TOXICOLOGICAL ASSESSMENT OF EFFECT OF MANCOZEB-TREATED LETTUCE (*Lactuca sativa*) ON WISTAR RAT LIVER

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Abstract

Vegetable contamination by pesticides presents current problem of public health. Previous studies have shown that 60% vegetables sampled collected in Lomé (Togo) have pesticide residues exceeding the tolerable limits. Because mancozeb, ethylene bis-dithiocarbamate, is mainly used, more than five times, during the growth of lettuce (Lactuca sativa), the aim of the present investigation is to evaluate the potential effect of mancozeb-treated lettuce on the rat liver physiology. Mancozeb-treated lettuce and two doses of mancozeb were administered during 28 days to rats. Along the study, animal behavior was assessed, and at the end of administration, some hepatic enzymes such as transaminases and alkaline phosphatase were studied. The decrease in rat body weight was noted and animals have soft feces. Plasmatic concentrations of transaminases, alkaline phosphatase, and total bilirubin are increased in rats administered with mancozeb-treated lettuce. The plasmatic concentration of total protein is not decreased significantly. Those results indicate that lettuce collected directly from gardens, without washing or less washed, are not fit for human consumption.

Keywords: *Lettuce, mancozeb, toxicity, liver, pesticide residues, public health*

Introduction

Pesticides are important and useful in agricultural production in spite of the increasing interest of the new concept called biological agriculture. This practice is not without risk on human and animal health and, in wide consideration, on environmental conditions due to the residues of pesticides that may persist many weeks or months after application (McConnell *et al.*, 1998; Sparling *et al.*, 2001). Evidences and data are continuously accumulated and updated on the relationship between chronic pesticide exposure and health condition impairment, including carcinogenesis, neurotoxicity, reproductive and growth disturbance, and immunological effects (Hernández *et al.*, 2006). Mancozeb, a fungicide belonging ethylene bis dithiocarbamate group, is used to protect and to give an attracting aspect to the vegetables. Despite its low acute toxicity, mancozeb has been shown to cause detrimental effects both humans and experimental animals' organ systems (Bindali and Kaliwal, 2002; Axelstad *et al.*, 2011). Mancozeb exposure was strongly associated with increased incidence of thyroid disease in pesticide applicators (Trivedi *et al.*, 1993; Goldner *et al.*, 2010). Reports are available on the toxicity of mancozeb on liver and kidney (Ksheerasagar and Kiliwal, 2003), on central nervous system (Nordby *et al.*, 2005; Tsang and Trombetta, 2007). Histological studies of the testis of the mice treated with high doses of mancozeb revealed spermatogenesis inhibition (Ksheerasagar and Kaliwal, 2010). This fungicide also inhibits implantations in pregnant mice, which exhibit a decrease in the diestrus and increase in the estrus phase (Bindali and Kaliwal, 2002). Topical application of mancozeb on mouse induced development of skin tumours (Shukla *et al.*, 1988; Gupta and Mehrotra, 1992) and at 250 mg/kg body weight this pesticide induced chromosomal aberrations in bone marrow cells of mice (Tripathi *et al.*, 2011). Throughout all those data, it is evidence that mancozeb exposure causes toxic effects. For this purpose, many countries and international recommendations had previously defined Maximum Residues Limits (MRLs) as threshold that may not be exceeded.

Unfortunately, many developing countries including Togo have difficulties to apply legislation about pesticide application. *Lactuca sativa* is one of the commonly consumed vegetables in Togo both in restaurants and in household, particularly at the littoral part where

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more than 80% of vegetable gardening activities are localized. This vegetable is currently treated with mancozeb over three times before harvest, and delay separating the last treatment and harvest was not often respected without consideration for consumers. In previous study, it has been demonstrated that mancozeb administered daily to albinos mice for 30 days has decreased significantly kidney and liver weight and moreover protein quantity in those organs (Ksheerasagar and Kiliwal, 2003). Mancozeb administered orally at 313.6 mg/kg to albinos mice for 10 days has increased significantly serum ALAT and ASAT (Sakr *et al.*, 2005). Apart from those discoveries, no study has examined the relationship between intakes of mancozeb-treated vegetable and the potential effect on liver. Therefore the present study aims to evaluate toxic effects of mancozeb-treated lettuce on the liver of wistar rat.

Material and Methods

Chemical and reagents

Mancozeb (WP 80% a.i.) was purchased from AGRIMAT (Manufactured by Rhom and Haas, France). Biochemical parameters such as ALAT, ASAT, Alkaline phosphatase, total protein, total bilirubin are essayed using commercial kits purchased from Human GmbH. D-65205, Wiesbaden, Germany. TPTZ (2,4,6-tripyridyl-S-triazin) was previously obtained from Avocado Research Chemical (Canada) with the assistance of the Professor John T Arnason (University of Ottawa, Ontario, Canada); Silymarin were obtained from Sigma Chemical (St. Louis, MO, United States). Iron sulphate, iron chloride, acetic acid, and sodium acetate were all analytical grade and manufactured BDH-Prolabo and purchased from VWR France.

Vegetable production

Field experiments to produce lettuce were conducted during August-October 2011 at vegetable garden perimeter in the area of Lomé harbor. Lettuce was planted on plots not used for farm purpose during one year, where organic manure collected from poultry farming was applied (150 kg/plot) before planting. Plot size was 6 m × 1.50 m each, with a spacing of 30 cm apart between plants. From seedbed to harvesting (eight weeks), every week, six plots were sprayed with mancozeb at dose 3.5 kg/ha, using a knapsack sprayer. Any other pesticide or chemical fertilizer was not used. Three untreated plots of lettuce was placed about 30 m from the test plots to serve as a control. Vegetable lettuce harvested weekly 72 hours after mancozeb last spraying, was separated into their various parts, and leaves were dried 5 days under cool condition at 16°C in room. The dried samples were then ground into powder using a Wiley mill.

Animals and Experimental Design

Adult male Wistar rats weighing 180 - 200 g were obtained from the animal facility of the Department of Physiology and Pharmacology of "Université de Lomé". They were kept under ambient temperature, and with a normal photoperiod of 12 h darkness and 12 h light. They were with free access to food and tap water. Animals were randomly divided into five groups of seven and blood was collected via orbital sinus bleeding on each animal under ether anesthesia before products administration. The rats of control group (group 1) received 5 mL of distilled water; the animal of group II received lettuce non treated with mancozeb at the dose of 5 g/kg (UL-untreated lettuce); animals of group III received lettuce treated with mancozeb (MTL) in the farm, as previously mentioned, at 5 g/kg (Mancozeb treated lettuce), animals of group IV and group V received mancozeb alone at 400 and 800 mg/kg respectively. All products were dissolved in 5 ml of distilled water and administered orally. During experimental period, animals were weighed two times weekly and were observed for their behaviors, rhythms and texture of feces. At the end of 28 days of products administration, blood was collected on each animal in the same condition as previously indicated.

Biochemical essays

Blood was centrifuged at 3000 rpm for 15 minutes at room temperature. Plasma was collected and kept in freezer (-20°C) until biochemical analysis. Transaminases (alanine aminotransferase and aspartate aminotransferase), alkaline phosphatase, creatinine, total protein and bilirubine were determined using appropriate kits for each. Data are expressed in international units (IU/L).

Ferric reducing activity of plasma (FRAP) was determined by measuring the total antioxidant potential of plasma from control and treated animals. A daily working reagent (prepared by mixing 25 mL of acetate buffer; 2.5 mL of 10 mM L^{-1} Fe^{3+}-TPTZ in 40 mmol of HCl; and 2.5 ml of $FeCl_3$- 6H2O) (300 µl) was mixed with 10 µl of plasma sample and 30 µl of distilled water (Nair *et al.*, 2007; Agbonon and Gbeassor, 2009). The change in absorbance at 593 nm was measured when the blue Fe^{2+}-tripyridyl-s-triazine (Fe^{2+}TPTZ) compound formed from colorless, oxidized Fe^{3+} after 20 minutes of incubation. Calibration curves were generated from aqueous solution of $FeSO_4$ at different concentrations ranging from 10 to 2000 µmole/L.

Statistical Analysis

Significant differences between groups were determined with analysis of variance (ANOVA) using Systat 5.0 software. Pairwise comparisons were done using the Fisher LSD at $p < 0.05$.

Results

Effect on Animals Behavior

Animals administered with lettuce treated with mancozeb have not shown any mortality. Animals administered with mancozeb alone at 400 and 800 mg/kg have shown signs of morbidity. Animals administered by mancozeb-treated lettuce have no shows evident signs of morbidity compared to mancozeb group; however those animals administered with mancozeb treated lettuce have ate less foods during 28 days of experimentation. Mancozeb-treated lettuce administered to animals for 28 days has induced soft feces which are more frequent in animals administered with mancozeb alone at 800 mg/kg. Animals administered with mancozeb treated lettuce or lettuce alone loosed weigh more importantly than animals treated with mancozeb alone at 400 and 800 mg/kg.

Effects on Biochemical Parameters

Mancozeb-treated lettuce has increased significantly the plasmatic concentration of alanine aminotransferase and asparte aminotransferase ($p < 0.01$) compared to control and untreated lettuce groups, however, these augmentations are less important than those resulting from mancozeb alone groups receiving the fungicide at 400 and 800 mg/kg (Figures 1 and 2).

Mancozeb-treated lettuce has not increased significantly the plasmatic concentration of alkaline phosphatase and creatinine ($p > 0.05$) when compared to the control. Only mancozeb at 800 mg/kg induced a significant increase of plasmatic concentration of creatinine ($p < 0.05$) (Figures 3 and 4).

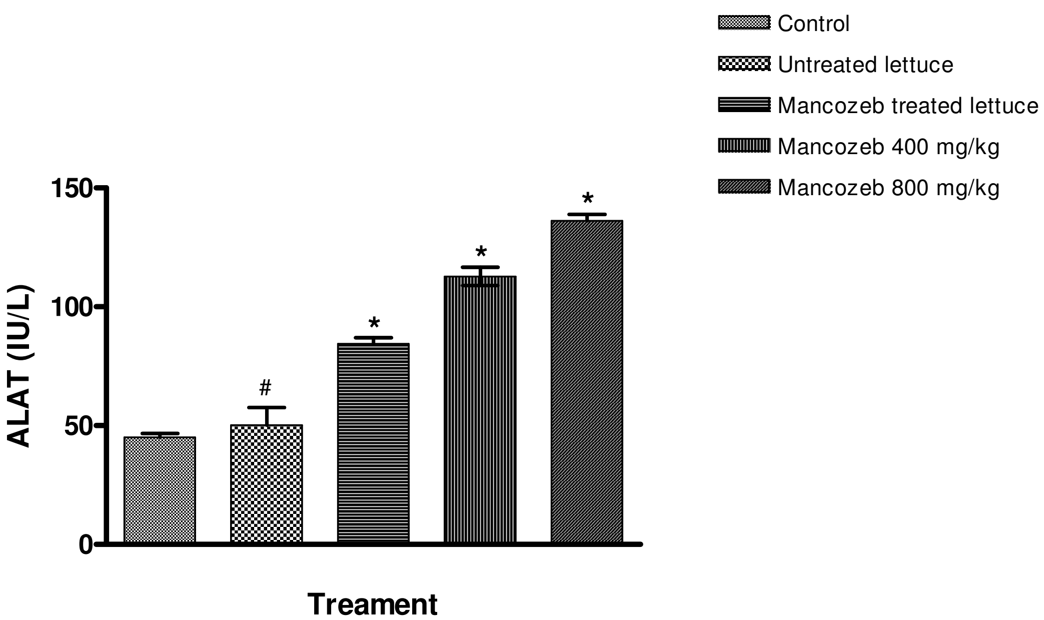

Figure 1 Effect of mancozeb-treated lettuce (MTL) on plasmatic concentration of Alanine aminotraferase.

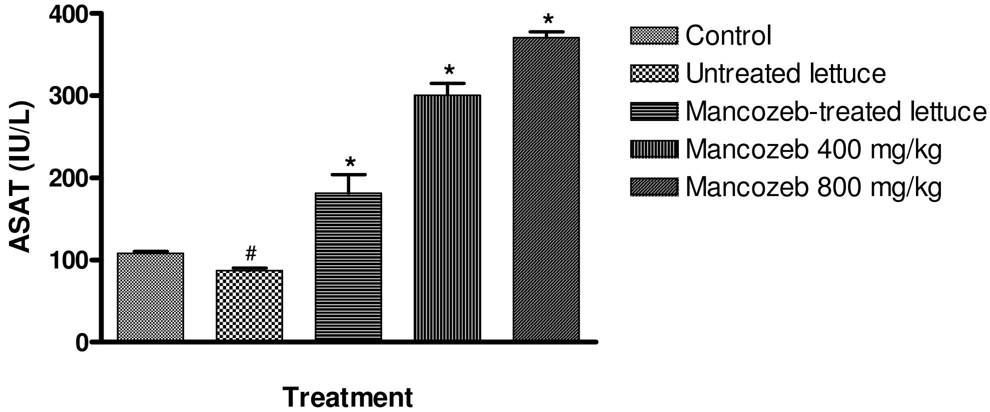

Figure 2 Effect of mancozeb-treated lettuce (MTL) on plasmatic concentration of Aspartate aminotransferase.

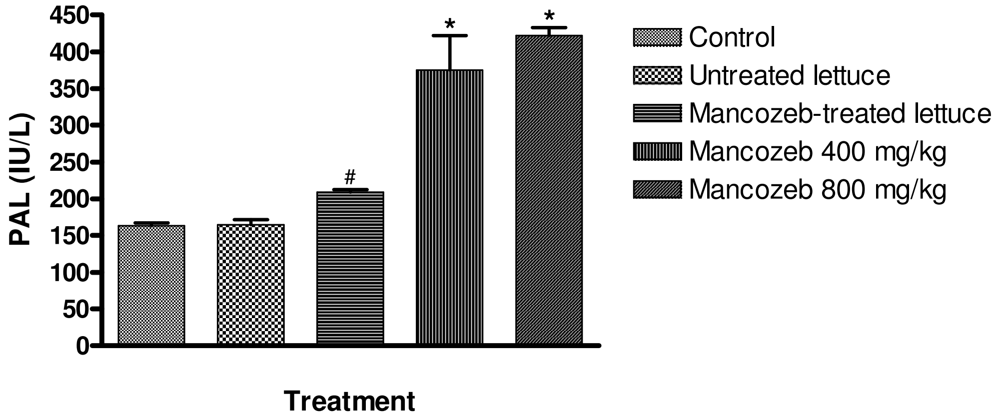

Figure 3 Effect of mancozeb-treated lettuce on plasmatic concentration of Alkaline Phosphatase.

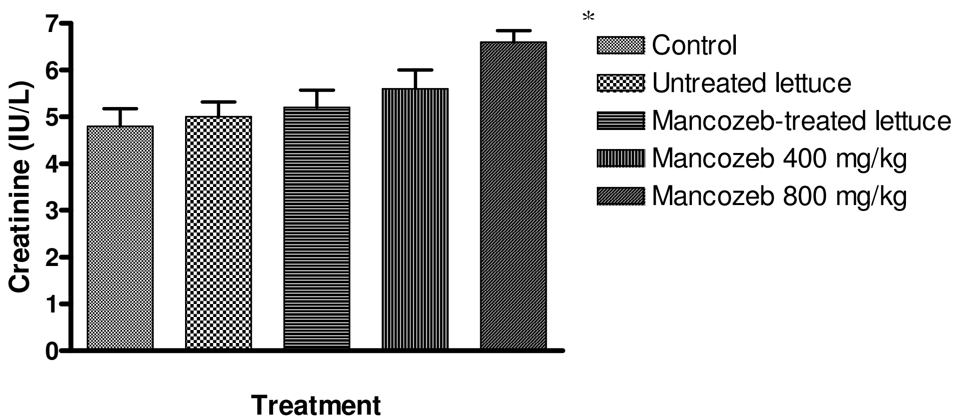

Figure 4 Effect of mancozeb-treated lettuce (MTL) on plasmatic concentration of Creatinine.

Mancozeb-treated lettuce (MTL), during 28 days of administration, does not affect plasmatic concentration of total protein ($p > 0.05$) (Table 1). Plasmatic concentration of total bilirubine is increased slightly in animals administered mancozeb-treated lettuce ($p < 0.05$); this augmentation is more importantly when animals received mancozeb alone at 400 and 800 mg/kg (Table 1). During 28 days of administration of mancozeb-treated lettuce, antioxidant potential of the plasma is impaired but this diminution is not significant as indicated in Table 2 through the diminution of Fe^{+2} concentration.

Table 1 Effect of mancozeb-treated lettuce (MTL) on plasmatic concentration of Total Protein and Total bilirubine

Treatment	Total protein mean±SD (n=5)	Total bilirubine mean±SD (n=5)
Control	72.37 ± 5.12	4.66 ± 0.88
Untreated lettuce	73.88 ± 7.04	4.58 ± 0.70
Mancozeb-treated lettuce	69.67 ± 25.91	6.46 ± 1.46*
Mancozeb 400 mg/kg	65.10 ± 13.32	10.58 ± 4.64*
Mancozeb 800 mg/kg	56.66 ± 4.38	11.54 ± 4.01*

Rats were administered orally during 28 days by lettuce previously treated by mancozeb.
*$p < 0.05$ when compared M400, M800 and Mancozeb-treated lettuce group to control

Table 2. Effect of mancozeb-treated lettuce on the FRAP values in the blood of rats administered during 28 days

Treatment	FRAP (µmol/l) at 20 min mean ± SD (n = 5)
Control	1853 ± 47.3
Untreated lettuce	1920 ± 23.8
Mancozeb-treated lettuce	1642 ± 41.5
Mancozeb 400 mg/kg	1315 ± 35.4
Mancozeb 800 mg/kg	1103 ± 27.6

Discussion

Lettuce is a daily food consumed currently by people both in home and in restaurant. The main objective of the present investigation was to evaluate the potential effect on the liver, in consumers, that may result from the consumption of this vegetable treated with mancozeb, fungicide currently used without any control during the growth of the plant. The main results may be summarized as fellow: administration of mancozeb-treated lettuce does not induce violent toxicity leading to mortality and evident morbidity in wistar rats, however, it induces the increase in plasmatic concentration of transaminases (alanine aminotransferase, aspartate aminotransferase), alkaline phosphatase, and slight increase in total bilirubine and moreover, evidence decrease in conjugate bilirubine. Previous studies have shown that mancozeb alone is toxic to the liver by increasing plasmatic concentration of hepatic enzymes such as alanine aminotransferase, aspartate amino transferase (Sunder and Rao, 1998; Kackar *et al.,* 1999; Hernández *et al.*, 2006). Increase in hepatic enzymes such as ALAT and ASAT in the plasma of rats administered by mancozeb-treated lettuce may result from mancozeb toxicity on the liver as shown in previous studies above mentioned. Moreover, mancozeb is known to generate reactive oxygen species (Calviello *et al.*, 2006) that contribute to liver dysfunction and justify increase of transaminases and alkaline phosphatase. When mancozeb-treated vegetable is administered to animal, the antioxidant potential of plasma is decreased.

It is well established that mancozeb inhibit spermatogenesis in animals (Ksheerasagar and Kiliwal, 2003); induces tumor genesis in human (Chrisman *et al.*, 2009). Based on these previous studies one may speculate that current consumption of mancozeb-treated vegetable such as lettuce may lead to perturbation in reproductive function and moreover the genesis of tumor in old human. The consumption of vegetable treated with mancozeb may induce, in addition to liver disturbance, the multi organs failure and generate pathological conditions such as ovary, and thyroid (Baligar and Kaliwal, 2001) and potentially the impairment of immune function. This hypothesis may be supported by the decrease of antioxidant potential of mancozeb-treated lettuce and mancozeb alone.

Mancozeb-treated lettuce induces change in plasmatic concentration of total protein. This impairment may result in liver dysfunction through diminution of protein synthesis (Mahadevaswami *et al.*, 2000); or from the decrease of intestinal absorption of nutrients leading to the frequent and soft feces induced by mancozeb alone or mancozeb-treated lettuce. One of the most biochemical dysfunction resulting currently form of pesticides exposition is the increase in free radical genesis. In the present investigation, results indicate that mancozeb-treated lettuce decrease slightly antioxidant potential of plasma. This effect can lead to liver dysfunction and in long term to pathogenesis of many diseases associated to pesticides exposition.

Conclusion

Results indicate that lettuce collected directly from gardens, without washing or less washed, are not fit for human consumption. The main finding in the present investigation is that the lack of control in fungicide such as mancozeb utilization in vegetable production may lead to public health problem. Further investigations are needed to quantify pesticides such as mancozeb in vegetables sold in the local market for consumption.

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