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ORIGINAL ARTICLE

Biochemical and Hematological Changes Following Long Term Exposure to Mancozeb

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ABSTRACT
The aim of this study is to evaluate the effects of mancozeb; a carbamate fungicide on some hematological parameters and some biochemical indices related to kidney and liver function. Male wistar rats were given different doses of mancozeb orally: 500 and1000 mg/body weight/day for 8 weeks. The obtained results showed an increase in the absolute weight of liver, spleen and kidneys, mainly in the group exposed to 1000 mg/ kg. Hematologic study revealed a decrease in red blood cells hemoglobin and hematocrit values in all treated groups. However, white blood cells witnessed an increase in all the treated groups. The biochemical tests revealed a rise in liver transaminases (AST / ALT), alkaline phosphatase and total bilirubin serum, while total protein levels and total lipids have significantly decreased mostly in group treated with 1000 mg/kg. Urea and creatinine have significantly increased in the group treated with 1000 mg/kg. In the light of these results, it appears that chronic mancozeb exposure produced biochemical and hematological changes of some parameters related to the liver and kidneys function.
Keywords*: Pesticide, Mancozeb, Toxicity, Rats, Liver, Kidneys*

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INTRODUCTION

Pesticides are widely used in the world to fight against pests and protect the public health; these chemicals are used in industry and agriculture for many purposes [1]. They have of great concern for their potential effects on human health since the intensive use of them leads to chronic exposure of the general population. Several studies have shown the presence of pesticides and their residues in food and human tissues worldwide [2]. Individuals who are exposed to these chemicals include agricultural workers, people living near farms/or chards or consumers [3]. Exposure to these products can cause health problems at long term or even at short term. A World Bank report issued in 2008 estimated that 355,000 people worldwide die poisoned unwittingly by pesticides each year. Ethylenebis-dithiocarbamates (EBDC) are widely used in agriculture as fungicides, mainly on fruit, vine and potato crops. Their extensive global consumption can be attributed to their low acute toxicity and short persistence in the environment. Mancozeb is a carbamate fungicide widely used in agriculture to protect crops from a range of fungal diseases [4]. Despite its low toxicity, mancozeb has been shown to cause adverse effects on human and animal health; it is classified as a carcinogen with evidence of genotoxic effects on animal laboratory [5]. In addition, exposure to mancozeb causes reduction in thyroxine level of wistar rats, which can harm the developing brain [6].The aim of our study was to test the hypothesis that mancozeb increases metabolic alterations: hematological including the biochemical parameters related to liver, spleen and kidney functions in male albinos rats.

MATERIALS AND METHODS

Chemicals

Mancozeb a Zn, Mn-ethylene bis-dithio-carbamate (commercial grade consisting of 80% wettable powder). TECH; CAS No: 8018-01-7 purchased from China Leading Manufacturer and supplier by INRAA

– ALGERIA. Mancozeb was dissolved in water for oral administration; doses were administered according to body weight. The quantity of solution administered never exceeded 2 ml/kg body weight/day.

Animals

All the experiments were carried out on adult male rats weighing 240 ± 20 g obtained from the Pasteur Institute – ALGERIA, and food was provided as special diet from the ONAB – ALGERIA. The rats were divided into 3 groups housed in appropriate and specific cages under laboratory conditions during 8 weeks of experimental period (free access to food and water, 12h L /12h D photoperiod, 60% of humidity and a temperature of 22 ± 2°C.

Experimental Design

The rats were randomly divided into three groups of eight, the first group served as a control, the second (Mcz1) was treated by 500 mg/kg/day and the last one (Mcz2) received 1000 mg/kg/day for 8 weeks. At the end of the experimental period, the animals were fasted overnight and sacrificed by decapitation; the arterio-venous blood was quickly collected into tubes. The liver, spleen and kidneys are removed by transverse abdominal incision and weighted. Samples of whole blood were collected in vacuum tubes containing EDTA as an anticoagulant for determining hematological parameters and the other dry tubes for biochemical assays. The liver transaminases (alanine transaminases /ALT and aspartate transaminases /AST) according to Murray & Kaplan [7,8] protocol, alkaline phosphatase (ALP) activity by Wenger & Kaplan [9], creatinine was determined by the colorimetric kinetic method [10], urea performed by enzymatic kinetic method [11], the total protein [12], the total bilirubin [13] and the total lipids [14] have been determined by the colorimetric method.

Statistical Analysis

The data have been expressed as mean values ± SD. The comparison of the data was done using Student's t test to compare means between different treatment groups. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS

Organs Absolute Weight

Oral administration of mancozeb for 8 weeks leads to a significant increase in the absolute weight of the liver, kidneys and spleen. This increase was remarkable especially in the group exposed to the highest dose (1000 mg/kg /day); while the group treated with a low dose (500 mg / kg / day) showed no change of absolute weight during treatment, including the kidneys and spleen. Table 1

Biochemical and Hematological Studies

Transaminases serum level (AST/ALT) and alkaline phosphatase (ALP) were increased significantly in group exposed to 1000 mg/kg while no significant difference was observed in group treated with 500mg/kg. The total protein level has decreased significantly at 500 mg/kg and 1000 mg/kg. This decrease has led to a significant increase of urea and creatinine levels in all treated groups with different doses. Total lipids that have significantly decreased in group treated with high dose as to500mg/kg showed no change. Total bilirubin increases significantly in groups treated with different doses compared to the control one (Table 2). The obtained results (Table 3) show a significant decrease in red blood cells (RBC) in groups exposed to mancozeb at different doses compared to the control group. Other components of the blood linked to the physiological state of erythrocytes such as hemoglobin (Hb) and hematocrit (Ht) have decreased significantly, while white blood cells (WBC) increased significantly in the groups treated with 500 and 1000 mg/kg respectively.

Table 1: Changes of absolute weight of liver, spleen and kidneys of rats exposed to mancozeb

Absolute weight(g)	Control	Mcz 1: 500mg/kg	Mcz2: 1000mg/kg
Liver	7.318 ± 0.902	10.6 ± 0.848 ***	11.99 ± 1.33 ***
Left kidney	1.28 ± 0.37	1.33 ± 0.17	1.42 ± 0.21 *
Spleen	0.8234 ± 0.0859	1.222 ± 0.203**	1.305 ± 0.245 **

Significant * *P<0.05* compared with control.

Table 2: Basic statistics of biochemical parameters of rats [a] exposed to mancozeb.

	Control	Mcz1: 500mg/kg	Mcz2: 1000mg/kg
AST (U/L)	153.16 ± 19.04	154.5 ± 32.30	195.75 ± 34.91*
ALT (U/L)	24.66 ± 2.65	39.25 ± 4.85**	59.18 ± 6.24***
PAL (U/L)	93.66 ± 25.57	120.33 ± 26.53	182.25 ± 14.52 ***
Urea (g/l)	0.39 ± 0.055	0.45 ± 0.050	0.49 ± 0.063**

Creatinine (mg/l)	4.75 ± 0.50	5.66 ± 0.51*	6.83 ± 0.40***
Total lipid (g/l)	2.86 ± 0.57	2.52 ± 0.51	2.11 ± 0.16*
Total bilirubin (mg/l)	1.16 ± 0.40	1.33 ± 0.51	1.75 ± 0.5*
Total protein (g/l)	76.5 ± 2.73	69.5± 2.16***	68.83 ± 3.31**

Significant * *p<0.05. **P<0.01. ***P<0.001.* [a] Value represents the mean ± SD of 8 animals.

Table 3: Hematological changes in male rats [a] exposed to different doses of mancozeb

	Control	Mcz1: 500mg/kg	Mcz2: 1000mg/kg
WBCs $x10^3$/ul	6.4 ± 0.4	8.91 ± 1.68*	10.72 ± 1.74**
RBCs $x10^6$/ul	10.58 ± 0.69	8.74 ± 0.38**	8.29 ± 0.31***
HCT %	54.67 ± 1.18	43.04 ± 2.13***	42.32 ± 1.22***
HB g/dl	13.92 ± 3.71	12.2 ± 6.54*	11.4 ± 2.84**

Significant *$p<0.05$. **$P<0.01$. ***$P<0.001$. [a] Value represents the mean ± SD. of 8 animals.

DISCUSSION

Human exposure to pesticides has been strongly associated with many physiological disorders and diseases. Mancozeb is widely used for its short persistence in the environment even though diverse chronic/sub-chronic toxicity effects have been reported [15]. In the present study, repeated exposure to mancozeb has caused a significant increase in the absolute weight of the liver, kidneys and spleen. Our results are similar to those obtained by Abdel-Tawab & Abbassy [16] who found an increase in the relative weight of the kidneys and liver after exposure to chlorpyrifos and methomyl for 90 days. In addition, El-Sayed [17] found that exposure to low dose equivalent to (1/20 LD50) and high dose equivalent to (1/10 LD50) of dimethoate, carbofuran and carbendazim administered for 30 days increased the relative weight of liver, kidney and spleen. The hematological study revealed a decrease in red blood cells (RBC) hemoglobin (Hb) and hematocrit (Ht) while the white blood cells (WBC) increased significantly. Patil & Govindwar [18] have reported that many biosynthesis steps of the heme are inhibited by the pesticide, which may be a physiological explanation of the obtained results. They also suggested that poisoning by pesticides residues induced anemia caused by interference of hemoglobin biosynthesis and shortening the lifespan of circulating red cells. Moreover, other results show that administration of Carbosulfan cause changes in hematological constituents by altering the production of red blood cells [19].Furthermore, a significant decrease of hemoglobin was recorded in all the treated groups. Low hemoglobin level is probably due to the decrease in oxygenation capacity or disruption of iron metabolism. These results correlate with those of Venees F *et* al [21] who observed lower rates in hemoglobin after diazinon treatment for three weeks. Also, our results showed a decline of hematocrit in all the treated rats, this is probably due to the synergistic link between blood parameters in all vertebrates [22]. Hence, we observed a significant increase in white blood cells; this may indicate an activation of the animals' immune system [23]. These results are in agreement with Mohamed *et* al [24] who recorded an increase in leukocytes of female mice after organophosphates exposure. The results of the biochemical analysis revealed a decrease in total protein, which might be due to the inactivation of various transcription factors [25]. These observations support the results obtained by Al-Amaudi [26] who observed a decrease in total protein of albino rats after exposure to metalaxyl. The lipid results showed a significant decrease in total lipids of rats. Similar findings showed that exposure to anti-cholinesterase such as carbamates can increase the degradation of lipids [27], however, other observations revealed an increase in rats` total lipids after 28 days of oral administration of methomyl. The results have shown an increase in the urea and creatinine concentrations: these parameters indicate a renal damage and their plasma levels can be attributed to the decrease of the kidney's filtration. Our results are in similarity with those previously obtained by El-Sayed et al [17] who found that the concentrations of urea and creatinine were increased in experimental animals after exposure to systemic pesticides. The same findings have been found on other pesticides [29]. Our data also showed an increase in enzymatic activity of liver transaminases (ALT, AST) and alkaline phosphatase (ALP), this increase indicate tissue damage after liver injury [30].These results confirmed those reported by many authors working on different pesticides at higher doses [30]. In addition, the increase in bilirubin may be the result of pathological changes such as liver necrosis causing rise in the permeability of liver cells' membrane, which leads to the release of bilirubin in the blood [31].

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