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Effect of cypermethrin-treated lettuce (*Lactuca sativa*) on wistar rat liver

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ABSTRACT

In this study, assessment of the potential effect of cypermethrin-treated lettuce on the rat liver physiology is carried out. Cypermethrin-treated lettuce and three doses of cypermethrin (CY) were administered during 28 days to rats. Along the experimental period, animal behavior was assessed, and at the end of administration, some hepatic enzymes were studied. The decrease in rat body weight was noted and animals have soft feces. Results showed plasmatic concentrations of ALAT, ASAT and total bilirubin increase in rats administered with cypermethrin-treated lettuce. The plasmatic concentration of total protein is not decreased significantly. Those results indicate that lettuce collected without waiting for the recommended pre-harvest intervals, might cause hazardous effects to vegetable consumers. Further investigations are needed to quantify pesticides such as cypermethrin in vegetables sold in the local market for consumption.

INTRODUCTION

There is increasing interest for vegetables consumption due to their antioxidant property and leading to human well being. However, vegetable production in tropical countries is currently attacked by pests and diseases. To reduce damages and prevent insect, fungus and bacterial attack, farmers use improperly insecticides. Current application of pesticides, fungicides and others chemicals may lead to food poisoning accidents and may impact environment including humans and animals health through soil, water and vegetable contamination. Pesticides applications are problematic concerned in Togo due to the lake of administrative control. During the growth of many vegetable, pesticides are applied more than ten times. Many investigations reported high contamination of vegetables and other edible food with pesticide residues in Togo. In previous studies, Djaneye-Boundjou *et al.* (2000) shown that sixty percent of vegetables and edible seeds sampled in Lomé contained aldrin with higher concentration than tolerable limits. Moreover, Mawussi *et al.* (2009) determined the high contamination of drinking water, maize and cowpea grains sampled in cash crop (cocoa, coffee, and cotton) cultivation areas in Togo by various organochlorine pesticides such as γ-hexachlorocyclohexane (HCH), dichlorodiphenyltrichloroethane (DDT), aldrin, dieldrin, endrin, heptachlor epoxide and endosulfan. FAO (2010) reported that more persistent and toxic pesticides intended for the same cash crop, were used on foodstuffs and vegetables (FAO, 2010). *Lactuca sativa* is among vegetables consumed by the Togolese in many household and restaurants. During its growth process, *L. sativa* gets damaged by many insects. Cypermethrin is one of the most extensively insecticides used by farmers to increase their production in south area of the country, where more than 80% of vegetable gardening activities are localized. Studies carried out by Chunyanuwat (2005) in Thailand shown that pesticide residues most often found on leafy vegetable were cypermethrin (CY) with concentration of 0-8.48 ppm. Many studies reported a lot of environmental and health problems due to continuous and excessive use of synthetic pyrethroid insecticides (Cuthbertson and Murchie, 2010; Cuthbertson *et al.*, 2010). Exposure to the cypermethrin is known to affect various vital organs and produce toxic signs in animals.

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Studies carried out by Khan and Fatima (2002) revealed that cypermethrin is toxic to *Agama lizard*. The exposition of cypermethrin 1% during 24 h decrease enzymatic activities of GOT up to 37% in liver, 66% in kidney and 61% in brain. The decreases of GPT activity in these organs were 33%, 44% and 46%, respectively. Toxicity studies of cypermethrin in rats on bloat, congestion of lungs, brain, and gastric mucosa, showed mild degenerative changes in the liver and kidneys (Nair *et al.*, 2011). Cypermethrin may alter the metabolism of lipids and proteins in rat liver (Aldana *et al.*, 1998). Mice exposed to diesel and cypermethrin (2%) aerosolization after six days, showed higher increased polymorphonuclear cells (eosinophils and neutrophils) in blood and lungs (Garcia *et al.*, 2009).

Experiments carried out by Giri *et al.* (2003) showed that cypermethrin has interactions with DNA activities in mice, indicating potential mutagenic effects. Application of cypermethrin to vegetable may lead to the accumulation of its residues in the plant organs and may affect later consumers. Although this potential risk, no study has examined the relation between intakes of cypermethrin-treated lettuce and liver physiology until to present in our community. The present investigation aims to evaluate potential toxic effects of cypermethrin-treated lettuce on Wistar rat liver.

MATERIAL AND METHODS

Chemical and reagents

Cypermethrin (EC 50%) was purchased from a local supplier AGRIMAT (manufactured by Arysta LifeScience, 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, and 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 cypermethrin at dose 1 L/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. The leaves of lettuce harvested weekly, 72 hours after cypermethrin last spraying, were dried 5 days in room under air conditioner. The dried samples were then ground into powder using a Wiley mill.

Animals and experimental design

The study was performed on male Wistar rats weighing 190 - 280 g, provided by the animal facility of the Department of Physiology and Pharmacology of “Université de Lomé”. Animals were housed five per cage and maintained under ambient temperature with a normal photoperiod of 12 h darkness and 12 h light. They have free access to food and water. Animals were divided into six groups (n = 5 per group). They were administered the vehicle (group I: 1 mL maize oil) or cypermethrin (groups II-IV: 10, 25 or 50 mg/kg in 1 mL maize oil) by oral route once a day for 28 consecutive days.

These doses of cypermethrin (CY) were expressed in mg/kg equivalent from active ingredient. The two last groups received untreated lettuce (group V: 5 g/kg body weight in 5 mL water) and cypermethrin treated lettuce (group VI: 5 g/kg in 5 mL water) by oral route for 28 consecutive days. All animals were weighed daily throughout the study. At the end of 28 days of products administration, blood was collected on each animal, after anaesthetized with ether.

Biochemical essays

Liver activity

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

In vivo antioxidant test

Ferric reducing activity of plasma (FRAP) was determined by measuring the total antioxidant potential of plasma from control and treated animals which are previously received lettuce or water during 28 days. Briefly, 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 $FeCl_3$- $6H_2O$) (300 µl) was mixed with 10 µl of plasma sample and 30 µl of distilled water [16,17]. The change in absorbance at 593 nm was measured when the blue Fe^{2+}-tripyridyl-s-triazine (Fe2+TPTZ) compound formed from colorless, oxidized Fe^{3+} after 10 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

Body weight variation, diarrhea signs and death

Body weight loss and diarrhea were most prominent clinical signs of the rats administered with different doses of cypermethrin alone or cypermethrin-treated lettuce. Except the control, maize oil and untreated lettuce groups, the others animals had soft feces during the experiment. The animal administered with cypermethrin alone presented frequently diarrhea, and two animals were died during the experiment. As summarized in Table 1, the insecticide at dose 50 mg/kg caused significant ($p < 0.01$) decrease in body weight compared to oil control-group, this weight fell from 204.6 ± 8.0 g to 184.3 ± 2.5 g. Either the untreated or the treated lettuce induced in animals significant weight loss ($p < 0.05$) compared to control. In this case, at the start of the study weights were 202.0 ± 3.7 g and 208.2 ± 4.9 g respectively, and at the end were 201.0 ± 9.3 g and 201.8 ± 6.3 g, respectively. Feed intake was less in groups where weight loss was noted.

Table. 1: Mean change in body weight of rats following consecutive daily oral administration at lettuce previously treated by cypermethrin for 28 days (Value are given as mean ± SD, n=5).

Treatment	Initial weight (g)	Final weight (g)	Change (g)
Control (water)	262.0 ± 34.7	267.8 ± 34.1	5.8
Maize oil	201.2 ± 7.5	225.0 ± 10.6	23.8
Untreated lettuce	202.0 ± 3.7	201.0 ± 9.3*	-1
CY-Treated lettuce	208.2 ± 4.9	201.8 ± 6.3*	-6.4
CY (10 mg/kg)	210.6 ± 17.0	226.8 ± 14.6	16.2
CY (25 mg/kg)	220.4 ± 16.6	209.4 ± 15.1	-11
CY (50 mg/kg)	204.6 ± 8.0	184.3 ± 2.5*	-20.3

* $p < 0.05$ in comparison with controls.

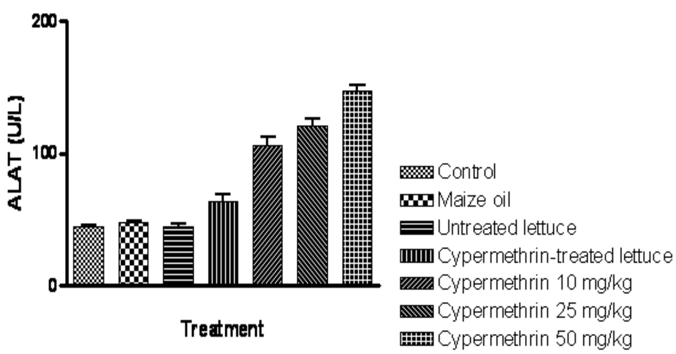

Fig. 1: Effect of cypermethrin-treated lettuce on plasmatic concentration of Alanine aminotraferase. Rats were administered orally during 28 days by lettuce previously treated by cypermethrin. Results are means ± SD; $p < 0.01$ when compared C50, C25, C10 and cypermethrin-treated lettuce to the controls and untreated lettuce groups.

Biochemical and antioxydants profile

Biochemical analysis concerning liver biomarkers showed that the concentration of alanine aminotransferase was increased significantly ($p < 0.01$) in the three groups of animals administered with cypermethrin (C50, C25 and C10) and cypermethrin-treated lettuce when compared to oil control and untreated lettuce groups (Figure 1).

The same three doses of cypermethrin caused significant increase in ASAT concentration, however, those activities did not differ significantly between cypermethrin-treated lettuce and control groups ($p > 0.05$) (Figure 2).

Plasma total protein levels were decreased significantly in cypermethrin treatment groups ($p < 0.05$). The effect of cypermethrin-treated lettuce was not significant ($p > 0.05$) (Figure 3).

As showed on Figure 4, total bilirubin concentrations significantly higher ($p < 0.01$) when compared all three doses of cypermethrin with controls. There was also a significant increase ($p < 0.05$) in the cypermethrin-treated lettuce when compared to controls groups. Lettuce sprayed with cypermethrin reduces significantly antioxidant potential of the plasma of animal (Table 2).

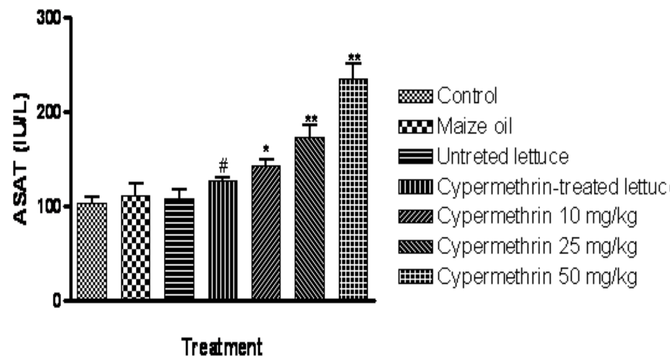

Fig. 2: Effect of cypermethrin-treated lettuce on plasmatic concentration of Aspartate aminotransferase. Rats were administered orally during 28 days by lettuce previously treated by cypermethrin. Results are means ± SD; **$p < 0.001$ when compared C50 and C25 to the control and untreated lettuce groups; *$p < 0.05$ when compared C10 to control and untreated lettuce groups; #$p > 0.05$ when compared cypermethrin-treated lettuce to the control and untreated lettuce groups.

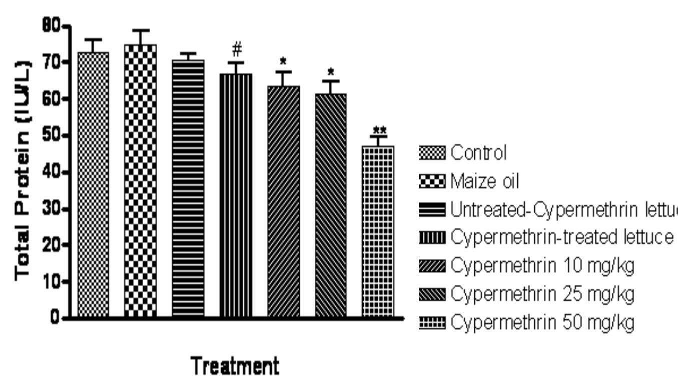

Fig. 3: Effect of cypermethrin-treated lettuce on plasmatic concentration of Total Protein. Rats were administered orally during 28 days by lettuce previously treated by cypermethrin. Results are means ± SD; **$p < 0.001$ when compared C50 to the control and untreated lettuce groups; *$p < 0.05$ when compared C25 and C10 to the control; #$p > 0.05$ when compared cypermethrin-treated lettuce to the control and untreated lettuce groups.

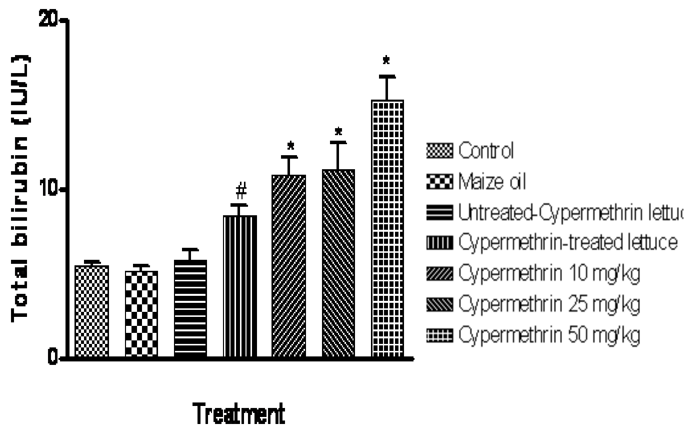

Fig. 4: Effect of cypermethrin-treated lettuce on plasmatic concentration of Total Bilirubin. Rats were administered orally during 28 days by lettuce previously treated by cypermethrin. Results are means ± SD; $*p < 0.01$ when compared C50, C25 and C10 to the control and untreated lettuce groups; $\#p < 0.05$ when compared cypermethrin-treated lettuce to the control and untreated lettuce groups.

Table. 2: Effect of cypermethrin-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 (water)	1100	±	85
Maize oil	1224	±	102
Untreated lettuce	1476	±	75
CY-Treated lettuce	957	±	96
CY (10 mg/kg)	722	±	63
CY (25 mg/kg)	628	±	38
CY (50 mg/kg)	537	±	59

DISCUSSION

There is increasing interest in the nutrition based on natural product and health protecting properties of vegetables particularly lettuce, that constitute basic raw fresh product for salad preparation, is well known. However, indiscriminate use of pesticides to control vegetable pests has become alarming in Lomé and may be a factor of risk. The drawback of cypermethrin residues, which remain on lettuce, may lead to the potential health degradation to consumers. This study focused on potential toxic effects of cypermethrin-treated lettuce on Wistar rat liver. Throughout the experimental period, the first evidence of health impairment is the significant body weight loss was recorded in administration high amount cypermethrin and lettuce groups when compared to controls. This is in contrast with the results found by Yavasoglu *et al.* (2006), who worked with cypermethrin and reported as Institóris *et al.* (1999) and Aziz *et al.* (2001) that some synthetic pyrethroids such as permethrin, deltamethrin had no effect on body weight of rats. Biochemical studies showed increase in plasmatic concentration of transaminases (alanine aminotransferase, aspartate aminotransferase) and total bilirubine. Alanine amino transferase (ALAT) and Aspartate amino transferase (ASAT) are more sensitive measures of hepatotoxicity. In Clinical chemistry, indicator parameters routinely implemented during nonclinical assessments for hepatotoxicity include: ALAT, ASAT (Hepatocellular leakage enzymes), bilirubin, ALP, GGT (Cholestasis indicators), albumin, urea, nitrogen (Function indicators), electrolytes, total CO_2, glucose, triglyceride, and cholesterol (Metabolism indicators) among others. Increases in these indicator parameters, even in the absence of histologic changes, are considered adverse, unless the pathogenesis indicates to the contrary. (Fokunang *et al.*, 2010 ; Samson *et al.*, 2012). Most previous studies reported liver alteration in animal after cypermethrin (CY) treatment. These investigations such as the impact of cypermethrin on enzyme activities in the freshwater fish *Cirrhinus mrigala* (Prashanth and Neelagund, 2008) showed the sub lethal dose exposure of cypermethrin induced elevated levels of ASAT and ALAT, but produced less change in the protein metabolism. Studies carried out by Tantarpale (2011) revealed that cypermethrin affected total proteins in muscles and liver of freshwater fish *Channa striatus.* These evidences, few studies, apart from those from Sakr *et al.*, (2001) have concerned effects of cypermethrin-treated vegetables on animals and less on human. It is well known that the different pyrethroids have been reported to cause significant effects on animals physiology and biochemical reactions (Kumar *et al.*, 2010), and especially cypermethrin causes hepatic and renal toxicity (Sushma and Devasena, 2010). In the present study, the significant increase of ALAT and total bilirubine concentration after rat administration with cypermethrin-treated lettuce for 28 days may be due to the effect of this pesticide on liver function. These results indicated that residue of cypermethrin remained on the lettuce, after food processes, in high level able to induce liver injury toxicity. Study showed previously that pesticide used to control pest persist in environment and may be accumulated in animals and human body by many ways such as through water, vegetables and fruits or other edible food (Karanth, 2000). Pesticides bioaccumulation is a serious problem along the littoral of the gulf of Benin because of the high load on field. Cabbage producers apply pesticides every 3 or 4 days for during three months before harvest. During the growing season of pepper (10 weeks), farmers also go up to 18 applications of pesticides (James *et al.*, 2010). In this situation, vegetables growers and consumers would be exposed to the harmful effects of pesticides, which are applied without caring about their persistent time. It had been shown that three days after spraying, 33.43% and 25.36% of pesticide residue persist in okra leaves and fruit sample respectively (Shinde *et al.,* 2012). These residues may persist during 17 days after spraying. Those reports support the finding in the present study where lettuce was treated every week during 8 weeks, and harvested three days after the last spraying. The present investigation shows that antioxidant capacity of lettuce, previously sprays with cypermethrin, is impaired. This loss lead to the disadvantage for the consumers because, vegetables are consumed for their antioxidant property conferring to them a well being. In conclusion, administration of cypermethrin-treated lettuce during 28 consecutive days leads to significant alteration of rats in liver. Further investigations are needed to quantify pesticides such as cypermethrin in vegetables sold in the local market and it is needed monitoring and regular evaluations of the quality of vegetables.

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