J. Agric. Sci. Mansoura Univ., 30 (3): 1701 - 1716, 2005

HAZARD ASSESSMENT OF MANCOZEB FUNGICIDE AFTER *IN VIVO* EXPOSURE: HEMATOLOGIC, CYTOGENETIC, AND ENDOCRINE TOXIC IMPACTS

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ABSTRACT

The hemato-cytogenetic and endocrine toxic effects of mancozeb (technical and formulated) have been studied in male Wistar rats. Sublethal doses administered orally for 28 day resulted in several alterations. Notable significant changes in organ weight ratio were recorded where the technical showed decreasing pattern, while the formulated showed increasing trend indicating alteraion of the physiological state of the exposed animals either in the whole body weight or in the function of internal organs. In the hemato-toxicologic context, blood indices were affected by exposure where technical compound exhibited increasing pattern of red and white blood cells while the formulated caused decreasing in red blood cells although the erythrocytes indices showed significant elevation after all dose regimens. Furthermore, technical mancozeb at 500 mg/kg resulted in slight significant increase ($p<0.01$) in the frequency of micronuclei in the polychromatic erythrocytes of bone marrow (10±0.816), while the same dose of the formulated induced a highly significant increase ($p<0.001$) in the frequency of micronuclei (14±0.816) when compared with the negative control raising concern for its mutagenic potential. Additionally, ultrastructural changes of the thyroid gland were recorded after exposure to both forms of the compound. It may be suggested that hormonal imbalance is the causative factor related to the thyroid follicular hyperplasia. In a conclusion mancozeb; particularly the formulated form, is suggested to be of toxicological concern due to a potential hazard posed to the effect on thyroid gland and its genotoxic effects. Further studies should delineate the respective pathways of mancozeb intoxication.
Keywords: Fungicide, Mancozeb, Toxicity, Micronuclei, Thyroid.

INTRODUCTION

Mancozeb, an ethylene-bis-dithiocarbamate (EBDC), has been one of the most commonly used fungicides. World Health Organization (WHO) has classified mancozeb as unlikely to present an acute exposure hazard under conditions of normal use (WHO, 1994). There is no evidence of neurotoxicity, nerve tissue destruction or behavioural changes, from the EBDCs (Morgan, 1982), however, dithiocarbamates are partially chemically broken down, or metabolized to carbon disulfide, a neurotoxin capable of damaging nerve tissues (Hallenbeck and Cunningham-Burns, 1985) through inhibition of ATP-dependent uptake of H^3-glutamate in rat cortical vesicles (Vaccari *et al*., 1999).

EBDCs can be broken down during the cooking process as well as by natural environmental processes (Wagner, 1983). A major toxicological concern, however, is ethylene-thiourea (ETU), a breakdown product of mancozeb which is classified according to Environmental Protection Agency (EPA) guidelines as a probable human carcinogen (EPA, 1992) and has the

potential to cause goiter and birth defects in experimental animals (Wagner, 1983).

Among the myriad of recent studies on endocrine-disrupting chemicals, relatively few involve thyroid disruption after fungicide exposure. Likewise, human exposure to fungicides may also pose problems related to the individual fertility (Bayley *et al.,* 2003) and mancozeb was found to inhibit implantation that may be due to hormonal imbalance or other toxic effects (Bindoli and Kalivsad, 2002).

There is a paucity of data on the potential adverse effects of exposure to low levels of mancozeb for prolonged periods (Roperto and Galati, 1998), although a recent study indicated that mancozeb must be considered as a multipotent carcinogenic agent (Belpoggi *et al.,* 2002). A data gap exists in the information available on the mutagenicity of mancozeb and ETU where mancozeb was found to be mutagenic in one set of tests, while in another it did not cause mutation (EPA, 1992) hence the evaluation of the genotoxicity of mancozeb is of immediate concern. The study presented herein was undertaken to determine, evaluate and link the potential of hemato-cytogenetic and endocrine toxic effects for animals undergoing oral exposure to mancozeb fungicide. The study has extended the findings to a comparison of the technical with the formulated form of mancozeb.

MATERIALS AND METHODS

Chemicals

Mancozeb (manganese ethylene bis(dithiocarbamate); polymeric) was provided from El-HELB Pesticides& Chemicals Co. (New Damietta, Egypt). The technical grade was 85% technical while the formulated one was Anadol Gold (80% WP). Both physical and chemical characteristics of the tested formulation were firstly examined and confirmed by GC-FPD compared with the technical grade. The median lethal dose (LD_{50}); oral and dermal of technical and formulated mancozeb were primarily tested. Furthermore, topical sensitization of the tested compounds was investigated. All studies were conducted in accordance with Good Laboratory Practice Standards and Oral/Dermal Toxicity Guidelines for Pesticide Testing (Organization for Economic Cooperation and Development, OECD, 1995).

Subjects and exposure regimen

Eighty male rats of Wistar strain, weighing 150±10g were allowed to acclimate to the environment {12h light/12h dark, temp (22±3C), humidity (50%±5)} and provided with commercial diet and water ad lib for one week prior to initiation of studies. The rats were divided randomly into 8 subgroups, each of ten, receiving 0, 125, 250 and 500 mg/kg body weight daily for 28 day of technical and formulated mancozeb; respectively. The doses administered represent a certain percentage of the oral LD_{50} of the tested compounds. The animals were dosed orally via a syringe with a feeding gavage needle containing the fungicide suspension at concentration providing a 0.5 mL dosing volume. All animals were weighed daily to ensure the maximum dose effect.

Bleeding regimen

Control and treated rats were anesthetized with light ether and blood was collected from the retro-orbital sinus vein (Herbert, 1973; Schalm, 1986) via heparinized microcapillary tubes (10 IU/mL blood). Rats were bled after

14, 21 and 28 day from the initiation of dosing and after 14 day from the last administered dose. Blood samples were withdrawn in a vacutainer tubes containing EDTA for hematological analysis. At the end of the exposure period (28 day) and the recovery period (14 day), all rat groups were sacrificed by excessive ether, examined grossly and blood was removed for hematological analysis. The internal organs were removed and weighed. Thyroid gland was removed, fixed in 10% formalin, mounted in paraffin, sectioned, stained and prepared for histopathological examination.

Hematology

The collected blood sample from each animal was analyzed via hemocytometer and counted microscopically for detecting the hematological profile which included white blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) (Drabkin & Austin, 1935; Wintrob, 1929).

Cytogenetic evaluation:Micronucleus test

The frequency of micronuclei in the polychromatic erythrocytes (PCE) as a premature RBC formed in the bone marrow, was examined according to Schmid (1976), Brusick (1978 & 1980) and Alder *et al., (*1991). Rats were killed and both femurs were immediately removed and cleaned of extraneous tissue. Bone tips were cut away so that a small syringe (containing 2 mL fetal calf serum) can be inserted and femoral contents flushed out in test tubes. The marrow was then isolated via centrifugation at 2000 rpm for 5 min. The supernatant was discarded and the suspended pellet was spread on a clean slide. The film was air dried, and then was immersed in methanol as a fixative for 20 min. The film was air dried again and then flooded with Wright stain followed by Giemsa stain. After 5 min, the slides were held with a stream of water and scanned for differential counting of PCE cells and micronuclei using a light microscope.

Histopathological examination

At the end of the exposure period, tissue samples of the thyroid gland from all the exposed and control animals were quickly cut into pieces and fixed in 10% formalin. These tissues were washed in normal saline followed by water and dehydrated in alcohol. Samples were then immersed in xylene and embedded in paraffin wax. Serial sections of 5 um in thickness were cut by microtome and stained with haematoxylin and eosin according to Carleton *et al. (*1967) and examined microscopically.

Statistics

Results were represented as mean± standard error. The significance was determined by t-test (Dixon and Massy, 1957; Winer, 1971) using Mystat computer software.

RESULTS AND DISCUSSION

Laboratory examination of physical and chemical characteristics of the formulated mancozeb revealed that this compound agreed with FAO standard specifications (based on suspensability, wettability, pH, and acidity& alkalinity tests). According to EPA's lists of pesticide product inert ingredients updated in July 1995, the identified inerts include calcium lignosulfonate, sodium lignosulfonate, magnesium sulfate, talc and others.

Acute oral toxicity study disclosed that the tested forms of mancozeb (both technical and formulated) are classified as category III of environmental pollutants (slightly toxic) according to EPA and WHO guidelines where their LD_{50} (oral and dermal) were more than 5000 mg/kg b.w. Clinical observations showed no evidence of compound-related effects and there was no compound-related mortality. Furthermore, no signs of dermal irritation like corrosion, erythma or Eshear formation were observed after 24, 48 and 72 hr of epidermal exposure to mancozeb (either technical or formulated).

The obtained findings agree with other studies investigating the acute toxicity of mancozeb (EPA, 1987; Berg, 1988; Hayes and Laws, 1990; Meister, 1992), where oral LD_{50} ranged from 4,500 to 11,200 mg/kg in rats, and when applied to the skin of rabbits, its dermal LD_{50} was 5,000 to 15,000 mg/kg.

Mancozeb is known as a mild skin irritant and sensitizer (DuPont, 1983) although agricultural workers handling crops treated with mancozeb have developed sensitization rashes (Hayes and Laws, 1990). Comparatively, other studies stated complete carcinogenic activity and tumor promoting activity of mancozeb following topical application on dorsal mouse skin in female Swiss albino mice exposed to mancozeb at a dose of 100 mg/kg after 31 weeks (Shukla *et al.*, 1990). Microscopic findings were limited to skin of treated and untreated animals and characterized by increased keratin production (hyperkeratosis) and thickening of the epidermis (acanthosis) *(EPA, 1992).*

As a part of series of studies investigating the hazard assessment of mancozeb, visual evoked potentials were accounted from wistar rats following repeated oral administration of mancozeb at different dose levels (125, 250, 500 mg/kg). From the results presented in Table 1, it could be observed that oral treatment with technical mancozeb has led to significant decrease in organ weight relative to the total body weight particularly for the kidneys, heart, brain and testes only after exposure to low dose schedule (125 mg/kg) while the higher doses (250 and 500 mg/kg) did not exhibit any notable changes compared to the control. After 14 day of recovery, no changes were observed in the overall mean body weight except a significant decline in weight of lungs and spleen was recorded particularly after exposure to the high doses (Table 2). On the other hand, exposure to formulated mancozeb (at 125 and 500 mg/kg) for 28 day caused a significant increase in organ weight particularly liver, heart, brain, lungs and testes. The effect on the liver (increase) lasted even during the recovery period (14 day). Exposure to 250 mg/Kg did not exhibit any significant change in organ weight except a significant increase in weight of brain and testes only after 28 days of exposure and a significant increase in weight of kidney and spleen after 14 day of recovery. Such enlarged spleen may likely due to sequestration of damaged erythrocytes. The change in organ weight may possibly be a part of a generalized increase in cellular metabolism.

The increase in the liver weight may be attributed primarily to hepatocytomegaly and excess lipid accumulation. According to FAO/WHO of ETU-related studies, the liver effects are due to stress-related liver growth from increased functional demand and these changes are reversible when exposures are brief or intermittent while the prolonged exposure may lead

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tumor information by a secondary mechanism (Jeffery *et al.*, 2000). In a conclusion, mancozeb alters the physiological state of the exposed rats either in the whole body weight or in the function of internal organs.

The obtained results coincide with previous studies where subacute toxicity of Dithane M-45 (80% mancozeb) in male Wistar rats for 12 wk resulted in significant increase in the relative weights of the liver and thyroid and those of the kidneys, adrenals and testes (Szepvolgyi *et al.*, 1989; Kackar *et al.,* 1999).

On the other hand, the results contradicted with the findings of Mahadevaswami *et al.* (2000) where there were no significant changes in the body and organ weight of albino rats treated with 500, 600, 700, and 800 mg/kg/day of mancozeb.

Data represented in Table (3) exhibited that technical and formulated mancozeb caused a noticed effect on the whole blood picture of the exposed animals. The number of RBCs increased with the lower doses of the technical even during the recovery period ($p<0.05$). On the contrary, formulated mancozeb caused significant drop in the number of RBCs after 21, 28 day of treatment with the high dose (500 mg/kg). But all the tested treatments caused significant increase in the number of RBCs during the recovery period (Table 4). No observed significant changes have been recorded in the level of Hb, PCV, and MCV throughout the exposure period to technical mancozeb. The exposed animals were recovered quickly during the recovery period in case of Hb while exposure to the high dose resulted in significant increase in PCV during the recovery period ($p<0.01$). There was a significant decrease in MCH level after 14 day of exposure to 250 mg/kg and during the recovery period of the lower dose (125 mg/Kg). The concentration of MCHC has not altered significantly except after 21 day of exposure to 250 mg/kg (decreasing trend).

Additionally, exposure to formulated mancozeb have altered the erythrocytes wintrob indices where it caused significant elevation in the level of Hb, MCH, MCHC after 21, 28 day of exposure to all dosage range during the experimental and recovery periods compared to the control group. The level of PCV was declined after exposure to all tested doses and during the recovery period. Only the dosage of 250 mg/kg caused a significant elevation after 28 day of exposure. There was also a detectable increase in MCV level after 28 day of exposure to the highest dose ($p<0.01$) followed by a sharp decrease ($p<0.001$) during the recovery period. The other dose (250 mg/kg) exhibited a similar pattern.

Furthermore, all tested dosage regimens of technical mancozeb had led to significant surge in the number of WBCs throughout the experimental period. The formulated compound caused significant increase in the number of WBCs only after 14 days of exposure to the high dose (500 mg/kg). The continuation of treatment with the dose of 250 mg/Kg for 28 day led to a significant decrease in the number of WBCs. The causes of such leucopenia are many and varied, ranging from decreased leucocyte survival time to bone marrow damage (Pietschmann, 1980). Therefore, a direct or indirect cytotoxic effect by formulated mancozeb or its metabolites on leucocytes may be suggested. A review of previous studies supports these suggestions.
According to EPA (1992), there were no treatment-related effects with regard to hematology after mancozeb exposure. Results of 13 week

exposure in rats indicated that some hematological and clinical chemistry changes were

noted but were within the normal range of values and were therefore not considered related to mancozeb exposure. Also, erythrocytes, hemoglobin and hematocrit were significantly decreased in male animals receiving 125 ppm at 18 months but not at other time periods while hematological findings were unremarkable in females.

In the bone marrow, the formation and incidence of micronuclei in the polychromatic erythrocytes (PCE) is an essential biomarker that can detect the genotoxic potential of chemical on animal models, therefore, an increase in micronucleus frequency is considered to an indication of induction of genotoxic damage as a response to external stimuli (Almassy *et al.,* 1987).

The effect of oral exposure to mancozeb on the frequency of micronuclei in PCEs is summarized in Table (5). *In vivo* treatment of technical mancozeb at 500 mg/kg resulted in a significant increase in the frequency of micronuclei (10±0.816) when compared with the negative respective control ($p<0.01$). Administration of the lower dose (125 mg/kg) showed slight increase in the frequency of micronuclei (7±0.577) when compared with the negative control. Comparatively, the two tested dose levels of formulated mancozeb (125 and 500 mg/kg) induced significant increase in the frequency of micronuclei (9.25±0.529 and 14±0.816; respectively) in a dose-dependent manner at a significance of $p<0.05$ and $p<0.001$; respectively when compared with the negative control. Thus, Anadol Gold (80% WP) can be highly considered as a mutagenic compound when tested on male Wistar rats using micronuclei test while the technical form under the same conditions was found to have slight mutagenic potential in the bone marrow at the tested dose levels.

Taking into account that micronuclei can arise both from acentric fragments (resulting from chromosomal breakage) and from complete chromosomes (fail to incorporate into the daughter nuclei during cell division), the ability of mancozeb fungicide to induce micronuclei (Fig. 1), appears to confirm the clastogenic and mutagenic activity of this compound, at least in rodents. However, such a conclusion must be considered as being circumstantial and further studies should delineate the respective pathways of mancozeb intoxication. These results complement previous data on the genotoxicity of this compound on rodents. In support of this finding, previous study have shown that exposure to mancozeb caused increase in sister chromatid exchange and in chromosome translocation for applicators of mancozeb (Steenland *et al.,* 1997).

According to EPA (1992), mancozeb has been tested for genotoxicity in a large number of *in vitro* and *in vivo* assays. It was negative in gene mutation and DNA damage tests. The compound could be considered as a weak clastogen: it is positive for chromosomal aberration tests *in vitro*, whereas conflicting data were obtained *in vivo*. There was no evidence for the induction of gene mutations or cell transformations.

For extrapolation purposes, the bone marrow of the rat is known to be so similar to that of human, but it is more hyperplastic, preponderantly erythroblastic, pronormoblastic, and normoblastic (Creskoff *et al.,* 1957).

Of particular interest is the effect of mancozeb on the thyroid gland. Mutagenicity does not seem to be a major determinant in thyroid

carcinogenicity, except for possibly some chemicals like acetochlor (Hurley, 1998). The histopathological findings in all treated cases were mostly similar, varying only in degree of each. Thyroid gland of the control group showed normal histological pattern with multiple follicles of different sizes and shapes lined with cuboidal epithelium. Some of their lumena were empty while others contained homogenous acidophilic colloid (Fig 2). Exposure to the 500 mg/kg of technical mancozeb led to several ultrastructural alterations. The alteration encountered were vacuolation, chronic inflammatory cells and degenerative changes. Most of the vacuoles of thyroid follicular cells were filled with a huge number of inflammatory cells, composed mainly of monocytes in addition to scaled submucosal cells (Fig. 3a). On the other hand, low dosage caused vacuolation and dilatation of follicular cells in some cases and hypertrophy in other cells. Hyperplasia of the interfollicular cells was apparent (Fig.3b).

As regards the rats which were treated with the high dosage of formulated macozeb (500 mg/kg), there were vacuolation, dilatation and interstitial congestion of most of the thyroid follicular cells accompanied with decrease and in some cases absence of colloid density in most of the follicles which were lined with low columnar epithelium (Fig 4a), whereas exposure to the lower dose (125 mg/kg) caused dilatation, hypertrophy of follicular cells, in addition to absence of the colloidal density (Fig. 4b).

Cellular infiltration in the lumen and in the connective tissue stroma and hyperplasia of the interfollicular cells were also evident The hormonal imbalance maybe the causative factor related to the onset of thyroid follicular hyperplasia and the elevation in thyroid stimulating hormone (TSH) represents the threshold for the remaining steps in the process. The prolonged and continuous elevation of TSH levels is responsible for the hypertrophy and hyperplasia of the thyroid follicular cells and ultimately in the development of nodular hyperplasia, adenoma and/or carcinoma in rats (Chhabra *et al*., 1992; Jeffery *et al*., 2000).

Our results matched with previous findings that illustrated the effect of mancozeb on the thyroid perturbation (Tomasi, *et al.,* 2001). Furthermore, the mancozeb metabolite, ETU, has been show to produce thyroid defects (EPA, 1987). ETU was found to cause decrease in thyroxine T_4 and increase in thyroid-stimulating hormone (TSH) in rodents and negatively affect the thyroid gland among heavily exposed human workers (Steenland *et al.,* 1997). In another study, exposure to mancozeb fungicide caused significant increase in thyroid/body weight ratio, histopathological changes, reduced iodine uptake and reduced activity of thyroid peroxidase (Kackar *et al.,* 1997).

In comparison with laboratory animals, humans are expected to exhibit a lesser degree of sensitivity to thyroid inhibitors including mancozeb (Costigan, 1998) since humans have a substantial reserve supply of thyroid hormone, much of which is carried in thyroxine-binding globulin, a serum protein that is missing in laboratory animals (Odell *et al*., 1967) . Additionally, the primary human response to prolonged thyroid insufficiency is goiter rather than neoplasia (Martindale, 1972). Thus a large uncertainty factor is found when extrapolation of the data to the human population is suggested.

All the above mentioned alterations can be rationalized in terms on the extent of absorption of the mancozeb and substantial degree of biotransformation of the compound and/or its metabolite; ETU. On the basis

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of these findings, these tests have been regarded as of great importance in the series of hazard assessment. In a conclusion we consider mancozeb (particularly the formulated form) and consequently its metabolite ETU to be of toxicological concern due to a potential hazard posed to the effect on thyroid and its genotoxic effects exerted in the current animal study.

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تقييم مخاطر التعرض *In Vivo* للمبيد الفطرى مانكوريب: التأثيرات السامة على مكونات الدم والتأثير الخلوى – الوراثى وعلى الغدد الصماء

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المعمل المركزى للمبيدات – مركز البحوث الزراعية – الإسكندرية

تم دراسة التأثيرات السامة للمبيد الفطرى مانكوزيب (فى صورته الخام والمجهزة) على مكونات الدم والخلايا الجينية والغدد الصماء فى ذكور الفئران من سلالة وستر. وقد أدى التعرض لجرعات فمية تحت مميتة لمدة ٢٨ يوم إلى العديد من التغييرات. فقد سجلت تغيرات ملحوظة فى وزن الأعضاء النسبى حيث أظهرت الصورة الخام للمبيد نمط متناقص بينما أظهرت الصورة المجهزة نمط متزايد مما يبين حدوث تغير فى الحالة الفسيولوجية للحيوانات المعرضة إما فى الوزن الكلى للجسم أو فى وظائف الأعضاء الداخلية. وفى إطار التأثير السمى على الدم فإن مكونات الدم قد تأثرت بالتعرض للمبيد حيث أظهر المركب الخام زيادة فى أعداد كرات الدم الحمراء والبيضاء بينما أظهرت الصورة المجهزة إنخفاضاً فى أعداد كرات الدم الحمراء على الرغم من حدوث زيادة معنوية فى ثوابت كرات الدم الحمراء بعد كل مستويات التجرع المختبرة. فضلاً عن هذا فقد أدى مبيد المانكوزيب الخام عند الجرعة ٥٠٠ ملجم/كجم إلى زيادة معنوية طفيفة فى معدل تكون الأنوية الصغيرة (١٠ ± ٠,٨١٦) فى خلايا الدم الحمراء عديدة الكروماتين (PCE) فى نخاع العظام بينما أدت نفس الجرعة من الصورة المجهزة لهذا المبيد إلى زيادة معنوية كبيرة فى معدل تكون الأنوية الصغيرة (١٤ ± ٠,٨١٦) عند مقارنتهم بالمجموعة الضابطة مما يرجح احتمال مقدرة المبيد على إحداث طفرات. بالإضافة إلى ذلك فقد سجلت تغيرات فى التركيب التشريحى للغدة الدرقية نتيجة التعرض لكلتا صورتى المبيد مما قد يرجع إلى الخلل الهرمونى كعامل مسبب لتضخم خلايا الغدة الدرقية. وفى المجمل يعد مبيد المانكوزيب (خاصة الصورة المجهزة) ذو تأثيرات تكسوكولوجية سامة نتيجة لتأثيره الضار على الغدة الدرقية والخلايا الجينية. ويجب عمل دراسات أخرى كى توضح مسار الفعل السام لمبيد المانكوزيب.

Table (1): Effect of mancozeb treatment on organ weight ratio after 28 days of oral administration (Data expressed as mean ± SE)

Treatmnt	Dosage (mg/kg)	Organ weight ratio						
		Liver	Kidney	Spleen	Brain	Lung	Heart	Testes
	0	11.23 ± 0.270	5.12 ± 0.087	3.52 ± 0.231	5.38 ± 0.024	4.42 ± 0.162	3.76 ± 0.173	6.79 ± 0.100
Mancozeb	125	11.59 ± 0.094	4.68 ± 0.100*	3.39 ± 0.145	4.89 ± 0.091*	4.41 ± 0.091	3.32± 0.051*	5.59 ± 0.245*
(85% technical)	250	11.20± 0.494	4.84 ± 0.196	3.63 ± 0.162	5.67 ± 0.402	4.35 ± 0.113	3.38 ± 0.103	6.46 ± 0.163
	500	11.95 ± 0.376	5.03 ± 0.071	3.71 ± 0.081	5.19 ± 0.122	4.60 ± 0.139	3.59 ± 0.122	6.71 ± 0.156
	0	10.70 ± 0.138	4.84 ± 0.067	3.73 ± 0.083	5.14 ± 0.071	4.34±0.108	3.48± 0.034	5.24 ± 0.296
Anadol Gold	125	10.19 ± 0.302	4.99 ± 0.049	3.76 ± 0.098	5.20 ± 0.067	4.48 ± 0.051	4.32± 0.356*	5.41 ± 0.239
(% 80 WP)	250	10.73 ± 0.084	4.84 ± 0.052	3.81 ± 0.085	6.01 ± 0.161**	4.59 ± 0.031	3.41 ± 0.034	6.36±0.137**
	500	11.32 ± 0.056**	4.81 ± 0.058	3.67 ± 0.074	5.39 ± 0.044*	4.83±0.106*	3.67± 0.064*	6.74±0.058**

***, **, and *** Significance difference at P< 0.05, P< 0.01, and P < 0.001 respectively.**

Table (2): Organ weight ratio in rats after 14 days of recovery from mancozeb treatment *(Data expressed as mean ± SE)*

Treatment	Dosage (mg/kg)	Organ weight ratio						
		Liver	Kidney	Spleen	Brain	Lung	Heart	Testes
	0	10.30 ± 0.183	4.63 ± 0.074	3.28 ± 0.122	4.62 ± 0.123	4.45 ± 0.043	3.41 ± 0.031	6.11 ± 0.224
Mancozeb	125	10.22 ± 0.082	4.53 ± 0.053	3.32 ± 0.062	4.59 ± 0.141	4.19 ± 0.112	3.27 ± 0.115	5.77 ± 0.310
(% 85 technical)	250	10.78 ± 0.108	4.69 ± 0.064	3.51 ± 0.097	4.70 ± 0.078	4.25 ± 0.071*	3.31 ± 0.059	6.2 ± 0.064
	500	10.25 ± 0.138	4.52 ± 0.105	2.94 ± 0.033*	4.48 ± 0.072	4.42 ± 0.048	3.31 ± 0.072	5.58 ± 0.074
	0	10.49 ± 0.098	4.65 ± 0.029	3.23 ± 0.047	4.93 ± 0.033	4.55 ± 0.045	3.34 ± 0.022	5.89 ± 0.995
Anadol Gold	125	10.89 ± 0.111*	4.63 ± 0.047	3.34 ± 0.116	4.62±0.043***	4.39 ± 0.143	3.33 ± 0.074	5.91 ± 0.123
(% 80 WP)	250	10.66 ± 0.139	4.81±0.042**	3.47±0.083*	4.85 ± 0.374	4.45 ± 0.135	3.41 ± 0.125	5.97 ± 0.048
	500	11.02 ± 0.179*	4.62 ± 0.106	3.38 ± 0.142	4.76 ± 0.022**	4.58 ± 0.065	3.38 ± 0.039	5.41 ± 0.174

***, **, and *** Significance difference at P< 0.05, P< 0.01, and P < 0.001 respectively.**

Table (4): Hematological profile of male wistar rats after 14 days of recovery ***(Data expressed as mean ± SE)***

Treatment	Dosage *(mg/kg)*	Blood indices/ Exposure period (day)						
		RBCs *(X 10^6 /µL)*	WBCs *(X 10^3 /µL)*	Hb *(g/dL)*	PCV *(%)*	MCV *(Ft)*	MCH *(Pg)*	MCHC *(%)*
Mancozeb *(%85 technical)*	0	6.84±0.32	7.00±0.61	11.48±0.29	51.67±0.85	77.73±3.82	18.09±0.99	22.27±0.89
	125	8.55±0.45*	6.17±0.47	13.13±0.15**	50.00±0.82	55.66±5.96*	14.57±1.39*	26.30±0.71
	250	6.78±0.16	11.22±0.22***	12.86±0.36*	53.67±1.31	79.25±1.68	19.04±0.79	23.99±0.58
	500	7.31±0.34	13.15±0.32***	14.31±0.35***	56.33±0.62**	77.76±3.94	19.74±1.03	25.38±0.37*
Anadol Gold *(% 80 WP)*	0	5.09±0.59	13.03±1.24	11.16±0.23	52.50±0.22	90.38±2.33	19.13±0.48	21.20±0.51
	125	7.62±0.44**	8.87±0.73*	19.76±0.55***	56.00±2.74	75.77±7.29	26.45±0.96***	36.24±2.51***
	250	7.28±0.54*	13.24±0.97	12.17±0.35*	50.75±1.88	71.23±5.14**	17.13±1.21	23.31±0.51*
	500	8.07±0.35**	10.19±1.64	10.64±0.43	50.00±0.948*	62.58±2.069***	13.26±0.71***	21.28±0.673

***, **, and *** Significance difference at P< 0.05, P< 0.01, and P < 0.001 respectively.**

Table (3): Hematological profile of male wistar rats after oral exposure to mancozeb ***(Data expressed as mean ± SE)***

Treatment	Dosage (mg/kg)	Blood indices/ Exposure period (day)																				
		RBCs *(X 10^6 /µL)*			**WBCs** *(X 10^3 /µL)*			**Hb** *(g/dL)*			**PCV** *(%)*			**MCV** *(Ft)*			**MCH** *(Pg)*			**MCHC** *(%)*		
		14	**21**	**28**	**14**	**21**	**28**	**14**	**21**	**28**	**14**	**21**	**28**	**14**	**21**	**28**	**14**	**21**	**28**	**14**	**21**	**28**
Mancozeb (%85 technical)	0	7.15 ± 0.50	6.90 ± 0.26	6.84 ± 0.32	7.26 ± 0.70	7.69 ± 0.39	7.69 ± 1.21	12.27 ± 0.62	12.11 ± 0.09	11..41 ± 0.35	50.50 ± 2.25	51.98 ± 2.46	51.98 ± 2.46	72.29 ± 7.49	77.73 ± 3.82	80.01 ± 5.01	19.92 ± 1.44	17.63 ± 0.76	18.09 ± 0.99	24.17 ± 1.71	25.84 ± 0.85	24.17 ± 1.71
	125	7.59 ± 0.28	7.22 ± 0.29	7.27 ± 0.68	10.65 ± 0.25**	11.57 ± 0.91*	12.29 ± 0.43*	12.24 ± 0.69	13.16 ± 0.84	11.55 ± 1.47	51.25 ± 4.14	55.75 ± 1.49	55.50 ± 0.87	67.60 ± 5.13	77.72 ± 4.09	79.07 ± 9.75	16.14 ± 0.71	18.24 ± 0.87	16.22 ± 2.14	24.37 ± 2.33	23.68 ± 1.77	19.26 ± 1.81
	250	7.65 ± 0.56	6.34 ± 0.42	6.15 ± 0.29	13.23 ± 1.37**	11.64 ± 0.59**	12.34 ± 1.31*	11.24 ± 0.74	10.09 ± 0.75	12.65 ± 0.48	49.00 ± 6.87	53.75 ± 3.09	54.00 ± 1.78	64.77 ± 5.66	85.67 ± 6.64	88.69 ± 6.81	14.71 ± 0.24*	15.96 ± 0.66	20.78 ± 1.37	23..13 ± 1.59	17.68 ± 1.33**	23.47 ± 0.92
	500	6.17 ± 0.14	7.14 ± 1.11	6.77 ± 0.73	10.80 ± 0.45**	12.11 ± 1.05*	10.50 ± 0.67	11.85 ± 0.37	11.69 ± 0.68	12.52 ± 0.50	52.00 ± 1.08	54.50 ± 2.06	56.00 ± 3.81	84.43 ± 2.78	74.44 ± 8.20	83.75 ± 3.83	19.23 ± 0.73	17.38 ± 2.47	18.88 ± 1.28	22.77 ± 0.32	21.67 ± 2.03	22.48 ± 0.62
Anadol Gold (% 80 WP)	0	6.22 ± 0.42	6.74 ± 0.52	6.52 ± 0.22	9.70 ± 0.97	12.53 ± 1.85	12.66 ± 0.28	12.85 ± 0.74	12.39 ± 0.19	10.67 ± 0.45	56.25 ± 2.31	51.75 ± 4.04	47.25 ± 0.86	91.73 ± 3.72	72.10 ± 2.91	72.66 ± 1.82	20.46 ± 0.76	18.55 ± 1.13	16.46 ± 0.87	22.15 ± 0.86	25.79 ± 0.72	22.60 ± 0.84
	125	5.58 ± 0.13	5.87 ± 0.57	6.77 ± 0.51	8.46 ± 0.69	10.68 ± 0.678	11.43 ± 0.91	13.25 ± 0.39	13.64 ± 0.32*	14.64 ± 0.62**	49.50 ± 3.12	47.00 ± 2.77	50.00 ± 2.47	89.22 ± 6.69	83.41 ± 8.52	71.57 ± 1.30	23.75 ± 0.30**	24.25 ± 2.14*	21.98 ± 0.72**	27.27 ± 1.97*	29.63 ± 2.14	29.87 ± 0.28***
	250	5.56 ± 0.12	6.19 ± 0.29	6.07 ± 0.38	10.72 ± 1.17	8.09 ± 1.02	9.13 ± 0.41***	14.83 ± 0.46	14.98 ± 0.49**	14.89 ± 0.97**	50.25 ± 1.59	50.50 ± 2.73*	52.00 ± 0.63**	90.68 ± 3.39	83.43 ± 7.73	87.63 ± 5.69*	26.70 ± 0.26***	24.43 ± 1.27**	25.30 ± 2.63*	29.63 ± 1.21***	30.05 ± 1.65*	28.55 ± 1.97*
	500	6.63 ± 0.18	4.81 ± 0.34*	5.62 ± 0.17*	17.60 ± 1.58***	10.66 ± 1.29	11.35 ± 0.78	13.31 ± 0.69	14.29 ± 0.07***	12.98 ± 0.78*	42.30 ± 2.33**	51.00 ± 2.39	47.75 ± 1.98	64.70 ± 5.02**	107.75 ± 6.96**	85.40 ± 2.22**	21.27 ± 0.43	30.48 ± 2.16**	24.05 ± 1.02***	34.33 ± 3.03**	28.40 ± 1.59	27.63 ± 2.38

***, **, and *** Significance difference at P< 0.05, P< 0.01, and P < 0.001 respectively.**