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Effects of Mixtures of Lambda-Cyhalothrin and Dimethoate on Liver and Antioxidant Enzyme Systems of Adult Male Albino Rats

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Abstract

Commercial mixtures of organophosphates and pyrethroids have become very common in the markets in developing nations and have resulted to increase in cases of mixed toxicity. This study was carried out to evaluate the toxic effects of commercial preparation of a pesticide mixture, Magicforce, which contains 30% Dimethoate (DM) and 1.5% Lambda-cyhalothrin (LC), compared to the individual commercial pesticides of 40% DM and 2.5% LC. Forty (40) adult male albino rats were divided into four (4) equal groups. Dimethoate group received 10.7 mg/kg b.w/orally/daily, Lambda-cyhalothrin group (LC) received 3.9 mg/kg b.w/orally/daily, Magicforce group (MF) received 1.2 mg/kg b.w/orally/daily, while the Control group (C) received only food and water daily. At the end of the treatment (28 days), the rats were sacrificed. The liver was excised and weighed, the tissue homogenate was prepared for biochemical analysis together with the blood samples. The estimation of liver function enzymes (AST, ALT and ALP) and oxidative stress markers; malondiadehyde (MDA), catalase (CAT), glutathione (GSH), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and superoxide dismutase (SOD) were determined. The results revealed that the activities of the liver function enzymes (AST, ALP and ALT) were significantly ($p<0.05$) higher in the LC-treated group when compared with the DM and MF treated groups as well as the control. The same trend was observed in the oxidative stress parameters (SOD, CAT, GSH, GST, GPx and MDA). It can be concluded that the Lambda-cyhalothrin (LC), singly had more toxic effect on the liver and antioxidant enzyme systems of the adult male albino rats than the Dimethoate (DM) and their combined commercial state (Magicforce). This suggests an antagonistic interaction in the mixture.

Keywords: Antioxidant, Dimethoate, Lambda-cyhalothrin, Liver enzymes, Magicforce.

1. Introduction

The use of pesticides in modern agriculture has inevitably resulted in substantial increase in food production and economic well being of the citizens in developing countries, thereby making its application the bedrock of world agriculture (Prakasam, Sethupathy & Kalitha, 2001). The unregulated use of these pesticides has caused serious toxic problems to non-target species such as birds, fishes, mammals and other beneficial soil microbes (Riebeiro, Guedes, Oliveira & Santos, 2003). According to World Health Organization, pesticide self-poisoning is a major public health concern which claims about 3 million people annually

(Varon, Özdemir, Çevik, Altun, Ibilog˘lu, Ekinci, Ibilog˘lu, Balduz, Demet, Tekin, Aktar, & Aluçlu, 2016).

Dimethoate is one of the most potent organophosphate insecticides. In agricultural practice, it is used against a wide range of pests and it is a class II insecticide with moderate toxicity (Hassanin & El Asely, 2015). The mechanism of dimethoate toxicity is due to its inhibition and accumulation of acetycholinesterase (AChE), at the nerve endings and the neuromuscular junctions (Sayim, 2007). Lambda-cyhalothrin (LC) is a type II pyrethroid, used mostly to control insect pest in agriculture, public facilities, homes and gardens. It is often considered a potent neurotoxic pyrethroid with the capacity to cause hepatotoxicity (Tu, Silvestre, Bernard, Douny, Phuong, Tao, Maghuin-Rogister & Kestemont, 2007; Manal, Elhalwagy & Nashwah, 2008).

Most toxicological investigations of chemicals have dwelt majorly on evaluation of single compounds exposures. In reality, humans are exposed to complex and variable interacting mixtures of chemicals, which may act independently as in a single exposure, but may also interact to modulate the effects of the mixture as a whole (Groten, Butler, Feron, Kozianowski, Renwick & Walker, 2000). Therefore, the purpose of this study was to evaluate the individual and combined toxic effects of dimethoate and lambda-cyhalothrin on liver and antioxidant enzyme systems of adult male albino rats.

2. Materials and Method

2.1. Insecticides

Dimethoate (DM) (40% EC; Jiangsu Tenglong Biological and Medicinal Co. Ltd. China.) and Lambda-cyhalothrin (LC) (2.5% EC; Bretmont Ltd. England) insecticides were used in the present research. A Mixture of both insecticides (20:1) was contained in MagicForce, a commercial EC formulation produced by Anhui Zhongshan Chemical Industries Co. Ltd. China. These chemicals were purchased from an Agro-chemical shop in Owerri, Imo state, Nigeria.

2.2. Experimental Animals

Forty male albino rats (3–4 month age; 160–180g b.wt.) were obtained from the breeding animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were housed in individual cages and allowed to acclimatize under laboratory conditions at room temperature for one week prior to commencement of the experiment. The rats were kept under hygienic and favorable conditions, and maintained under a 12 h light/12 h dark cycle, in accordance to the care and wellbeing of research animals (NIH, 2011). They were fed with commercial pelletized animal feeds and water available *ad libitum.*

2.3. Treatment of experimental animals

The animals were divided into 4 groups of 10 animals each with the mean weights equalized as nearly as possible. Dimethoate group (DM) received 10.7 mg/kg body weight; Lambda-cyhalothrin group (LC) received 3.95 mg/kg body weight; Magicforce group (MF) received 1.2 mg/kg body weight while the control group (C) received food and distilled water only. At the end of the 28 day feeding period, an overnight fast was imposed on the animals before sacrificing.

2.4. Blood and Tissue Sampling

At the end of the post feeding fast, the rats were subjected to light diethyl ether to induce anesthesia. Blood was collected by cardiac puncture into clean and dry test-tubes without anticoagulant, allowed to stand to separate serum and then preserved in a refrigerator until used for analyses. Then the liver was collected, cleaned, weighed (absolute weight) and homogenized in 5mL cold buffer (0.1 M-phosphate buffer, pH 7.4) per gram tissue. The homogenates were further vortexed for 15 minutes, allowed to stand and the supernatant collected into sterile tubes and preserved in a refrigerator until used for biochemical analyses.

The relative organ weight of each animal was calculated as follows:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Final body weight of the rat (g)}} \times 100$$

2.5. Biochemical Analyses of Samples.

Serum aspartate aminotransferase (AST) activity was measured by using optimized UV-test according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), as reported by Thomas (1998). Serum alanine aminotransferase (ALT) activity was measured based on the method of Reitman and Frankel (1957) as reported by Sherif *et al*. (2014). The activity of alkaline phosphatase (ALP) was determined according to the method described by Englehardt *et al*. (1970). The levels of lipid peroxidation in samples were measured as malondialdehyde (MDA) according to the method of Buege and Aust (1978). Superoxide dismutase (SOD) activity was determined following the method reported by Sherif *et al*. (2014). Catalase activity in the blood was determined by the method of Aebi (1983). Glutathione peroxidase activity was assayed using the method of Hafemann *et al*. (1974). Glutathione-S-transferase (GST) was measured by the method of Habig *et al.,* (1974). Reduced glutathione in the samples was determined according to the method of Moron *et al*. (1979).

2.6. Statistical Analysis

The biochemical data was analyzed with one-way analysis of variance (ANOVA) using the Statistical Package for Social Science (SPSS) programme version 11 and least significant difference (LSD) was used to compare significance between groups.

3. Results and Discussion

There was general increase in the average weight of the liver of rats exposed to the pesticides (Figure 1). The treated groups (DM, LC and MF) had 4.67g, 5.50g and 4.66g respectively as against the control group (C) 4.25g . The data obtained in the organ weight shows that there was significant increase in the liver weight of all the pesticide treated groups compared to the control. The LC treated group had the highest weight followed by the DM group and the MF. This agrees with the observations of Waggas (2013) that the liver is an important vital organ in the animal body as it is the site of detoxification and elimination of toxic materials. A foreign body in form of a chemical stress is sufficient enough to cause severe hepatic dysfunction such as inflammation of the liver.

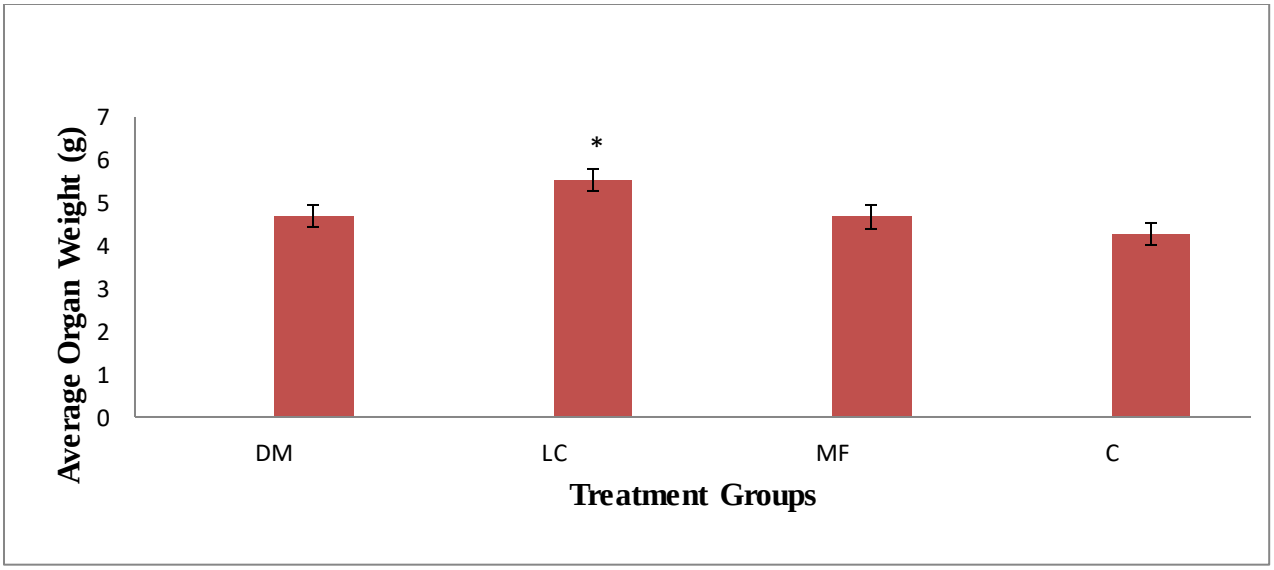

Fig 1.0 Average Liver Weight of Male Albino Rats exposed to pesticide mixtures

From the results of the liver function tests as (Figure 2), there were variations in the activities of liver function enzymes due to pesticide exposure. There were significant increases ($p<0.05$) in the activities of ALT, AST and ALP compared to the group. The labda-cyhalothrin (LC) treated group showed higher activities of these marker enzymes followed by the dimethoate (DM) treated group and MagicForce (MF) group, which is a mixture of labda-cyhalothrin and dimethoate. The Aminotransferases (ALT and AST) are sensitive indicators of liver cell damage for both acute and chronic hepatocellular injury (Barth, Steven & Robert, 2009). Therefore levels of these enzymes reflect the state of hepatic function (Konan, Bacchia, Lincopan, Varelac & Varandac, 2007). The elevated levels of ALT indicate a possible hepatotoxicity which would have resulted in the leakage of the enzyme into the serum. Similarly, Shakoori, Ali & Saleem (1994) reported that the increase in the activity of AST is mainly due to the leakage of this enzyme from the liver cytosol into the blood stream, which reflects liver damage and disruption of normal liver function. On the other hand, Al-Haj, Nasser & Anis (2005) observed that ALP is often employed to assess the integrity of the plasma membrane of the liver. The significant increase in serum activity in ALP due to treatment with 1/20 LD_{50} of the pesticide may be as a result of disruption of the plasma membrane.

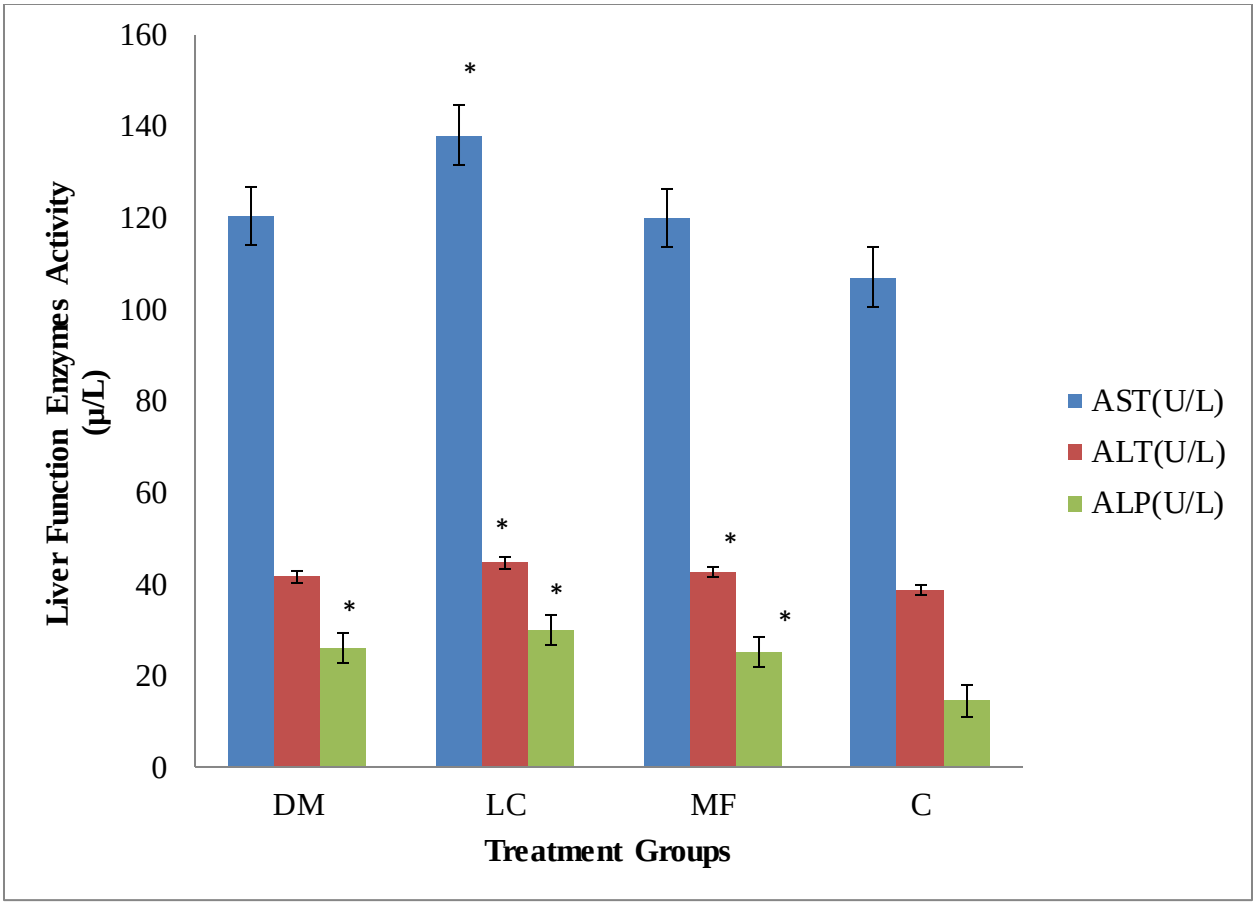

Fig 2.0. Effects of the Pesticides and their Mixture on Liver Function Enzymes

Table 1 showed the results of oxidative stress parameters of the male albino rats exposed to pesticides and their mixture. The study revealed that the exposure of the rats to the pesticides resulted in decrease in antioxidant defense mechanisms due to a state of oxidative stress in the liver. There were reductions in the activity of SOD, CAT, GP_x and the levels of GSH and MDA in the treatment groups compared to the control. The LC treated group had more decreased activity of SOD, CAT, GP_x and levels of GSH and protein followed by the DM treated group, then the MF. MDA was most elevated in DM-treated group followed by the MF, then the LC treated group compared with the control. On the other hand, GST activity in the liver was insignificant compared with control. Pesticides have been implicated to cause oxidative stress and change in antioxidant status system (Solati, 2013). Effects of organophosphate pesticides in the liver have been previously reported (Yousef, El-Deerdash, Kamel & Al-Salhen, 2010). They may induce oxidative stress through their "redox-cycling" activity, where they generate superoxide anions and hydrogen peroxide, or through ROS generation via change in normal antioxidant homeostasis that results in depletion of antioxidants (Altuntas, Kilinc, Orhan, Demirel, Koylu & Delibas, 2011). Most pesticides have been shown to induce inflammation and cell infiltration (Elhaway & Zaki, 2009). Decrease in GSH concentrations may be through low production or non-enzymatic oxidation of GSH to glutathione disulfide (GSSH) due to oxidative stress in the pesticide-treated rats' liver (Banerjee, Seth, Bhattacharya, Pasha & Chakraborty, 1999). Oxidative stress can be monitored by observing the elevation of lipid peroxidation products (Goel, Dani

& Dhawan, 2005). The elevated MDA level in the intoxicated rat livers was in agreement with the above statement. The oxidative stressed state of the liver had led to the fall in protein synthesis.

Table 1: Oxidative Stress Parameters of liver of male albino rats exposed to pesticides and their mixture.

PARAMETER	DM	LC	MF	C
SOD(IU/L)	4.16E-05±1.9E-06*	3.92E-05±1.2E-07	4.36E-05±4.4E-06*	8.12E-06±3.3E-08
CAT(U/L)	7.14E-06±6.8E-07	5.31E-06±2.7E-07*	7.77E-06±2.1E-07	8.82E-06±1.1E-06
GSH(mg/dL)	0.92±0.3*	0.67±0.1*	1.58±0.1*	2.62±0.7
GST(µmol/g.tissue)	5.42E-06±1.6E-07	4.27E-06±2.5E-07	3.96E-06±0.0	4.14E-06±6.3E-07
GPx(mg/g.tissue)	4.03±0.2*	3.32±0.4*	4.50±0.5*	6.59±0.6
MDA(nmol/g.tissue)	0.50±0.0*	0.30±0.0	0.42±0.0*	0.28±0.0
Protein(g/L)	64.5±5.4*	59.6±3.1*	65.3±7.6*	83.8±0.5

All values were expressed as mean ±SEM. LSD* shows the significant difference of treatments compared to control (C) at p≤0.05. Dimethoate(DM), Lambda-Cyhalothrin(LC), Magicforce(MF), Control(C). SOD = Superoxide dismutase, CAT = Catalase, GSH = Glutathione, GST = Glutathione-S-transferase, GPx = Glutathione peroxidase, MDA = Malondialdehyde.

4. Conclusion

According to the results obtained from the present study, it can be concluded that chronic exposure to sublethal dose (1/20 LD_{50}) of dimethoate and lambda-cyhalothrin singly and in combination (magic force) cause significant hepatotoxic effects in rats while lowering the antioxidant defense system significantly due to high oxidative stress of the toxicants. However, the singly effects of the pesticides were higher when compared with their combined effects. This suggests that there is an antagonistic interaction in the novel commercial product (Magicforce).

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