CHRONIC CYTOTOXICITY OF MANCOZEB IN ALBINO RATS

Sleem, F. R. A.; Abou Elmagd, M. M. and Elalfy, M. M.

Department of Forensic Medicine and Toxicology,

Faculty of Veterinary Medicine, Mansoura university.

ABSTRACT

The oral LD_{50} of mancozeb was determined. The obtained results showed that, LD_{50} of mancozeb was 2143.6 mg/ kg. B. W. For studying the chronic cytotoxic effects, eighty male albino rats weighting 90 to 100 gm., were divided into four groups (20 for each). The first group used as control. Second, third and fourth given mancozeb orally through stomach tube at doses of 1/10, 1/20, 1/40 of calculated oral LD_{50} respectively twice weekly. After 20 weeks the animals were sacrificed, blood were collected and sera were separated for biochemical determination. Bone marrow was extracted from the femurs and prepared for detection of chromosomal aberrations. Liver, kidney, spleen, and brain were preserved in buffered formalin for histopathological changes. One gram of fresh livers were homogenized in 10% distilled water for determination of DNA and RNA contents. All used doses of mancozeb caused doses dependent significant increase in γ glutamyl transferase activity . Both doses of 1/10 and 1/20 LD_{50} showed significant increase in serum alkaline phosphatase activity. All doses showed significant increase in serum aspartate aminotransferase activity. All doses showed dose dependent significant increase in serum urea level. All doses showed dose dependent significant decrease in serum glucose level. All doses showed dose dependent significant increase in serum cholesterol level. Mancozeb showed no significant in serum bilirubin level. Chromosomal aberrations showed a dose dependent significant increase which appear in the form of fragments, gap, ring and sticked chromosomes. Doses of mancozeb (1/10 and 1/20) LD_{50} showed significant increase in quantity of RNA. There is no significant changes observed in DNA of liver contents of all treated groups and control. Histopathological changes revealed that, liver showed focal subcapsular coagulative necrosis infiltrated with numerous leukocytes as mononuclear cells and giant cells. Hyperplasia of epithelial lining of bile ductules besides newly formed bile ductules surrounded with few mononuclear cells and fibroblasts in the portal area and the adjacent hepatocytes showed apoptosis. Congestion of the portal blood vessels surrounded with mononuclear cells and large vesicular nuclei and the

adjacent hepatocytes showed pressure atrophy. Brain showed congestion, hemorrhage and focal encephalomalcia. Kidney showed congestion of the cortical blood vessels besides focal coagulative necrosis of some renal tubules. Spleen showed moderate depletion of lymphocytes from white pulp besides numerous siderocytes and congestion in red pulp.

The main conclusions of this study are mancozeb has chronic cytotoxic effects manifested by either histopathological or biochemical changes in addition to deleterious effects on nucleus as chromosomal aberrations and apoptosis. all these changes may lead to mutagenesis and probable carcinogenesis which should be considered and need extensive studies since the mancozeb is widely used effective fungicides either in agriculture field or fresh food preservatives.

INTRODUCTION

The extensive use of pesticides and the risks they pose to human health and the environment are now the focus of the world concern. All living creature tested throughout the world are polluted with pesticides such as birds, fish, wild life, domestic animals, live stock and human being including newborn babies **(Moses, 1992 and Davis, 1993)**.

The use of pesticides over the past 50 years has been resulted in the pollution of the soil, water, plant, and animal species. This pollution has created a long lasting environmental problem especially the members of the organchlorine class of pesticides where they resistant to environmental degradation and have been labeled as persistent accumulator **(Rought et al., 1999)**.

Mansour (2004) showed that Pesticides have contributed to great increases in crop yields and in the quantity and variety of the diet. Also, they have helped to limit the spread of certain diseases, but pesticides have harmful effects, they can cause injury to human health as well as to the environment. The range of these adverse health effects includes acute and persistent injury to the nervous system, lung damage, injury to the reproductive organs, and dysfunction of the immune and endocrine systems, birth defects, and cancer.

The use of pesticides has been increased dramatically in both developed and developing countries during the last few decades, with doubling every 10 years, between 1946 and 1985 about 600000 tons of pesticides annually were exported and used in developing countries, about 50000 tons of these were used for public health problems **(Jan et al., 1997)**. Dithiocarbamates are widely used as fungicides because of their efficacy against a broad spectrum of fungi and their associated plant diseases. Dithiocarbamates are also used in industry as slimicides in water-cooling systems, in sugar, pulp, and paper manufacturing, and as vulcanization accelerators

and antioxidants in rubber. Because of their chelating properties, they are also used as scavengers in waste-water treatment. (**Paul et al 1995**).

Mancozeb is used to protect many fruit, vegetable, nut and field crops against a wide spectrum of diseases, including potato blight, leaf spot, scab (on apples and pears) and rust (on roses). It is also used for seed treatment of cotton, potatoes, corn, safflower, sorghum, peanuts, tomatoes, flax and cereal grains (**Hayes and Laws 1990**). Mancozeb has been classified as unlikely to present acute hazard in normal use by the WHO Recommended Classification of Pesticides by Hazard, when handled in accordance with instructions (**WHO 1992**).

Mancozeb, is one of the most commonly used fungicides in commercial use for several decades. Nevertheless, up to now, no adequate published experimental studies on the carcinogenicity of Mancozeb have been published. Because of the importance of the compound and of the number of people potentially exposed (workers engaged in the production and use of the fungicide, people living in agricultural areas where the compound is sprayed, and people consuming polluted products (**Belpoggi et al 2002**).

There are a wide range variation of oral LD50 of mancozeb which determined in rats in many researches which ranging from 5000 to 14000 mg/kg. B.W. (Ivanova and Chemishanka 1969, **Watts and Chan 1984 and Chapman 1996**).

The aim of this study was evaluate the chronic cytooxic effects of mancozeb in addition to detect the LD50 due to a wide range variation of oral LD50 of mancozeb in rat.

MATERIALS AND METHODS

Pesticide: Dithane M 45 WP (Mnacozeb), was kindly obtained from EL-Nasser Company of Intermediate Chemicals, Egypt.

Common name: mancozeb

Trade name. Dithane^{R} M-45

Chemical name: Manganese ethylene bis (dithiocarbamate) complex with zinc salts

Chemical formula: $(C_4H_6MnN_2S_4) \times (C_4H_6N_2S_4Zn)$

Molecular weight: 541.064

Experimental animals:

Male albino rats weighted from 90 to 100 gm obtained from experimental unit, Faculty of vet-

erinary medicine, Zagazig University. Animals were apparently clinical healthy and were housed in stainless steel cages with wood shavings as bedding. Animals were accommodating to laboratory condition for two weeks before being experimented. Rats were maintained on balanced ration of barley and dry milk. Water and feed were given *ad libitium* throughout the experimental period.

Determination of median lethal dose (LD_{50})

Twenty five male albino rats weighting 90 to 100 gm were randomly distributed into five groups for determination of median lethal dose (LD_{50}). The groups given mancozeb at doses of 0, 1000, 2000, 4000, 8000 mg./Kg. B. W. respectively orally using stomach tube. The experimental animals were observed for 24 hours. The clinical signs, mortalities, and gross lesions were recorded through the experimental period. The LD_{50} value was calculated according to the method described by **Weil (1952)** using the following formula:

log m = Log D + d (k-1)/2+ df

Where m = median effective dose or exposure.

D = the lowest dose tested.

d = the logarithm of the constant ratio between dosages levels.

f : constant value obtained from special tables, for the proper k (the total number of level tested = k +1)

The confidence interval 95% was determined according to the same method using following formula.

$\text{Log } m \pm 2.179\ \delta_{\log m}$

Where log m = log LD_{50}

$\delta \log m = d.\ \delta f$

d : the logarithm of the constant ratio between dosages levels

f : a constant value obtained from special tables **(Weil, 1952)**

Experimental design

Eighty male albino rats weighting 90 to 100 gm., divided into four groups (20) for each. The first group used as control. Second, third and fourth given mancozeb orally through stomach tube at doses of 1/10, 1/20, 1/40 of calculated LD_{50} respectively twice weekly. All rats weight-

ed weekly to maintain the dose constant all over the period of experiment. after 20 weeks the animals were sacrificed, blood were collected and serum were separated for biochemical determination. Bone marrow were extracted from the femurs and prepared for detection of chromosomal aberration. Liver, kidney, spleen, and brain were preserved in buffered formalin for histopathological changes. One gram of fresh livers were homogenized in 10% distilled water for determination of DNA and RNA contents.

Biochemical analysis :

The activity of serum gamma-glutamyl transferase **Gendler and Kaplan (1984)**, Serum alkaline phospatase **(Kind and King, (1954)**, the activity of aspartate aminotransferase **(Reitman and Frankel (1957)**, serum glucose level **Kaplan (1984)**, total serum bilirubin **(Jendrassik and Grof (1938)**. serum urea **(Patton and Crouch (1977)** and serum cholesterol were estimated **(Naito and Kaplan (1984)**.

Chromosomal aberrations detection :

Chromosomal aberrations study was performed according to the method of **Choudhury et al. (2004)**.

Determination of Deoxyribonucleic acid (DNA) :

Liver DNA contents were determined colourmetrically by the diphenylamine procedure described by **Dische and Svhwarz (1937)**.

Determination of ribonucleic acid (RNA) :

Liver RNA contents were measured calorimetrically using orcinol procedure described by **Mejbam (1939)**.

Clinical signs and necropsy findings:

Clinical signs were observed in poisoned rats throughout the experimental period. Autopsies were performed in all rats and tissues were examined. macroscopically and histopathologically. For histopathological examination the tissues were fixed in 10 % neutral buffered formalin and were processed for routine histopathological examination **(Carson and Freida, 1990)**.

Statistical analysis:

Data obtained in this study were subjected statistically analyzed for variance (ANOVA), and least significant difference (LSD) as described by **Snedecor and Cochran (1989)**.

RESULTS

Determination of LD_{50} of mancozeb :

Biochemical findings :

Regarding to the effect of mancozeb on γ glutamyl transferase activity, all used doses of mancozeb 1/10, 1/20 and 1/40 LD_{50} caused doses dependent significant increase in γ glutamyl transferase activity. Both doses of 1/10 and 1/20 LD_{50} showed significant increase in serum alkaline phosphatase activity as compared to the control group. All doses of mnacozeb (1/10, 1/20 and 1/40 LD_{50} showed doses dependent significant increase in serum aspartate aminotransferase activity as compared to the control group. All doses of mancozeb (1/10, 1/20 and 1/40 LD_{50} showed doses dependent significant increase in serum urea level as compared to the control group. All doses of mancozeb (1/10, 1/20 and 1/40 LD_{50} showed doses dependent significant decrease in serum glucose level as compared to values of the control group. All doses of mancozeb (1/10, 1/20 and 1/40 LD_{50} showed dose dependent significant increase serum cholesterol level as compared to values of the control group. All doses of mancozeb (1/10, 1/20 and 1/40 LD_{50} showed no significant changes in serum biltrubin level as compared to the control group. All this results are summarized in table 2.

Chromosomal aberrations :

Chromosomal aberrations were a doses dependent and showed significantly increase in all doses of mancozeb (1/10, 1/20, 1/40 LD_{50}) which appear in the form of fragments, gap, ring and sticked chromosomes. This results summarized in table 3, and fig. 1A, B, C and D.

Effect of mancozeb on nucleic acid (DNA and RNA) :

Doses of mancozeb (1/10 and 1/20 LD_{50}) showed significant increase in quantity of RNA as compared to the control group but there is no significant was observed between dose 1/40 LD_{50} and control. There were no significant were observed in DNA of liver contents of all treated groups and control. The results are illustrated in Table 4.

Histopathological findings :

Liver showed focal subcapsular coagulative necrosis infiltrated with numerous leukocytes as

mononuclear cells and giant cells. Hyperplasia of epithelial lining of bile ductules besides newly formed bile ductules surrounded with few mononuclear cells and fibroblasts in the portal area and the adjacent hepatocytes showed apoptosis. Congestion of the portal blood vessels surrounded with mononuclear cells and large vesicular nuclei and the adjacent hepatocytes showed pressure atrophy necrosis at dose of 1/10 and 1/20 LD_{50} of mancozeb Liver showed congestion of the hepatic blood vessels and hepatic sinusoids with mononuclear cell infiltration at dose of 1/40 LD_{50} of mancozeb. This result illustrated in fig. 2A, B and C. Brain showed hemorrahagic congestion and focal encephalomalcia at doses of 1/10 and 1/20 LD_{50} of mancozeb But group 3 intubated with 1/40 LD_{50} of mancozeb showing congestion. This result illustrated in fig. 2D. Kidney showed congestion of the cortical blood vessels besides focal coagulative necrosis of some renal tubules at dose of 1/10 and 1/20 LD_{50} of mancozeb Kidney showed congestion of renal blood vessels at dose of 1/40 LD_{50} of mancozeb. This result illustrated in fig. 3A and B. Spleen showed moderate depletion of lymphocytes from white pulp besides numerous siderocytes and congestion in red pulp at doses of 1/10 and 1/20 LD_{50} of mancozeb. But group 3 intubated with 1/40 LD_{50} of mancozeb showed mild depletion of lymphocytes from white pulp besides congestion of the red pulp This result illustrated in fig. 3C and D.

DISCUSSION

There is a wide range variation of the oral LD_{50} of mancozeb in rats in many researches, for this reason we determined LD_{50} before starting the experimntal study. The present study investigated the oral median lethal dose (LD_{50}) of mancozeb in male albino rats was 2143.6 mg/ kg. B. W. which disagree with **Ivanova and Chemishanka (1969)** who reported that oral LD_{50} in male rats was 14000 mg/kg B.w. and in female rats 12000 mg/kg b.w. And also disagreed with **Watts and Chan (1984)** who determined the median lethal dose of mancozeb administered orally to male mouse, rat, and rabbit, the dose was recorded more than 5000 mg/kg. B. W. **Chapman (1996)** reported that LD_{50} of mancozeb in rats was 5000 mg/K.g. B. W. The oral LD_{50} in rat was acceptable and attributed the difference due to different strains of rat..

Chromosomal aberrations were dose dependent with (1/10, 1/20 and 1/40 of LD_{50}) and were in the form of fragments, gap, ring and sticked chromosomes. Significant increase in the frequencies of cells with structural chromosomal aberrations and sister-chromatid exchanges in short-term culture of peripheral lymphocyte of workers occupationally exposed to mancozeb during its production (**Jablonicka et al. 1989**) . Dithane caused genotoxic effect of bone marrow cells of male albino mice **Gautam and Kapoor 1991**. The chromosomal aberrations observed were fragments, rings, dicentric chromosomes, terminal chromatid deletions, chromatid gaps

and breaks. In addition to these chromosomal aberrations, physiological effects such as uneven stretching of chromatin material, end-to-end chromosomal associations, exchange configurations, clumping, stickiness and centromeric associations were also observed **(Gautam and Kapoor 1991. Soloneski et al 2002** studied the mutagenicity of both zineb and azzurro in Chinese hamster ovary cells. Concentrations of 0.1-25.0 microg/ml of zineb or azzurro induced a significant dose-dependent increase in sister chromatid exchange frequency over control values. The mutagenic effect of mancozeb in two strains of salmonella typhimurium was recorded **(Shukla et al 2004)** and postulated that the true mutagenic potential of mancozeb may be masked by its toxic effect. **Vasudev and Krishnamurthy (1994)** investigated the cytogenecity of both Dithane M-45 and Baygon in mice, neither pesticide induced a significant increase in the number of chromosomal aberrations in germ cells or in the percentage of erythrocytes micronuclei. In addition to these reports which indicated the mutagenicity of mancozeb, other reports indicated the direct damage effects of mancozeb on both DNA and RNA which constitute the basic structure of chromosomes. **Nicolau (1982)** found that when rats exposed to mancozeb with a dose of 100 ppm/day, the circadian rhythms of RNA, DNA and proteins in the thyroid and adrenal slightly affected but was statistically significant and also the testicular RNA rhythm shows multiple peaks. **Perocco et al, (1989)** studied the toxic and DNA-damaging activities of the fungicides mancozeb and thiram on human lymphocytes cultured in vitro with or without an S-9 mix microsomal metabolizing system. **Gupta and Mehrotra (1992)** studied the effect of mancozeb on mouse skin ornithine decarboxylase activity and DNA synthesis. Ornithine decarboxylase activity was exhibited a peak level at 5 hours but when cycloheximde was used , an inhibitor of protein synthesis, ornithine decarboxylase induction was inhibited. The rate of DNA synthesis also increased by mancozeb, as indicated by thymidine [3H] in coroporation into skin DNA. Induction of ornithine decarboxylase DNA synthesis was among the events probably involved in tumorigenic action of mancozeb in mouse skin .

YAO et al. (2004) found highly positive correlation between total GGT activity and the total RNA level of rats liver. The observed significant increase of quantity of DNA and RNA due to mancozeb administration attributed to genotoxicity of mancozeb. **Calviello et al. (2006)** studied DNA damage and apoptosis induction by Mancozeb in fibroblasts cultured in vitro and in peripheral blood mononucleated cells isolated from Wistar rats.

The results obtained from the present study revealed that chromosomal aberrations when rats exposed to mancozeb for twenty weeks, also RNA contents in the liver was affected and apoptosis in the hepatocytes in addition to alteration of liver function enzymes especially gamma glytamyl transferse activity. **Kovalszky et al, (1996) and Lopez et al. (1996)** showed that the induction of gamma glytamyl transferse in altered hepatocytes may permit these cells to utilize

extracellular glutathione to preserve their internal glutathione levels. Glutathione S-transferase induction allows glutathione utilization for the protection of the altered hepatocyte after exposure to xenobiotics, such as promoting agents. Thus, the combined effects of gamma glytamyl transferse and Glutathione S-transferase, in a toxic environment, may provide for the enhanced proliferation observed in pre neoplastic hepatocytes **(Hendrich and Pitot 1987)**.

Also **Yao et al. (2004)** found that fetal liver-type gamma glytamyl transferse in sera and the liver of rats is closely related to hepatotumorigenesis. It can be used as a sensitive enzymatic marker for the early diagnosis of liver cancer. The observed increase in gamma glytamyl transferse activity could be attributed to chronic sublethal exposure and need for additional detoxification mechanism as mancozeb decreased detoxicating capacity of liver **(Szepvolgyi et al. 1989)**. The oxidative effect of mancozeb suggest that its prooxidant action may be involved in the proapoptotic effect exerted by this compound in rat cells. It appears possible that the observed oxidative and genotoxic damage may be involved in the pathogenesis of various pathologies associated with the chronic exposure to mancozeb, including cancer **(Calviello et al 2006)**.

According to the previous reports and present study , mancozeb has direct effects on the cells either the cellular membrane, cytoplasm and nucleus which detected in histopathlogical changes as especially vesicular nuclei, DNA and RNA and caused chromosomal aberrations which lead to mutagenicity and apoptosis. All these changes could be considered the pre steps for cancer formation. The absence of cancer formation in the present study mainly attributed to short period of exposure and small doses used in addition to low number of doses administered (two doses weekly). The biochemical and histopathological changes of mancozeb were also cited on the cells by **Kackar et al (1999)** who found that mancozeb produced significant changes in the enzyme activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase and acetylcholinesterase throughout the period of study in a dose dependent manner. The alterations in the activity of enzymes associated with pathomorphological changes suggest that the chronic exposure of mancozeb produced significant toxicological effects in rats. The slight biochemical and histopathological changes in the present study mainly attributed to the small doses used and the time between the two successive doses administration which give chance of animal to tolerated and detoxificated of the most mancozeb.

The main conclusions of this study is that mancozeb has cytotoxic effects which manifested either by histopathological or biochemical changes, in addition to deleterious effects on nucleus which lead to chromosomal aberration and apoptosis, all these changes may lead to mutagenesis and probable carcinogenic effects which require extensive studies since the mancozeb is widely used as effective fungicides either in agriculture field or as fresh food preservatives.

Table 1. Results of LD_{50} of mancozeb in albino rat.

Group	No. of rats /group	Dose (mg/Kg. B.W.)	No. of mortalities
1	5	0	0
2	5	1000	0
3	5	2000	2
4	5	4000	5
5	5	8000	5

Table 2. Serum biochemical changes due to the effect of mancozeb on albino rat with different doses for 20 weeks (Mean ± SE).

Parameters / Groups	SGT (u/l)	ALP (u/100 ml)	AST (u/l)	UREA (mg/dl)	glucose (mg/dl)	cholesterol (mg/dl)	bilirubin (mg%)
Control	17.16^{c} ± 0.98	89.46^{b} ± 1.09	66.33^{b} ± 3.15	37.76^{b} ± 1.01	79.86^{a} ±2.27	73.46^{c} ± 4.78	12.76^{ab} ±0.16
1/10 of LD_{50}	45.33^{a} ± 2.46	107.83^{a} ± 2.61	116.5^{a} ± 4.75	48.33^{a} ± 2.28	39.116^{c} ± 1.15	103.8^{a} ± 6.65	13.66^{a} ± 0.38
1/20 of LD_{50}	41^{a} ± 1.02	102^{a} ±5.20	108.16^{a} ±4.69	43.33^{ab} ±2.64	30.38^{b} ± 0.54	98.6^{ab} ± 3.28	13.33^{a} ± 0.69
1/40 of LD_{50}	31^{b} ± 1.56	66.66^{c} ±4.83	93.33^{a} ± 3.20	42.66^{ab} ±1.50	30^{b} ±1.62	84.83^{bc} ± 3.13	11.5^{b} ±0.39

Means in the same column having the same superscripts were not significantly different ($p > 0.05$)

Table 3. Chromosomal aberrations induced in bone marrow cells of albino rat given different doses of mancozeb for 20 weeks

Groups	Number of examined cells	No. of Aberrant cells	Structural Aberrations				Numerical Aberrations (polyploidy)
			Fragment	Gap	Ring	Stick	
Control	100	2	0	0	0	2	0
1/10 LD_{50}	100	19	9	3	3	6	0
1/20 LD_{50}	100	11	6	3	1	5	0
1/40 LD_{50}	100	8	4	1	0	3	0

Table 4. Effects of different doses of mancozeb on DNA and RNA contents of rat liver mg/g. wet tissues (means ± SE)

Groups / Parameters	Control	1/10 LD_{50}	1/20 LD_{50}	1/40 LD_{50}
DNA	15.875^{a} ± 0.32	16.875^{a} ± 0.27	16.125^{a} ± 0.48	16^{a} ±0.40
RNA	7.125^{b} ± 0.275	8.50^{a} ± 0.15	8.25^{a} ± 0.153	7.25^{b} ±0.46

Means in the same column having the same superscripts were not significantly different ($p > 0.05$).

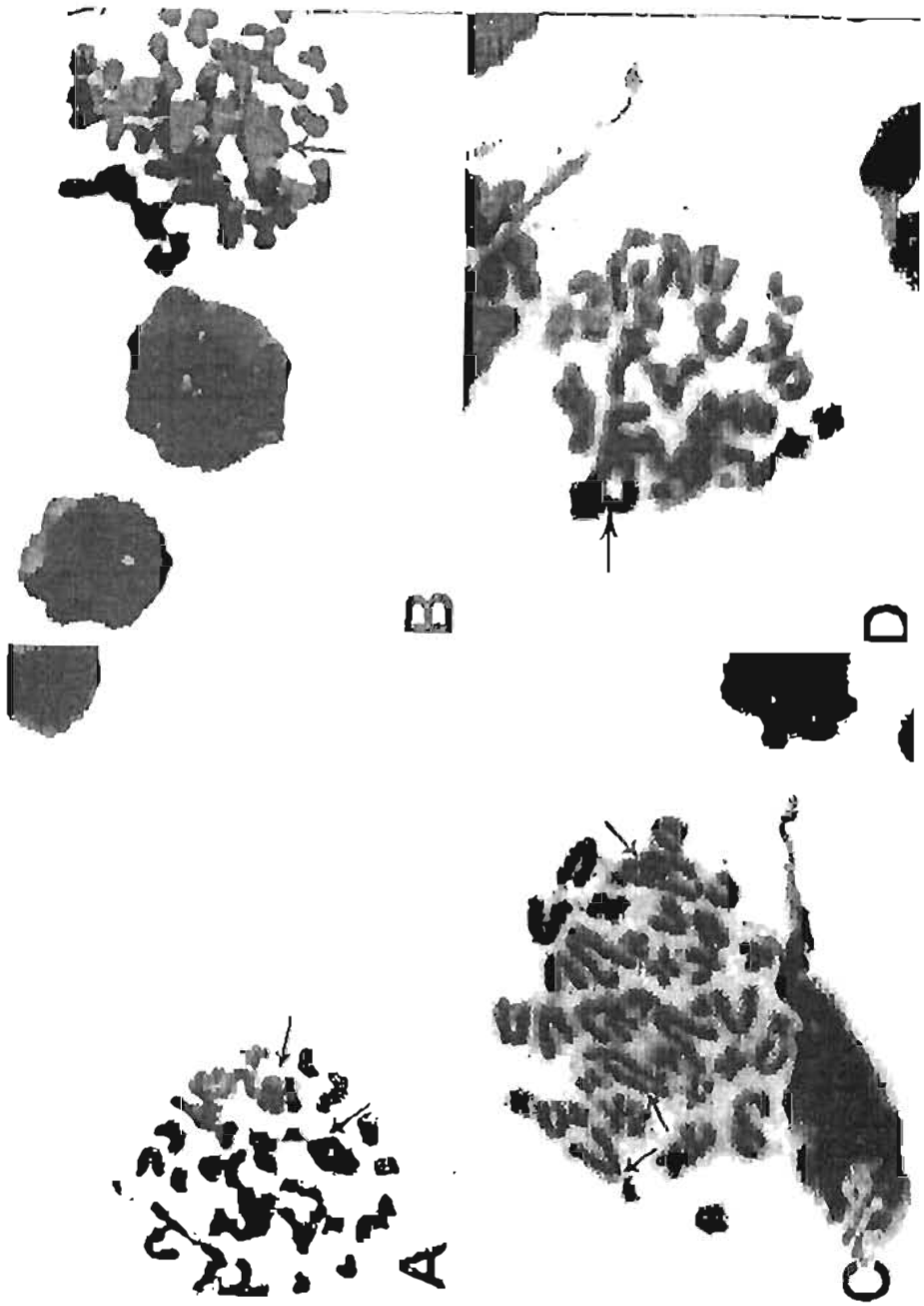

Fig. (1): Bone marrow cells of rats in metaphase spreading administered of mancozeb for 20 weeks with a showing (A): Ring (dosage of 1/10 LD_{50}). (B): gap and sucked (dosage of 1/10 LD_{50}). (C): fragment (dosage of 1/10 LD_{50}). (D): Ring (dosage of 1/20 LD_{50}).

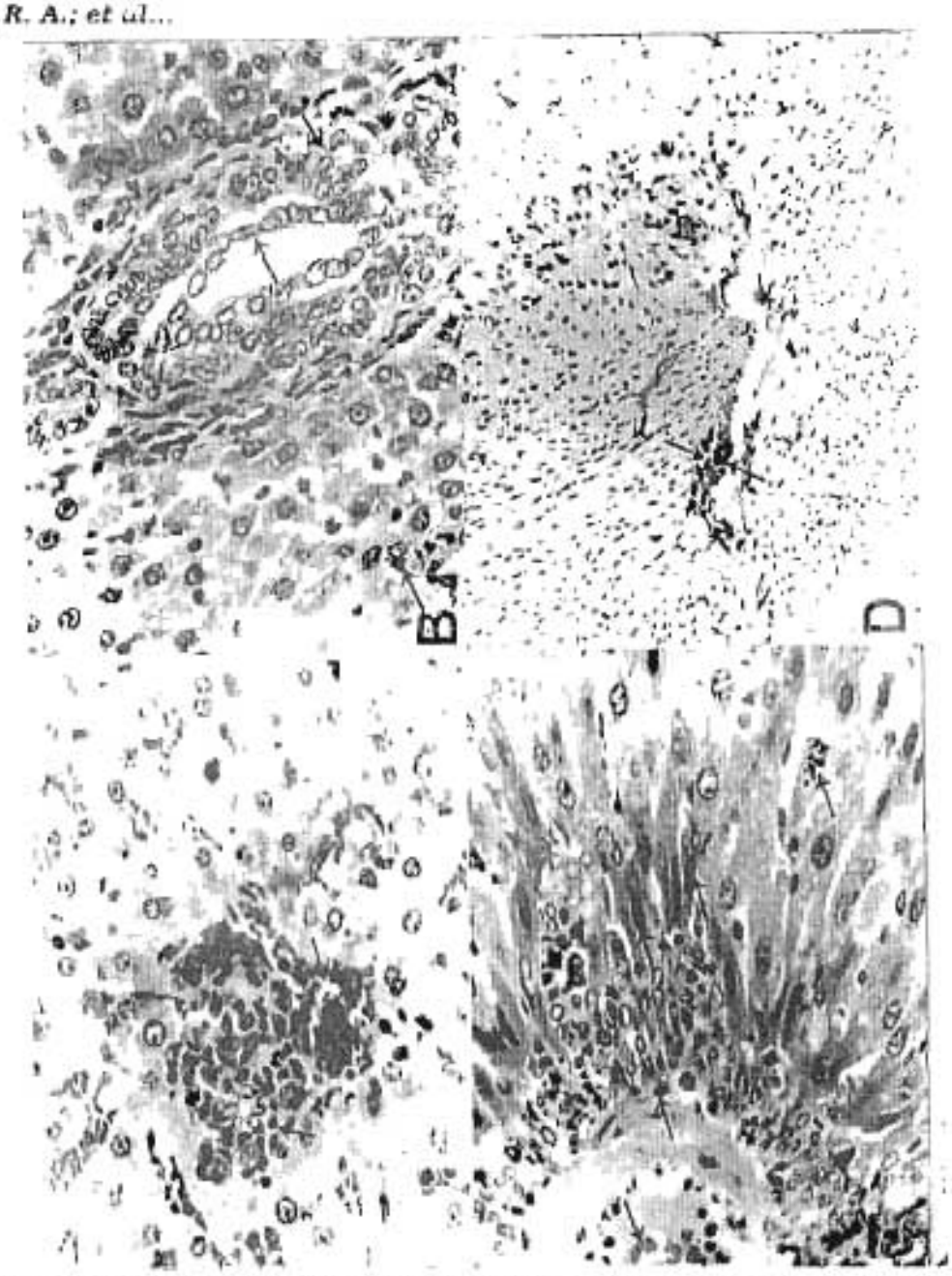

Fig. (2): **(A):** section of Liver from rat treated orally with 1/40 LD_{50} of mancozeb showing congestion of hepatic blood vessels and hepatic sinusoids with mononuclear cell infiltration (H&E. x 520). **(B):** section of Liver from rat treated orally with 1/10 LD_{50} of mancozeb showing hyperplasia of the epithelial lining of bile ductules besides newly formed bile ductules surrounded with few mononuclear cells and fibroblasts in the portal area. The adjacent hepatocytes showed apoptosis (H&E. x 520). **(C):** Section of Liver from rat treated orally with 1/10 LD_{50} of mancozeb showing congestion of the portal blood vessels surrounded with mononuclear cells and large vesicular nuclei. The adjacent hepatocytes showed pressure atrophy necrosis (H&E. x 520). **(D):** Section of brain from rat treated orally with 1/10 LD_{50} of mancozeb showing hemorrhage besides focal encephalomallcia (H&E. x 130).

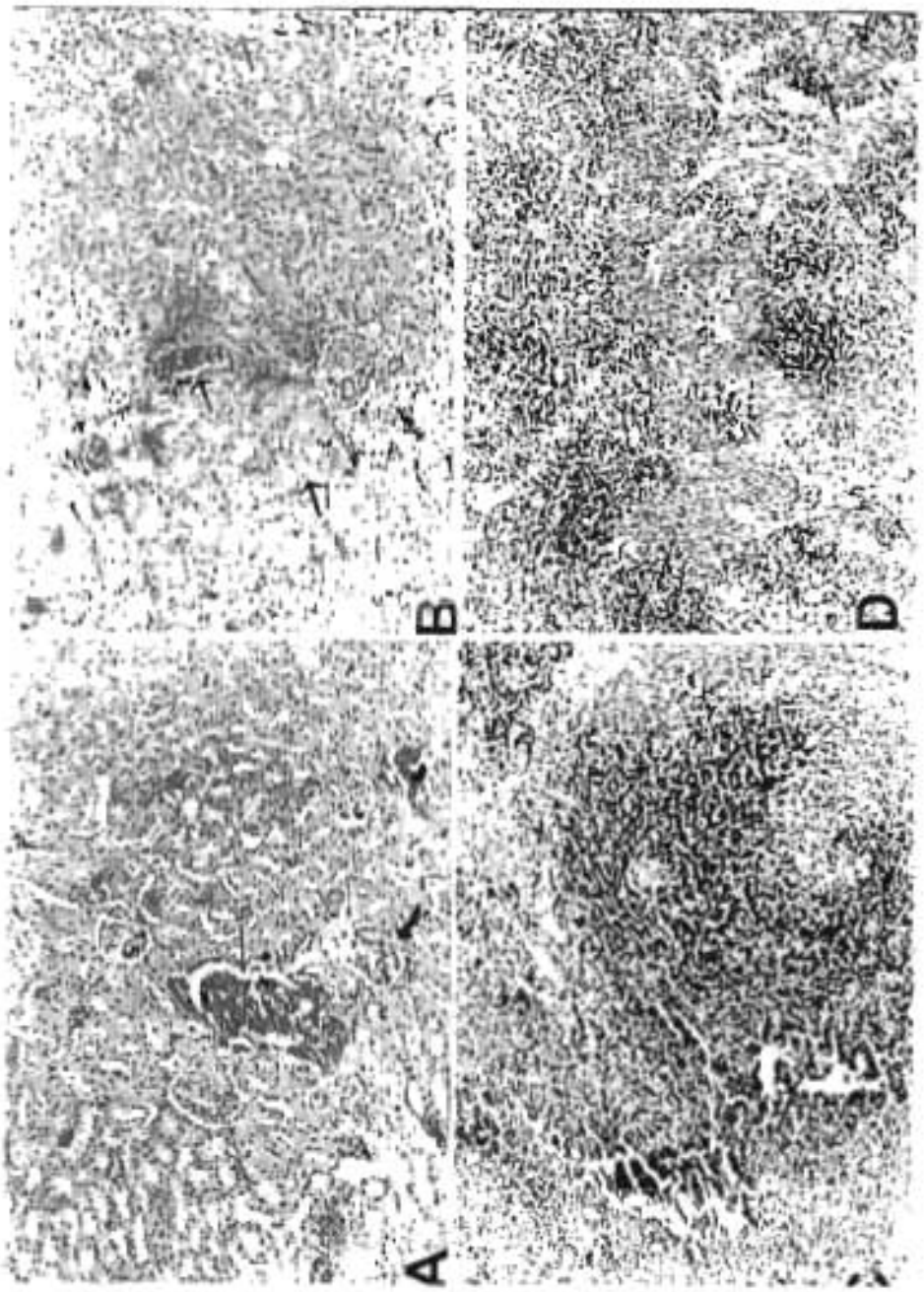

Fig. (3): (A): Section of kidney from rat treated orally with 1/40 LD_{50} of mancozeb showing congestion of renal blood vessels. encephalomalicia (H&E. x 130). (B): Section of kidney from rat treated orally with 1/10 LD_{50} of mancozeb showing congestion of the cortical blood vessels besides degenerated renal tubules . (H&E. x 130). (C): Section of spleen from rat treated orally with 1/40 LD_{50} of mancozeb showing mild depletion of lymphocytes from white pulp besides congestion of the red pulp. (H&E. x 130). (D): Section of spleen from rat treated orally with 1/10 LD_{50} of mancozeb showing moderate depletion of lymphocytes from white pulp besides numerous siderocytes in the red pulp. (H&E. x 130).

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الملخص العربى

التأثير السمى الخلوى المزمن لمركب مانكوزيب فى الفئران البيضاء

فتحى رضوان على سليم ممدوح محمد أبوالمجد محمود محمد الألفى

قسم الطب الشرعى والسموم - كلية الطب البيطرى - جامعة المنصورة

المبيدات من أهم وأكثر الملوثات فى البيئة المصرية مما يهدد حياة وصحة الإنسان ويعرضها للخطر. لهذا تم إختيار أحد المركبات الضادة للفطريات الأكثر إستخداماً وهو مركب مانكوزيب، تم تحديد الجرعة المميتة لنصف عدد الحيوانات (الفئران) وقد وجد أنها 2143.6 مج/كجم من وزن الحيوان، لدراسة التأثير السمى تم تقسيم الفئران الذكور إلى أربع مجموعات تحوى كل مجموعة عشرون فأراً، المجموعة الأولى مجموعة ضابطة، المجموعة الثانية والثالثة والرابعة تم إعطائها 40/1، 20/1، 10/1 من الجرعة المميتة لنصف عدد الحيوانات المحسوبة على التوالى مرتين فى الإسبوع لمدة عشرون إسبوع، تم ذبح الفئران فى نهاية التجربة تم فصل السيرم من الدم لتقدير التغيرات الكيماوية، وعينات الأنسجة تم حفظها فى الفورمالين 10% للفحص الهستولوجى وعينات النخاع العظمى لتعيين التغيرات الكروموسومية وعينات الكبد للتقدير الكمى للحمض النووى DNA، RNA أظهرت النتائج تغيراً طفيفاً فى وظائف الكبد واتضح ذلك من الزيادة فى البيلبروبين واسبرتيت أمينو ترانسفريز وكذلك كل من الكوليسترول واليوريا بصورة معنوية مقارنة بالنسبة للمجموعة الضابطة، وأيضاً وجود زيادة فى نشاط إنزيم الجاما جلوتاميل ترانسفريز بصورة معنوية، بينما إنخفض كل من إنزيم الالنين أمينو ترانسفريز الجلوكوز فى جميع الجرعات بصورة معنوية مقارنة بالنسبة للمجموعة الضابطة، لقد خلصت هذه الدراسة إلى أن المانكوزيب أدى إلى زيادة معنوية فى التغيرات الكروموسومية مثل النقطة والمسافة والدائرة والالتصاق الكروموسومى مقارنة بالنسبة للمجموعة الضابطة، ولقد خلصت هذه الدراسة أيضاً إلى أن المانكوزيب أدى إلى زيادة كمية الريبونيكليك أسيد فى الكبد بصورة معنوية مقارنة بالنسبة للمجموعة الضابطة، وقد أظهر الفحص المجهرى وجود إحتقان بالأوعية الكبدية مع وجود تجمع للخلايا الليمفاوية فى المنطقة البابية والخلايا الكبدية المجاورة مضمحلة مع وجود نخر أو تنكرز فى أسفل غطاء الكبد وبه بعض الخلايا وحيدة النواة، وأيضاً يوجد تجمع من الخلايا وحيدة النواة حول القنيات الصفراوية التى بها نشاط خلوى وبعض الخلايا الكبدية أظهرت ابتلاع الخلايا الكبدية المجاورة والميتة، وجد إحتقان وتهتك فى بعض القنوات الكلوية، وأيضاً وجد إحتقان وتجمع للخلايا وحيدة النواة فى المخ.

من هذه الدراسة يتضح أن مركب المانكوزيب له تأثير ضار على الخلايا مما يؤدى إلى تغيرات فى السيتوبلازم والنواة مثل التغيرات الكروموسومية وقد تؤدى إلى طفرات مما يؤدى بدوره إلى إحتمال حدوث تأثيرات سرطانية لذا يجب تقنين إستخدام هذا المركب وإجراء دراسات مكثفة للتأكد من تأثيره السرطانى بصفة قاطعة.