2009) showed that ginger scavenging free radicals by its potent antioxidant activity. These authors recorded that ginger reduced the level of serum malondialdehyde acting as lipid peroxidation marker, and increased the serum level of antioxidant enzymes, 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 defence (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 defence system or by their direct free radical scavenging activity. Amin and Hamza (2006) demonstrated that Z. officinalis increased the activities of testicular antioxidant enzymes, superoxide dismutase, glutathione and catalase and reduced level of malondialdehyde.

Although several mechanisms have been postulated for interpreting the ameliorative action of ginger as alternative medicine, this work suggested that Z. officinale mediates its effects on adrenal damage induced by mancozeb via its potent antioxidant action.

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تأثير الزنجيب على التغيرات الهيستوكلينية والهستوكيماوية الحادثة في العدة الكظرية للجرذان

صلح محجوب
قسم علم الحيوان، كلية العلوم، جامعة المنوفية، مصر

نوضج في المحتوى الكلي للعدة الكظرية من المواد المنكوبة "الزنجيب" في العدة الكظرية للجرذان، Nombre de las especies de Zingiber officinale que se han utilizado en la literatura, Nombre de las especies de Zingiber officinale que se han utilizado en la literatura, Nombre de las especies de Zingiber officinale que se han utilizado en la literatura, Nombre de las especies de Zingiber officinale que se han utilizado en la literatura, Nombre de las especies de Zingiber officinale que se han utilizado en la literatura.

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