SUBCHRONIC MANCOZEB TREATEMENT INDUCED LIVER TOXICITY VIA OXIDATIVE STRESS IN MALE *WISTAR* RATS

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SUMMARY

Mancozeb is a manganese/zinc ethylene-bis-dithiocarbamate fungicide that is widely used in agriculture to control a broad variety of fungal infections of both vegetables and ornamental plants. The present study has been carried out to investigate the possible effect of mancozeb on animal the oxidative stress and some of the biochemical markers in male *wistar* rats. In this experiment, adult male rats weighing between 200 and 250 g were treated *per os* for 4 weeks with two different doses of 800 and 1200 mg/kg per day. Reduced glutathione (GSH) levels were decreased in all treated groups compared to control ones. It has been observed a significant increase in the fresh weight of liver in individuals of both doses. Moreover, mancozeb exposure caused a significant ($p<0.05$) fall in aspartic aminotransferase (AST) and alanine aminotransferase (ALT) in group treated with 1200 and 800 mg/kg/day. Similarly, alkaline phosphatase (ALP) activity underwent a significant ($p<0.05$) increase in both groups. The obtained observations clearly reveal hepatotoxic effects of mancozeb in rats and constitute, therefore, an environmental health risks to living organisms.

Key words : Mancozeb, rat, toxicity, oxidative stress, liver

INTRODUCTION

The widespread use of pesticides over the last decades has become a real health concern in our modern society. Their development has not only improved agricultural products, to meet the increasing world populations, but also represents a major health threats The World Health Organization (WHO) has estimated that there are approximately 3 million cases of pesticide poisoning each year, with 220,000 deaths worldwide (Jaga and Dharmani, 2003 ; who, 1988) the majority of poisonings occur in developing countries (Mbakaya *et al*., 1994; Smith, 2001). The study of the impact of pesticides in the carbamate family on public health are of a great importance in toxicological studies. Carbamates are selected on the basis of their biodegradable property and to their supposed low toxicity to mammals. Mancozeb (manganese/zinc ethylene-bis-dithiocarbamate) belongs to the carbamate class of fungicides used against a variety of fungal diseases of field crops (O`Neil and Marshal, 1984) Exposure to mancozeb has been tested on animals, inducing, histopathological changes in the adrenal gland, thyroid and kidney (Belpoggi *et al*., 2002 ; Sakr, 2007).

MATERIALS AND METHODS

Experimental design

Adult male Wistar rats weighing between 200 and 250 g were divided into 3 groups. The animals were housed in appropriate and specific cages under laboratory conditions (food and

water were available ad libitum) during the four weeks experimental period. The treated individuals of both 2 groups received the fungicide (800 and 1200 mg/kg body weight) by gavage, while the last one served as control.

Biochemical assays

At the end of the experimental period, blood samples were collected into dry tubes containing heparin solution by decapitation, the serum was obtained by centrifugation at 3000 ×g for 15min. The transaminases (alanine transaminase—ALT and aspartate transaminase—AST) and alkaline phosphatase (ALP) activities were assayed using commercial kits from (Spinreact, Spain, refs: GOT-1001165, GPT-1001175 and ALP-1001131).

Reduced glutathione level

Reduced glutathione concentrations were evaluated following the method described by Ellman, (1959) as modified by Jollow et al. (1974) and based on the development of a yellow color when DTNB is added to compounds containing sulfhydryl groups. In brief, 0.2 ml of liver supernatant was added to 3 ml of 4% sulphosalycylic acid and tubes were centrifuged at 2500 g for 15 min. Supernatant (0.2 ml) was mixed with 0.4 ml of 10mM DTNB and 1 ml phosphate buffer (0.1M, pH 7.4). Finally, absorbance at 412nm was recorded. Total GSH content was expressed as nmol GSH/mg protein.

Protein assay

Total protein contents were estimated according to the method of Bradford, (1976) using bovine serum albumin as standard.

Statistical analysis

All the results were expressed as mean values ± SEM. Comparison between mean values were made using the Student's t-test. Differences were considered significant at $p \leq 0.05$.

RESULTS

Effect of mancozeb on Liver weight

The obtained results are shown in Figure 1. They reveal a very significant increase in liver weight. The rate of increase was 45 and 62 % in the Mancozeb treated groups at 800, 1200 mg/kg/day, respectively compared to controls.

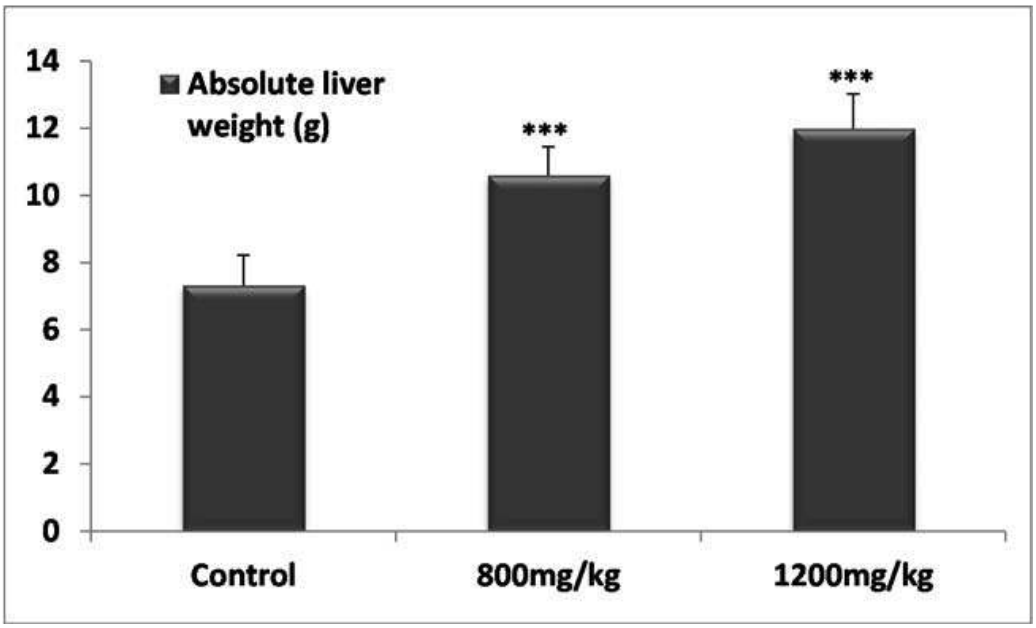

Figure 1. Variation of absolute liver weight of rats treated with Mancozeb at : 800 and 1200 mg/kg/day (mean ± SEM, n = 8)

Effect of mancozeb on oxidative stress index

The results that are summarized in Figure 2 show that the treatment of rats with mancozeb decreases significantly hepatic glutathione content rate (GSH) in animals of both treated groups compared to those of the control one.

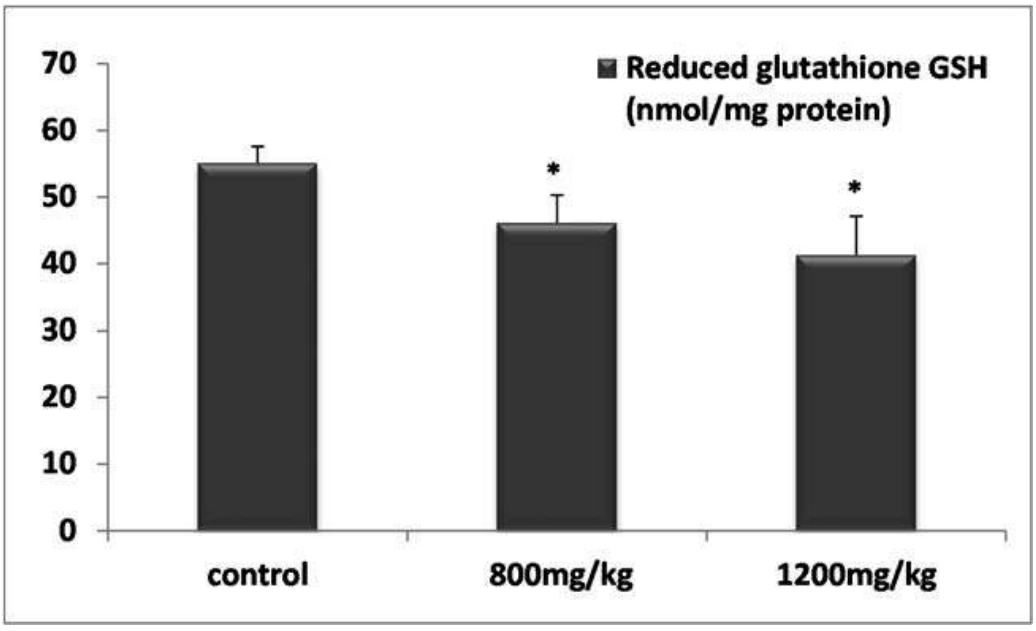

Figure 2. Reduced glutathione (nmol/mg protein) level in liver of control and rats treated with mancozeb (mean ± SEM, n = 8)

Effect of mancozeb on Biochemical parameters

The enzymatic activity of alkaline phosphatase ALP are illustrated in figure 3. They reveal a significant increase only in animals that had received the highest dose of mancozeb.

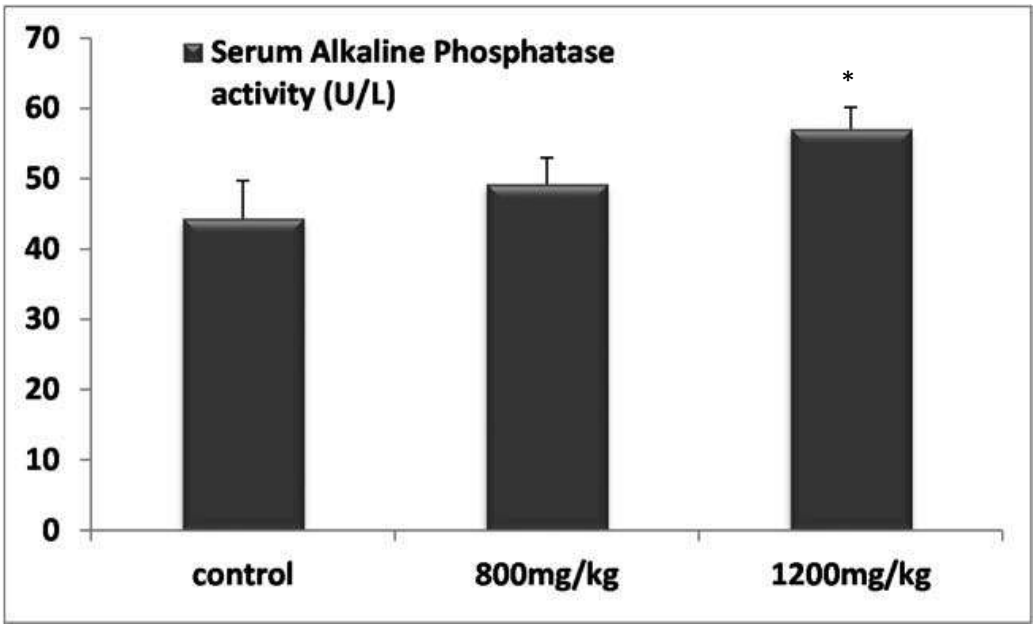

Figure 3. Effect of increasing concentrations of mancozeb on serum alkaline phosphatase in male rats (mean ± SED, n = 8)

Moreover, the mean values of inflammatory markers of liver function, figures 4, 5, such as serum transaminases alanine aminotransferase and aspartate aminotransferase (ALT/ AST) were remarkably increased in treated groups with 800 1200 mg/kg/day compared to controls.

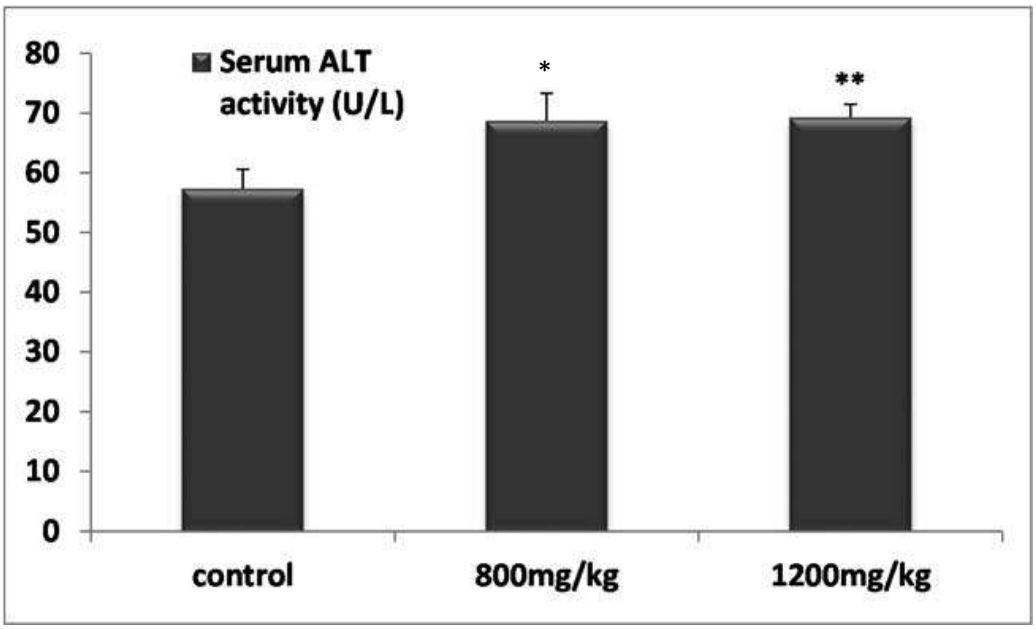

Figure 4. Changes in ALT activity after treatment with mancozeb in male rats (mean ± SEM, n = 8)

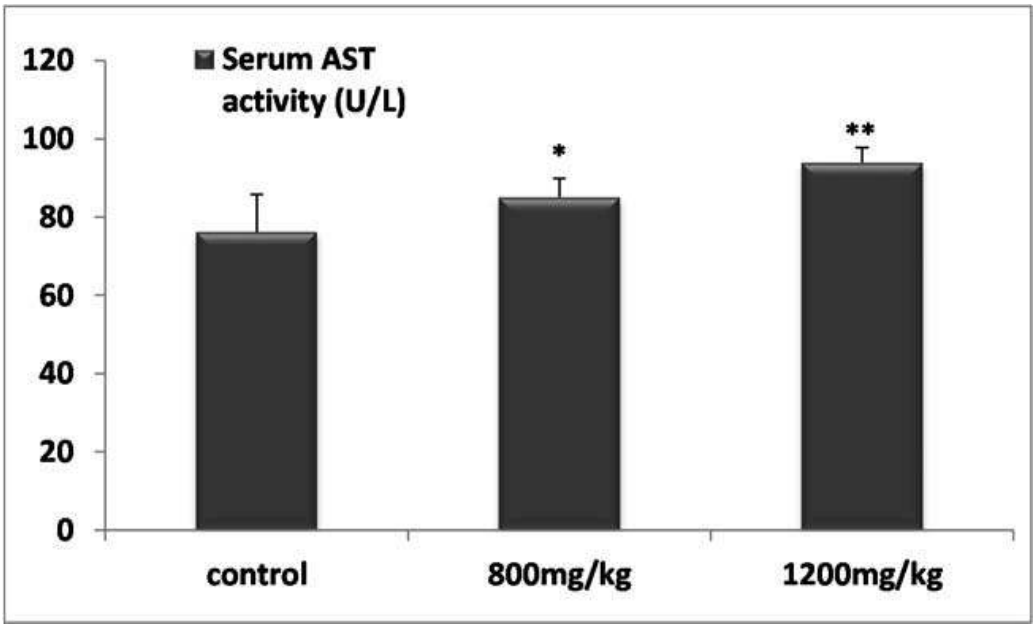

Figure 5. Changes in AST activity after treatment with mancozeb in male rats (mean ± SEM, n =8)

DISCUSSION

The current investigations were carried out to study the possible toxic effects of mancozeb, a commonly used pesticide in agriculture, on liver function in male rats.

The collected data reveal that exposure of rats to such a pesticide provokes an increase in liver weights compared to that of controls. These observations are similar to those of Mallem *et al*. (2007), where a remarkable increase in liver weight of rabbits treated with maneb were recorded . This may be explained by the accumulation of the toxic molecule in hepatocytes for detoxification or effect of enzyme induction (Wayland and Edward, 1991). In contrast, other investigations have demonstrated that repeated exposure to mancozeb reduced liver mass (Raghavendra *et al*., 2003) and causes hepatocellular morphological changes (kackar,1997)

Liver is the target organ involved in detoxifying processes (Guha Mazumder, 2005) and the leakage of hepatic enzymes such as ALT,AST and ALP are commonly used as an indirect biochemical index of hepatocellular damage (Klaassen and Watkin, 1984). Hepatic dysfunction followed by high levels of serum enzymes, indicating the cell leakage. Observations arising from our present investigation are in total agreement of those mentioned above , where increased activities of `AST, ALT and ALP in the serum of treated rats with mancozeb were registered. Similar findings have also been made in other previous studies carried out on pesticides toxicity (Bhatti *et al*., 2011; Singh *et al*., 2011; Choudhary., 2003). Lavric *et al*. (1990) reported that the fungicide bithionol sulfoxide when applied at high doses caused hepatotoxicity including an increase in serum AST. Elevations in the serum levels of these enzymes were mostly attributed to acute hepatocellular damage, extra hepatic obstruction, or both (Takaori, 1993). A study on the transaminases (AST / ALT) and the alkaline phosphatase(ALP) suggests that this increase can be due to a mutation of genes responsible for the synthesis of these enzymes (Azmi, 2006). Moreover, the increase in ALP concentrations might be due to the increase in macrophages activity including Kupffer cells. In contrast, other studies report a fall in theses enzymes activities While other research conducted by Oruc and Uner. (1999); Basanta and Mukherjee. (2003).

Finally, GSH is a tri peptide that is known to play a major role in oxidative stress (Masella *et al*, 2005). GSH can act directly with activated-oxygenated species but it is mainly used as a substrate for glutathione peroxidase which ensures the elimination lipid peroxides (Satsangi and Dua, 2000). It, thus, forms the first line of defence by acting as a non-enzymatic antioxidant (Giles, 2006). Data arising from our current study show that treatment with mancozeb exposure increased oxidative stress and altered antioxidant status in the treated groups. Liver GSH contents in the mancozeb treated rats were significantly decreased, compared to control ones. Changes in glutathione levels may be an important indicator of the capacity of the detoxification ability of the organism (Cheung, 2001). Its depletion can result in cell degeneration due to oxidative stress caused by pollutants (Zhang *et al*., 2008). Similar observations were made by Chiali *et al*., (2013) where chronic exposure to low doses of metribuzin were founded to decrease GSH content in liver.

In summary, our results demonstrate that chronic exposure to two doses of mancozeb may have adverse effects on liver functions leading to physiological impairments. In addition, mancozeb exposure resulted in increased oxidative stress and altered antioxidant status in liver. In the light of these observations, it is recommended that mancozeb should be used with caution.

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