ABSTRACT
Mancozeb is an ethylene-bis-dithiocarbamate (EBDC) fungicide. It is used in a wide range to control several of fungal diseases in field crops and fruits. Administration of mancozeb at a dose level of 1/10 LD50 3 times / week for six weeks induced marked histological and histochemical changes in thyroid gland of albino rat. The histological changes included dilation and congestion in blood vessels of the interstitium, atrophy of many follicular cells and hyper trophy of the parafollicular cells, decrease in the amount of colloid and cytoplasmic vacuolation in the follicular cells. The histochemical changes appeared as decrease in both PAS positive materials and protein contents. Ginger (Zingiber officinale) is a monocotyledonous plant with many pharmacological properties. Treating animals with ginger extract induced improvements in both the histological and histochemical changes induced by mancozeb in the thyroid gland. These results proved that ginger had inhibitory effects against thyroid toxicity induced by mancozeb.

INTRODUCTION
The most important class of fungicides for controlling the fungi of agricultural crops was known to be ethylene-bis-dithiocarbamate (EBDC) forms. This class include nabam , maneb , mancozeb , meteriam and zeineb (Seiler, 1974 , Graham et al., 1973) . Mancozeb is applied used as a protective fungicide to control a wide range of pathogens in field crops, fruits, ornamentals and vegetables (Worthing, 1991). Despite all the good beneficial properties of mancozeb it has a lot of hazardous effects on human and animal health. Debbarh et al. (2002) reported that cases which exposed to short-term exposure to (EBDC) exhibited variable neurological symptoms including headache, dizziness, confusion and a few cases experienced seizures. Long-term exposure has been associated with Parkinsonism. However epidemiological studies indicated an increased risk of neurocognitive impairment associated with long term-exposure to pesticides in general and EBSC in particular. Regarding effect on fertility, mancozeb exerted many toxic effects. Kacker et al. (1997) studied the gonadal toxicity signs in male rats after chronic exposure to mancozeb and found signs of toxicity. A significant increase in the testes and epididymis weights, degeneration in seminiferous and epididymis tubules also loss of sperms were recorded. Mancozeb induced histological and ultrastructural changes in liver (Sakr et al., 2005) such as congestion of blood vessels, leucocytic infiltration, cytoplasmic vacuolation of the hepatocytes and pyknosis. Ultrastructurally the hepatocytes showed irregular nuclei with abnormal chromatine, as well as swollen and degenerated mitochondria.

Belpoggi et al. (2002) confirmed that mancozeb caused an increase in total malignant mammary tumours, ear duct carcinomas, hepatocarcinomas, malignant tumour of pancrease, malignant tumour of the thyroid gland, osteosarcomas of the bones of
the head, and haemolymphophoreticular neoplasia in rats. In addition, mancozeb was found to induce chromosome aberration and increase the frequency of sister chromatid exchange (Jablonicka et al., 1989).

Today many of drugs include a natural product prototype which were originally discovered through the study of traditional cures and folk knowledge of indigenous people. Ginger is an example of botanicals that gained popularity amongst modern physicians (Gilian and Rahman, 2005). A considerable number of researches were carried out on ginger and its pungent constituents, fresh and dried rhizome. Among the pharmacological effects demonstrated are anti-oxidants, anti-tumour, anti-rhinoviral and anti-hepatotoxicity (Gujral et al., 1978, Sambaiah and Srinivasan, 1991, Wohlmut, 1999). Ginger was found to have hypcholesterolaemic effects (Bhandari et al., 1999). Ginger was found to have ameliorative effect of ginger on toxicity with motion sickness, surgery and pregnancy symptoms of nausea and vomiting associated (Fisher-Rasmuseen et al. 1992, Sharma et al., 1994). One of the most popular uses of ginger is to relief the arthritis effect of ginger was demonstrated by many investigators like as Mascolo et al., 1989, Srivastava and Mustafa, 1992, Sharma et al., 1994. One of the most popular uses of ginger is to relief the symptoms of nausea and vomiting associated with motion sickness, surgery and pregnancy (Fisher-Rasmuseen et al., 1991). The present work was conducted to study the possible ameliorative effect of ginger on toxicity induced by the fungicide, mancozeb on thyroid gland of albino rats.

**MATERIAL AND METHODS**

Healthy adult male rats (Rattus norvegicus) approximately three months old and each weighs 130 ±5 g, were used in the present study. The animals were selected randomly and kept in cages under laboratory constant condition of temperature 25ºC±3 with a reverse natural light-dark cycle for at least one week before starting the experimental work. Animals were maintained on a standard synthetic diet, manufactured especially for laboratory purposes and tap water were available ad libitum. Animals were divided into four groups.

Group (1): Animals of this group served as control.

Group (2): Animals of this group (20 rats) were orally given one ml of final aqueous extract of ginger (24 mg/ml) 3 times a week, then sacrificed at 2nd, 4th & 6th week post-treatment.

Group (3): Animals of this group (20 rats) were orally given 1/10 LD50 of mancozeb, 313.6 mg/kg bw (Sakr et al., 2005) 3 times a week, then sacrificed at 2nd, 4th & 6th week post-treatment.

Group (4): Animals of this group (20 rats) were orally given mancozeb (1/10 LD50) followed by 1 ml of final aqueous extract of ginger (24 mg/ml) 3 times a week, then sacrificed at 2nd, 4th & 6th week post-treatment.

The thyroid gland of the treated and control animals were removed and fixed either in alcoholic Bouin’s (for histological study and general carbohydrate) or 10% neutral formalin for histochemical demonstration of total protein. Fixed materials were embedded in paraffin wax and sections of 5 µm thickness were cut on rotary microtome. Sections were stained with haematoxylin and counterstain with eosin for histopathological examination. For demonstration of carbohydrates, the periodic acid Schiff’s (PAS) technique was applied (Hotchkiss, 1948). Total protein was demonstrated by using mercury bromophenol blue method (Mazia et al., 1953). For lipid peroxidation and antioxidant enzyme determination, sera were obtained by centrifugation of the blood samples and stored at -20ºC until assayed for the biochemical parameters. Serum malandialdehyde (MDA) (Ohkawa et al., 1979), superoxide dismutase activity (Rest and Spitznagel, 1977) and catalase activity (Aebi et al., 1974) were determined. The result was analyzed statistically using student’s “t” Test.

**RESULTS**

1-Histological results:

The control animals and those orally administered with ginger showed normal thyroid gland structure (Fig.1). The gland is formed of oval or spheroidal follicles; each represents the gland’s functional unit. The follicles are lined with simple cuboidal epithelium. The cavity of each follicle is filled with homogeneous colloid which is a pink-staining homogenous proteinaceous material rich in thyroglobulin. The thyroid gland is enveloped in a fibrous capsule from which fine collagenous septa extend into the gland, dividing it into lobules. The septa convey a rich blood supply together with lymphatic and nerves. The parafollicular cells are separated from the lumen, they are large than the follicular cells, oval or spherical in shape, and their cytoplasm displays lighter staining than that of the follicular cells.
The thyroid glands of rats that received mancozeb 1/10LD50 (313.6 mg/kg) 3 times a week showed noticeable histological evident. After 2 weeks of treatment, the thyroid gland showed dilated and congested blood vessels in the interstitium, decreased size in many follicles, reduced amount of colloid and vacuolation in the cytoplasm of the follicular cells (Fig. 2).

Examination of the thyroid gland after four weeks thyroid gland exhibited many follicles that were filled with desquamated cells and others showed foamy Lumina. Also there were decreases in the amount in the follicular colloid and vacuolation in cytoplasm of the follicular cells. The interstitium between the follicles became wider (Fig. 3). The histopathological alterations in thyroid were increased after 6 weeks of treatment. There were numerous small atrophic follicles with remnants of colloidal material. Most nuclei of follicular cells were pyknotic. Highly congested blood vessels in the interstitium were demonstrated, and the interfollicular spaces were dilated. Hypertrophy of the parafollicular cells was shown at the basal lamina of the thyroid follicles (Fig. 4).

The thyroid gland of rats treated with mancozeb followed by ginger 3 times a week showed marked improvement compared with the same periods of mancozeb group only. The thyroid gland showed marked increase size of its follicles with widening of their lumina. The colloid material filled the follicle lumina and the follicular cells exhibited...
normal shape in all periods of treatment (Figs 5, 6 & 7).

Fig. 5. Section in thyroid gland of a rat treated with mancozeb followed by ginger after two weeks showing widening of the follicle lumina and marked increase in the size. The cytoplasm of some cells show cytoplasmic vacuolation (arrow) H.E. × 400

Fig. 6. Section in thyroid gland of a rat treated with mancozeb followed by ginger after 4 weeks showing restoration to normal structure with cytoplasmic vacuolation of follicular cells (arrow) H.E. × 400

2- Histochemical Results:  

A-General carbohydrates:  

The thyroid gland of the control animals and those which were orally given ginger showed a strong PAS reaction of colloid material in the follicles while the basement membrane showed moderate reaction. The follicular cells showed faintly stained inclusions in their cytoplasm (Fig. 8).

Fig. 8. Section in thyroid gland of a control rat showing strong PAS reaction of colloid material in the follicles (F) and faintly stained cytoplasm of follicular cells (arrow), PAS × 400

After two weeks of treatment with dose of 1/10 LD50 of mancozeb, the colloid material in the follicle was decreased and exhibited a moderate PAS reaction, also the basement membrane showed moderate PAS reaction (Fig. 9).

Fig. 9. Section in thyroid gland of a rat treated with mancozeb (1/10 LD50) for two weeks showing a decrease in the colloidal material of follicle (F) with moderate PAS reaction, PAS × 400

After 4 weeks, the colloidal material of the follicles was obviously decreased and became less PAS positive and there were
faintly stained basement membrane and follicular cells (Fig. 10).

After six weeks of treatment, there was almost complete lack of carbohydrate in the thyroid follicles and the follicular cells were almost devoid of carbohydrates (Fig. 11). The thyroid glands of rats treated with mancozeb followed by ginger day after day showed a marked degree of improvement with tendency towards restoration of carbohydrate contents (Figs 12, 13, & 14).
**B-Total protein:**

Examination of the thyroids gland of control rats and those which were orally given ginger showed a strong positive reaction of colloid material and also in the follicular and Parafoolicular cells (Fig. 15).

Fig. 15. Section in thyroid gland of a control rat showing positive reaction of colloid material, follicular (arrow) and parafoolicular cells (double arrow) Bromophenol blue × 400

Thyroid gland of animals that received mancozeb (1/10LD50) 3 times/week showed a gradual decrease in the proteinic contents showed a decrease in colloidal material and also there was a decrease in follicular cells contents of protein. A moderate reactivity with mercury bromophenol blue after two and four weeks of treatment were detected (Figs 16 & 17).

Fig. 17. Section in thyroid gland of a rat treated with mancozeb for four weeks showing a marked decrease in proteinic content of follicles (F) and cytoplasm of follicular cells (arrow) Bromophenol blue × 400

After six weeks of treatment, there was a complete absence of proteinic contents in follicles. The follicular cells exhibited weak reactivity (Fig.18).

Fig. 18. Section in thyroid gland of a rat treated with mancozeb for six weeks showing a complete absence of proteinic contents in follicles (F) and exhibited a weak reactivity of the cytoplasm of follicular cells (arrow) Bromophenol blue X 400

Examination of thyroid gland of rats treated with mancozeb was evident (1/10LD50) followed by ginger (24 mg/ml) 3 times a week showed a marked degree of improvement with tendency towards restoration of proteinic contents (Figs 19, 20, & 21).
C- Oxidative stress and antioxidant:

1- Malondialdehyde (MDA):

Data in Figure 22 revealed that there was a significant increase (P<0.05) in the level of Malondialdehyde (MDA) in the sera of animals treated with mancozeb (1/10 LD50) in all periods of treatment compared with those of control. On the other hand, animals treated with mancozeb followed by ginger showed a decrease of the (MDA) level in serum after 2 weeks of treatment which become significant after 4 and 6 weeks of treatment in comparison with the mancozeb treated group.

![Graph showing changes in serum MDA (nmol/ml)]

2- Catalase activity (CAT):

Animals treated with mancozeb (1/10 LD50) 3 times / weeks showed a significant decrease in the level of catalase (CAT) in all treated groups of treatment compared with the control one, while animals treated with mancozeb followed by ginger showed a significant increase in the level of CAT compared with mancozeb treated group, (Fig. 23)

3 - Superoxide dismutase (SOD) activity:

Figure 24 showed that the level of superoxide dismutase (SOD) exhibited 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 of serum.
DISCUSSION:

The present study examined the effect of mancozeb fungicide on thyroid gland of rats using histological and histochemical as well as biochemical parameters. Concerning the histopathological alterations, the thyroid gland showed numerous small atrophic follicles with remnants of colloidal material. Most nuclei of follicular cells were pyknotic. Fully congested blood vessels in the interstitium were demonstrated, and the interfollicular spaces were dilated. There was hypertrophy of parafollicular cells lying at the basal lamina of the thyroid follicles. O’Hara and Didnto (1985) reported that mancozeb causes vacuolization, interstitial congestion and decrease colloid density in the thyroid gland of rats. Jacobsen et al. (2004) reported a significant increase in thyroid gland weight and hypertrophy of the follicular cells after treatment with mixture of five pesticides including mancozeb. Belpoggi et al. (2002) reported that administration of mancozeb at the concentration of 1000, 500, 100, 10 and 0 pm in food supplied ad libitum for 104 weeks, caused malignant tumour of thyroid gland. WHO (1993) announced in a long-term toxicity and carcinogenicity study on rats fed 0, 20, 60, 125 or 750 ppm mancozeb for two years, caused a significant increase of follicular cell adenomas in the thyroid gland especially in the highest dose. Tomasi et al. (2001) examined the effect of mancozeb on thyroid function of rats. They found that the thyroid hormones concentration was generally decrease. Also, Trivedi et al. (1993) demonstrated that mancozeb at a dose level of 500, 1000, and 1500 mg / kg for 90 days decreased the level of serum thyroxin and TSH in rats. Furthermore, Szepvolgyi et al. (1989) indicated a significant decrease in thyroid iodine content after rats exposed to mancozeb mixed in the food.

Concerning histochemical results in the present work, the thyroid gland showed severe reduction in the carbohydrate content. Also, there was a complete absence of the proteinic contents in the colloid of the follicles. The follicular cells exhibited weak reactivity after treatment with mancozeb.

The histochemical results in this work are almost similar to those reported in different organs under the effect of mancozeb. Baligar and Kaliwal (2001) and Mehadevaswami et al. (2001) reported that mancozeb at dose levels of 600, 700 and 800 mg / kg day induced a significant decrease in the level of glycogen in the liver and ovary of albino rats. Sakr et al. (2005) reported a decrease in the liver content of protein and glycogen in albino mice treated with mancozeb. Mancozeb treated rats at doses of 500, 700, and 800 mg / kg body weight / day for 30 days revealed a significant decrease in the levels of protein and glycogen (Baligar and Kaliwal, 2001).

Result of the oxidative stress and antioxidant enzymes in this work revealed that there was a significant increase in the oxidative stress, malondialdehyde (MDA) which is lipid peroxidation marker. On the other hand, there was a significant decrease in the level of serum antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) activity in mancozeb-treated group.
Malondialdehyde (MDA) 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. (2004) confirmed the oxidative effect of mancozeb which caused post-apoptotic and necrotic alteration in cell membrane integrity. These results come with agreement with the findings of the present study. Similarly, Fabra et al. (2004) demonstrated that mancozeb produced biochemical alteration in membrane composition, polysaccharides and polyamines. Also, Leiphon and Picklo (2007) demonstrated that maneb and benomyle inhibit the fungicide of whole respiring rat brain mitochondria using the oxidation of neurotoxic lipid-aldehyde trans-4-hydroxy-2nonenal (NHE) as a biomarker for mitochondria - aldehyde dehydrogenase (ALDH) activity in situ. Thus, the maneb and benomyle can decrease the detoxification lipid peroxidation derived aldehydes derived from neurotransmitters.

Animals treated with the mancozeb and ginger in the present work exhibited a marked degree of improvement in histological and histochemical alterations compared with the group treated with mancozeb only.

Apart from the effect on thyroid, ginger was found to have a nephroprotective effects against cisplatin-induced renal damage in mice (Ajith et al., 2007). Ansari et al. (2006) reported that ethanolic extract of ginger alleviates isoproterenol-induced myocardial necrosis in rats. Amin and Hamza (2006) found that ethanol extracts of ginger prevents histological and biochemical alterations induced by cisplatin in testis of rats. Ginger was found to have a hepato protective effect against CCL4 and acetaminophen-induced liver damage in rats (Yemitan and Izeqbu, 2006).

With respect to the results of oxidative stress and antioxidant enzymes in this work, the results obtained from mancozeb - and ginger - treated groups showed that ginger was scavenging free radical by its potent antioxidant. These results were cleared by the data in which ginger reduced the level of serum malondialdehyde (MDA) acting as lipid peroxidation marker and increase the serum level of antioxidant enzymes, exemplified in catalase (CAT) and superoxide dismutase (SOD). These results were similar to those shown by Siddaraju and Dharmesh (2007) illustrated that ginger-free phenolic (GRFP) and ginger hydrolysed phenolic (GRHP) fractions of ginger (Zingiber officinale) exhibited free radical scavenging, inhibition of lipid peroxidation, DNA protection and reducing power abilities indicating strong antioxidant prosperities. Ansari et al. (2006) showed that the ethanolic Z. officinale extract (200 mg/kg) pre-treatment for 20 days in isoproterenol treated rats induced oxidative myocardial necrosis in rats, enhances the antioxidant defence (catalase, superoxide dismutase and tissue glutathione) and exhibited cardioprotection property.

Z. officinale (250 mg / kg body weight) was found to be better in elevating the reduced activity of superoxide dismutase, catalase, glutathione peroxidase and decrease the high level of malondialdehyde (MDA) in cisplatin-treated group (Ajith et al., 2007). The same authors also reported that Z. officinale ameliorated cisplatin-induced nephrotoxicity and this protection is mediated either by preventing the cisplatin-induced decline of renal antioxidant defence system or by the its direct free radical scavenging activity. This result was conformed by Amin and Hamza (2006) who demonstrated that Z. officinale increased the activities of testicular antioxidant enzymes, superoxide dismutase, glutathione and catalase and reduced level of malondialdehyde. Ginger is, therefore, suggested to have highly protective and inhibitory effects due to its potent antioxidant action.

Hirahara (1974) showed that many cellular lipids and especially polyunsaturated fatty acids are vulnerable to attack by reactive oxygen species resulting in the formation of lipid peroxidation. The peroxidized lipid can cause cellular damage such as a cross-linking of proteins and DNA. Also, oxidized low density lipoproteins can contribute to the formation of atherosclerotic plaques. The same author also added that water and alcoholic extract of ginger have been shown to possess potent antioxidant activity on fats and oils and prevent lipid peroxidation.

Although several mechanisms have been postulated for interpreting the protecting action of ginger as alternative medicine. This work suggested that Zingiber officinale mediates its preventive effects on tissue damage induced by mancozeb via its potent antioxidant action.

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111


Tأثير الزنجبيل على الغيرات النسيجية والكيميائيـة التي بحـثت منها مبيد الفطريـات "المكودب" على الغدة الهرمية للجرادين البيضاء

صاحب عبد الرحمن صفار، يسري على عَدَد، إيمان كمال العادلي

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للzugbaraithكسة وكذلك غرام الألمولني حيث قُل نشاط إرذ وغيرييرموز في مصل الدم كما حدّ ارتفاع مِلْحَوَت لمستوي الغليايني الدهني. كما أشارت النتائج إلى أن نبات الزنجبيل من النباتات التي تشيع استخدامه في علاج كثير من الأمراض، أظهرت العدد الهرمية للحيوانات المعالمة بالصيد ومستخلص المائي نبات الزنجبيل مما تسهم ملحوت في كل من الغيرات النسيجية والكيميائية والبيولوجية للمادة المكودب، مما سيتيح أن نبات الزنجبيل أثر علاجية ووقائية ضد النسيجية المعالمة بالصيد الفطري المكودب.

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