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EFFECT OF GINGER (*Zingiber officinale*) ON MANCOZEB-INDUCED HISTOCHEMICAL CHANGES IN THE LIVER OF ALBINO RATS.

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ABSTRACT

Mancozeb, is an ethylene-bis-dithiocarbamate fungicide applied against a wide range of fungal diseases of crops and vegetables. The effects of mancozeb on the histochemical contents of the hepatocytes of albino rats were investigated. Treating rats with mancozeb at a dose level of $^{1}/_{10}$ LD_{50} three times/week for six weeks induced various histochemical changes .The hepatic cells revealed reduction in total carbohydrates, total proteins and nucleic acids (RNA,DNA). These alterations were time-dependent and were obvious in animals treated with mancozeb for 6 weeks. Treating animals with mancozeb and ginger *(Zingiber officinale)* led to an improvement in the histochemical alterations induced by mancozeb alone. These results proved that ginger had a therapeutic effect against liver injury produced by mancozeb and this effect is attributed to its antioxidant properties.

Keywords: Mancozeb, Ginger ,Rats, Histochemistry.

INTRODUCTION

Fungicides are extensively used against a wide range of fungal diseases of many field crops fruits and ornamentals. These chemicals were shown to be present in fruits products prepared for human consumption (Cabras and Angioni 2000). Mancozeb (Diathan-M) is an ethylene-bis-dithiocarbamate fungicide used against a wide range of fungal diseases of many plants (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 bengin skin tumours.

It is increasingly clear that certain botanicals natural products can mimic or antagonize the actions of different toxicants and in this manner they 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 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) . 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) . Sakr (2007) reported that ginger has an ameliorative effect against mancozeb fungicide-induced liver injury in albino rats. The present work was extended to study the effect of ginger on histochemical changes induced by mancozeb in hepatocytes of albino rats.

MATERIALS AND METHODS

Animals and treatments:

Adult male rats (*Rattus norvegicue*) 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 groups:

Group1: animals of this group (20 rats) were given orally the fungicide mancozeb dissolved in water at a dose level of 1/10 LD_{50} (313.6 mg/kg body weight) (Sakr 2007), 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).

Group 3: animals in this group (20 rats) were given ginger only.

Group 4: these animals (10 rats) were given water and were served as normal controls.

Histochemical studies:

The treated animals and their controls were killed by cervical dislocation after 2 ,4 and 6 weeks , quickly dissected and small pieces of liver were fixed in 10% neutral formalin, dehydrated, embedded in wax and 5 micrometers thick sections were cut. Sections were stained with the PAS-technique for the demonstration of general carbohydrates (Hotchkiss, 1948) and mercuy bromophenal blue method for the identification of total proteins (Mazia et al., 1953). Feulgen-methylene blue method was used for identification of both DNA and RNA (Garvin et al., 1979) and DNA was detected using Feulgen reaction (Stowel.1945).

Results

Total carbohydrates

The control animals which orally given water or ginger showed hepatocytes with normal histochemical contents. Liver sections of control rats stained with PAS showed normal total carbohydrates appeared in the form of deeply stained reddish granules in the cytoplasm of the hepatocytes, but the nuclei gave a negative stain (Fig.1).Hepatocytes of animals treated with mancozeb and examined after 2 and 4 weeks showed noticeable decrease in total carbohydrates in the cytoplasm of most cells (Fig. 2). Such reduction of total carbohydrates markedly appeared in the liver of animals examined after 6 weeks (Fig.3). Animals treated with manozeb followed by ginger revealed an improvement in total carbohydrates contents of the hepatocytes when compared with the schedule treatment of mancozeb group and a marked restoration of total carbohydrates was seen after 6 weeks (Fig. 4).

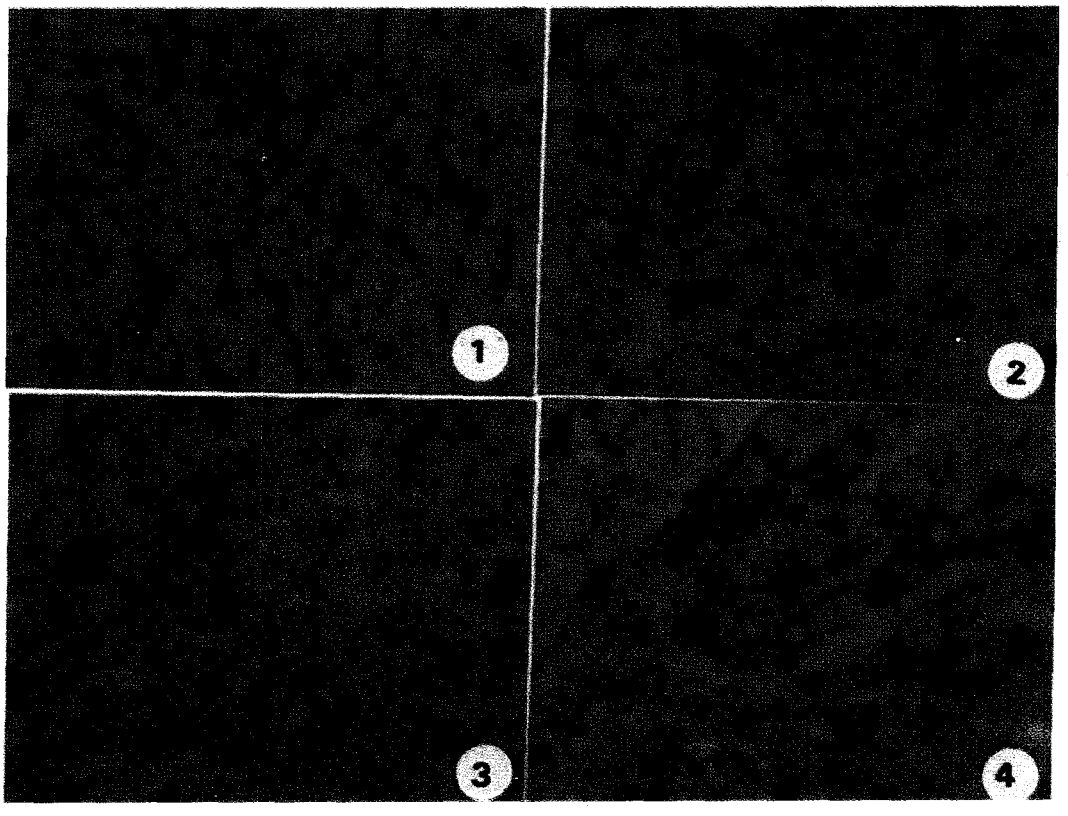

Fig.1. Liver section of a control rat showing distribution of total carbohydrates in the cytoplasm of the hepatocytes, X 400.

Fig.2. Reduction of total carbohyrates in hepatocytes of a rat treated with mancozeb and examined after 4 weeks, X 400.

Fig.3. Marked reduction of total carbohyrates in hepatocytes of a rat treated with mancozeb for 6 weeks, X 400.

Fig.4. Restoration of total carbohyrates in hepatocytes after 6 weeks post-treatment with mancozeb and ginger,X400.

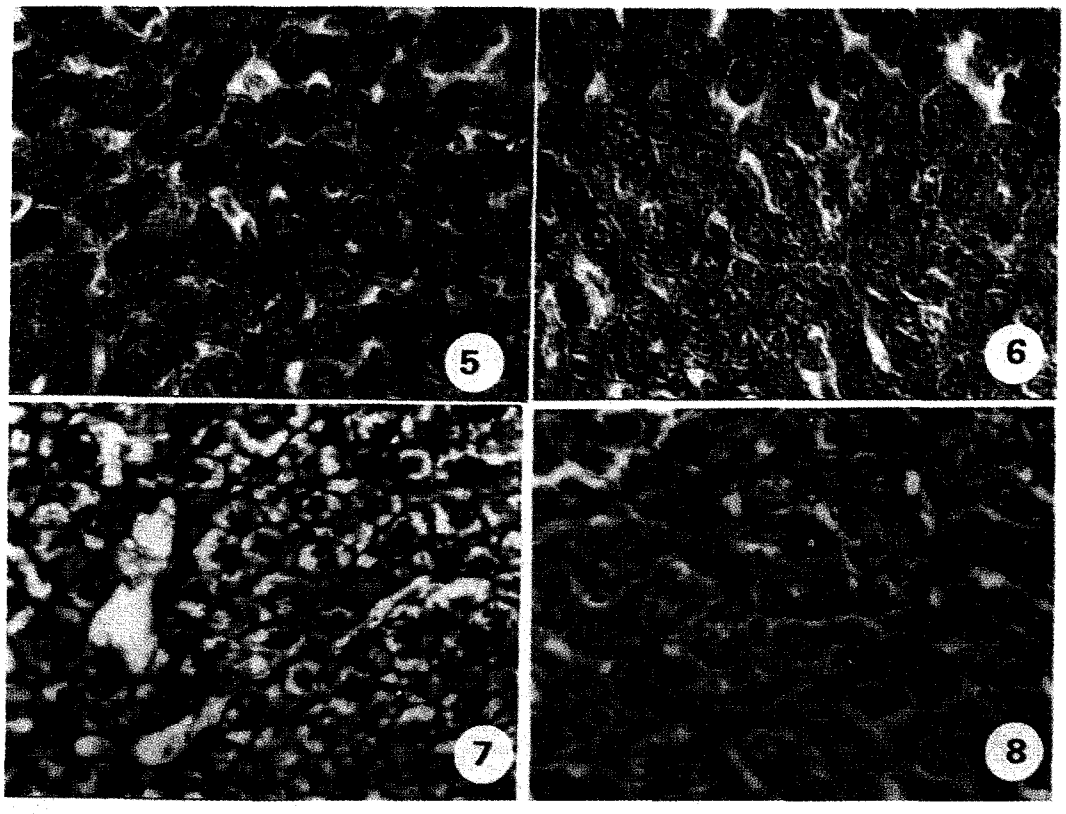

Fig.5. Normal protein content in the liver of a control animal , X 400.

Fig.6. Liver cells with loss of proteins in an animal treated with mancozeb for 4 weeks, X 400.

Fig.7. Marked reduction of proteins illustrated after 6 weeks following treatment with mancozeb, X 400.

Fig.8. Improvement of total protein contents in the hepatocytes after 6 weeks of treatment with mancozeb and ginger ,X 400..

Total proteins

Total proteins appeared in the hepatocytes of control rats as intensely dark blue coloured inclusions in the cytoplasm. The chromatin bodies and the nucleoli exhibited blue colouration with the bromophenol blue stain (Fig.5). Examination of liver of rats after 2 and 4 weeks of treatment with mancozeb showed a large number of cells were nearly devoid of proteins (Fig.6). After 6 weeks most of the hepatocytes appeared with cytoplasmic vacuolization and showed a reduction of their protein content (Fig. 7). Animals treated with mancozeb followed by ginger and examined after 2 and 4 weeks revealed improvement of protein content and a large number of the hepatocytes contained considerable amounts of proteins while others showed moderate amount .A marked increase of proteins was detected after 6 weeks (Fig. 8).

Ribonucleic acids (RNA , DNA)

Using Feulgen methylene blue method, RNA- containing particles appeared in the hepatocytes of control rats as small bluish-coloured particles distributed in the cytoplasm and the nuclei exhibited red colour indicating their DNA contents (Fig.9). Examination of hepatic cells after 2 and 4 weeks of treatment with mancozeb showed a decrease in their RNA centent (Fig.10). This decrease became marked in animals examined after 6 weeks (Fig.11). Hepatocytes of animals treated with mancozeb followed by ginger and examined after 2 and 4 weeks showed an increase in RNA content in comparison with those treated with mancozeb alone . After 6 weeks most of the cells have regained their normal contents of RNA (Fig.12).

DNA - containing particles (chromatin) appeared in the form of densely stained red particles distributed in the nucleoplasm and the peripheral rim of the nuclei (Fig. 13). Hepatic cells of rats examined 2 and 4 weeks after treatment with mancozeb showed a weak Feulgen reaction of their chromatin granules indicating a reduced amount of DNA (Fig.14). The decrease in DNA became obvious in animals examined after 6 weeks (Fig.15). Six weeks following mancozeb treatment in addition to ginger most of the nuclei acquired normal amount of DNA containing particles (Fig.16).

DISCUSSION

The present results showed that mancozeb induced histochemical changes in the liver of rats. The magnitude of these changes appeared to be time-dependent. Total carbohydrates and total proteins decreased in hepatocytes of mancozeb-treated rats. Similarly, Mehadevaswami et al. (2001) reported that mancozeb induced a significant decrease in the levels of protein, glycogen, total lipid, phospholipid and neutral lipid in the liver, uterus and ovary of albino rats.

Baligar and Kaliwal (2000) further demonstrated reduction in glycogen and protein contents in the liver and ovary of rats. The decrease of carbohydrates by the fungicide mancozeb seems to be achieved through modifying the activities of the enzymes of glycolytic pathway, TCA cycle, glucogenesis and the oxidative phosphorylation (Sherlock and Doely,1993). It was also reported that some insecticides may affect the carbohydrate metabolism through their effects on the endocrine system, especially by modifying the secretion of glucocorticoids and insulin (Pilo and Mehan, 1987). However, one or more of such factors could be considered as the causal agent of carbohydrate reduction observed in the liver of mancozeb-treated animals. The effect of fungicides on protein contents was studied by some investigators. Sakr et al. (2004) found that benomyl fungicide reduced total carbohydrates and proteins in hepatic cells of rats. Igbedioh and Akinyele (1992) proved that proteins decreased in liver of benomyl-fed rats. Oral administration of the fungicide maneb inhibited protein synthesis in liver and testis of rats (Ivanov and Izmirova, 1977). The reduction in protein content observed in this work may be attributed partially to the decreased level of protein synthesis in hepatic cells suffering from mancozeb toxic effect (Shakoori *et al.*, 1988).

Reduction of the nucleic acids, RNA and DNA was observed in the hepatocytes of rats exposed to mancozeb In this concern, Nicolau (1982) reported that exposure to mancozeb affected RNA, DNA and protein content in the thyroid and adrenal. It has been speculated that the decrease in DNA and RNA could be attributed to disruption of lysosomal membranes under the effect of various toxicants leading to freeing their hydrolytic enzymes (DNase & RNase) in the cytoplasm and resulted in marked lysis and dissolution of the target materials, DNA and RNA. This result confirmed that of Awasthi et al. (1984) who found elevated lysosomal enzymatic activity accompanied by a decrease in protein and nucleic acids contents in response to organophosphate insecticide with release of nucleases and proteases affecting RNA, DNA and protein metabolism.

Animals treated with mancozeb followed by ginger revealed an improvement in the histochemical changes induced in the liver by mancozeb. In agreement with this results , ginger was found to have a hepatoprotective effect against mancozeb (Sakr ,2007) , CCl_4 and acetaminophen(Yemitan and Izeqbu, 2006) induced liver damage in rats. It also have a nepheroprotective effects against cisplatin induced renal damage in mice (Ajith *et al.*2006). 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 ands biochemical alteration induced by cisplatin in testis of rats.

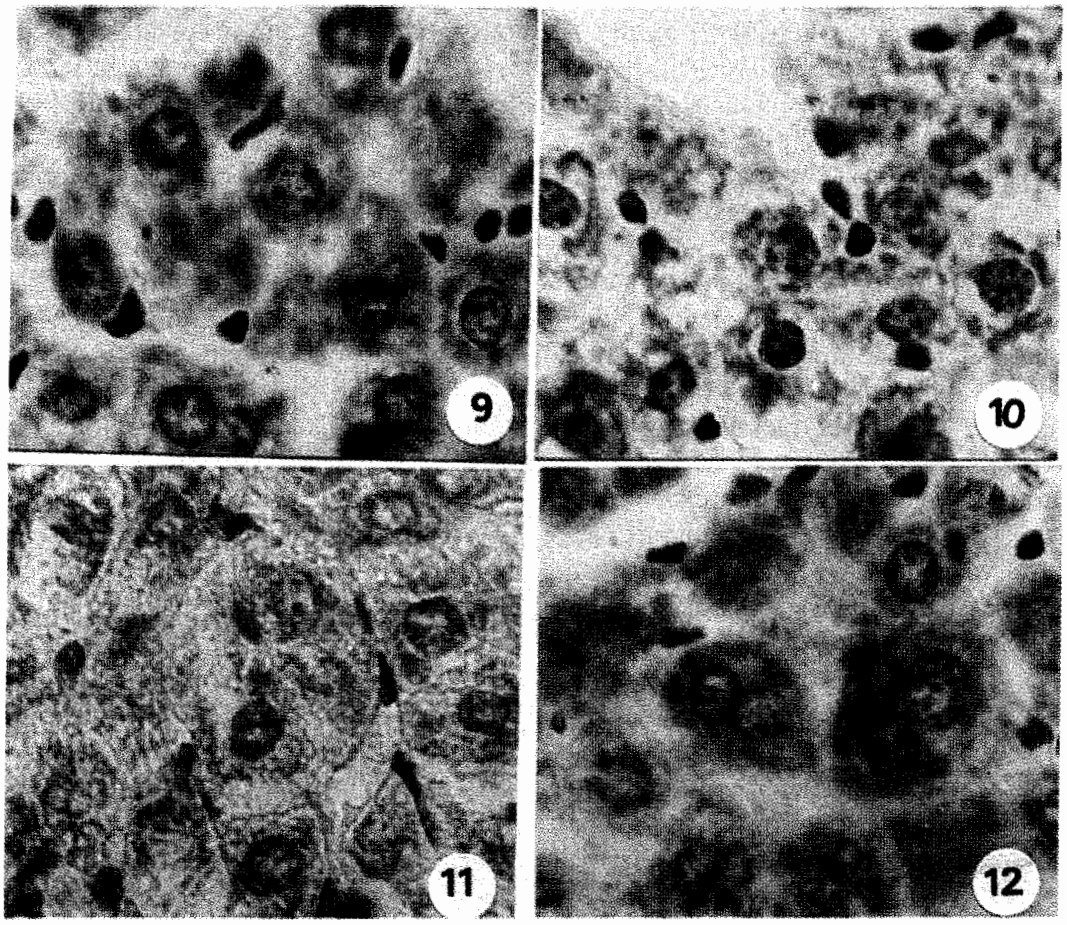

Fig.9. Normal content of RNA-containing particles in the cytoplasm and nucleoli of the hepatocytes , X 1000.

Fig.10. Decrease of RNA-containing particles in the hepatocytes of an animal examined 4 weeks after treatment with mancozeb, X1000.

Fig.11. Marked loss of RNA-containing particles in the hepatocytes of an animal examined 6 weeks after treatment with mancozeb , X1000.

Fig.12. restoration of RNA-containing particles in the hepatocytes of a rat examined 6 weeks after treatment with mancozeb and ginger ,X 1000.

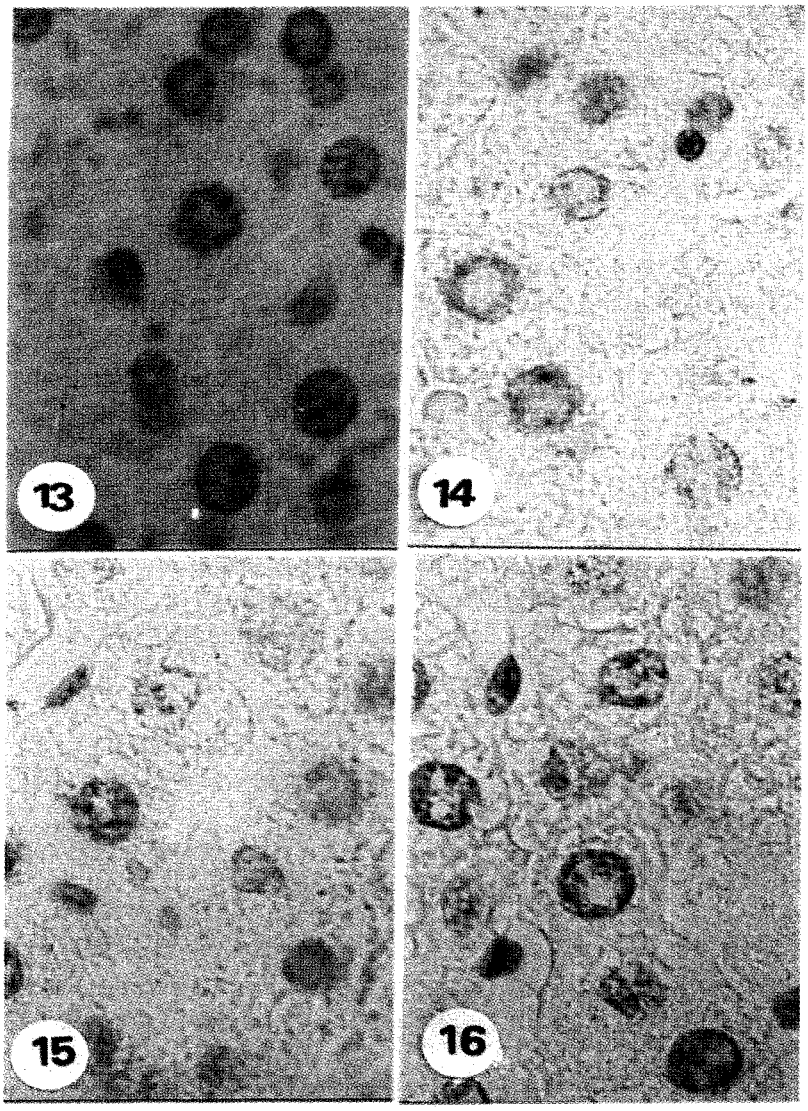

Fig.13. Normal distribution of DNA-containing particles in the nuclei of hepatocytes of a control animal,X1000.

Fig.14.Diminution of DNA-containing particles in the nuclei of hepatocytes of an animal treated with mancozeb for 4 weeks,X1000.

Fig.15. Hepatocytes of a rat treated with mancozeb for 6 weeks showing marked reduction of DNA particles, X1000.

Fig.16. Hepatocytes of an animal treated with mancozeb and ginger and examined after 6 weeks showing increase of DNA particles in some cells, X1000

The mechanism(s) of ginger action were investigated by some authors. Siddaraju and Dharmesh (2007) elucidated 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 properities. Ansari *et al.* (2006) showed that the ethanolic *Z. officinale* extract (200 mg / kg) pre treatment for 20 days in isoproternol treated rats induced oxidative myocardial necrosis in rats, enhances the antioxidant defense (catalase, superoxide dismutase and tissue glutathione) and exhibited cardioprotection property . *Z. officinal* (250 mg / kg body weight) was found to be better in elevating the reduced activity of superoxide dismutase, catalse, glutathione peroxidase and decrease the high level of malondialdhyde (MDA) in cisplatin treated group (Ajith *et al* ., 2007). The same authors also reported that *Z. officinale* was ameliorated cisplatin- induced nerphrotoxicity and this protection is mediated either by preventing the cispaltin – induced decline of renal antioxidant defense system or by 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 malondialdhyde.

Thus, it suggested that the effect of *Z.officinale* against mancozeb-induced histochemical changes in the rat hepatocytes may be attributed to its potent antioxidant activities.

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تأثير نبات الزنجبيل على التغيرات النسيجوكيميائية المتكونه فى كبد الجرذان بالمبيد الفطرى المنكودب"

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تناول هذا البحث دراسة التأثير العلاجى لنبات الزنجبيل عى الأضرار المحدثه بواسطة المبيد الفطرى المنكودب فى كبد الجرذان البيضاء. عند معاملة الحيوانات بجرعة مقدارها ١/١٠ من الجرعة نصف المميتة (٣ مرات أسبوعيا) عن طريق الفم لمدة ستة أسابيع أظهر فحص الكبد كثير من التغيرات النسيجوكيميائية حيث نقصت كمية الكربوهيدرات الكلية والبروتينات الكلية وكذلك الأحماض النوويه RNA;DNA وازدادت هذه التأثيرات مع زيادة مدة التعرض للمبيد. عند تعرض مجموعة الحيوانات لجرعة مقدارها ١/١٠ من الجرعة نصف المميتة من المبيد ثم معاملتها بنبات الزنجبيل بجرعة مقدارها ١ مل من المستخلص المائى لنبات الزنجبيل عن طريق الفم ثلاث مرات أسبوعيا لمدة ٦ أسابيع أوضحت المعالجة بالنبات تحسن فى المحتوى الهستوكيميائى لنسيج الكبد. وأتضح من هذه النتائج أن لنبات الزنجبيل آثار علاجية ضد التأثيرات الهستوكيميائية لمبيد الفطرى المنكوذب فى كبد الجرذان البيضاء.