

Exposure to uppercott induces hepatotoxicity in male albino wistar rats

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Abstract

Background: The use of herbicides and pesticides in agriculture and for public health purposes has led to harmful effects in many non-target species including man.

Methods: In this study, the hepatic effect of exposure to cypermethrin and dimethoate mixture (uppercott) was investigated in male albino wistar rats. Forty five (45) male wistar albino rats were distributed into five (5) groups of nine (9) animals each. Groups 1 and 2 served as the normal control and oil control respectively, groups 3, 4 and 5 received 2.5%, 5% and 7.5% LD₅₀ of uppercott orally for 28 days.

Results: Results obtained revealed that uppercott exposure significantly ($p < 0.05$) increased serum aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity and alkaline phosphatase (ALP) activity when the test groups were compared with control. Also, uppercott exposure raised serum bilirubin concentration slightly insignificantly compared to the control. Superoxide dismutase activity was significantly reduced across all test groups as compared with control. Histology of liver tissues revealed patchy necrotic sessions in the liver tissues of the test experimental groups (2.5%, 5% and 7.5%).

Conclusion: The results obtained from this study are strongly indicative of the hepatotoxic effect of uppercott pesticide and hence, caution during usage is advised.

Keywords: Alanine Transaminase (ALT); Alkaline Phosphatase (ALP); Aspartate Transaminase (AST); Hepatotoxicity; Uppercott Pesticide.

1. Introduction

Pesticides are substances meant for attracting, seducing, destroying, or mitigating any pest [1]. They are either chemical or biological agents which slow, weaken, or deter pests. Those targeted are insects, weeds, plant pathogens, mollusks, mammals, fish, birds, microbes, and nematodes. The enormous beneficial uses of pesticides notwithstanding, pesticides could be potentially toxic to man and other desired species. Pesticides are most commonly used as plant protection products, which generally guide against attacks from weeds, plant diseases or insects. Pesticides encompass insecticides, herbicides, termiticide, molluscicide, piscicide, avicide, fungicide, disinfectant, and sanitizer to mention but a few [2]. Pesticides have been shown to help in the prevention of sickness in man which possibly is food related, like food infected with molds or infected produce. Uppercott is a type of pesticide composed of two main components namely cypermethrin (30g/l) and dimethoate (250g/l).

The liver is an organ situated in the upper and right part of the abdominal cavity, below the diaphragm. It is the largest organ in the body weighing between 3% and 4% of the total body weight. It has four lobes consisting of 50,000 to 100,000 individual hepatic lobules [3]. According to [4], the liver, the largest organ in the body, is often the target organ for chemically induced injuries. There are a number of reasons why the liver is particularly susceptible. Firstly, it is not surprising that the liver is the first point of call when xenobiotics are involved. This is because, most xenobi-

otics actually get into the body using the gastrointestinal (GI) tract and, once completely absorbed; they are moved to the liver through the help of the hepatic portal vein. Secondly, as mentioned earlier, the liver has a very high concentration of the enzymes responsible for metabolising xenobiotics, notably the CYP P450-dependent monooxygenase system. Even though most biotransformations usually happen to be detoxification reactions, the oxidative reactions end up producing highly reactive metabolites capable of inducing liver necrosis. The areas often damaged are mostly in the centrilobular region [5], this is because there is a reasonably higher concentration of cytochrome P450 in that section of the liver [5]. The liver could be affected by different types of injury ranging from fatty liver, necrosis, apoptosis and cholestasis [6].

Knowing that the liver is the major organ involved in xenobiotic metabolism, this study was therefore carried out to investigate the effect of Uppercott pesticide on liver integrity in Wistar albino rats.

2. Methods

2.1. Chemicals and reagents

All chemicals and reagents used were of analytical grade. Uppercott pesticide was purchased from Agro chemical company in Calabar, Cross river state, Nigeria.

2.2. Experimental animals

Forty-five (45) male wistar albino rats weighing between 150-180g were used for the experiments. They were gotten from the animal grooming section of the department of Biochemistry, University of Calabar and allowed to acclimatize for one week. All animals were maintained under standard conditions and were given normal pellet diet ad libitum.

2.3 Experimental design and treatment of animals

Prior to the study, the LD₅₀ of the Uppercott pesticide was determined to be 14.14mg/kg body weight adopting Lorke's method [7]. The experimental animals were divided into five (5) groups of nine (9) rats each as shown below:

Table 1: Distribution of Animals into Experimental Groups

Groups	Number of animals	Treatments
Group 1	9 males	Control (untreated) (NCM)
Group 2	9 males	Control administered only oil (OCM)
Group 3	9 males	2.5% (0.35 mg/kg b.wt) of LD ₅₀
Group 4	9 males	5% (0.71 mg/kg b.wt) of LD ₅₀
Group 5	9 males	7.5% (1.06 mg/kg b.wt) of LD ₅₀

Treatments were by oral administration and lasted for 28days.

2.4. Collection of blood samples and procedure

At the end of treatments, 5 rats were selected at random from each group and sacrificed. Blood samples were collected by cardiac puncture into plain screw-cap sample bottles for the liver function tests (LFTs) and antioxidant assay. The blood samples were spun with the aid of MSE model (England) table-top centrifuge at 2000 rpm for 5 minutes. The sera collected were used for biochemical analyses. All biochemical analyses were carried out within 24 hours of serum separation.

2.4.1. Determination of bilirubin concentration

Bilirubin was estimated using Span assay kits [8].

(a) Total bilirubin

To 50 ml of sodium nitrite solution 29 mM, 200 ml sulfanilic acid (29 mM in 170 mM HCl), 1ml caffeine (130mM in 156mM sodium benzoate and 460 mM sodium acetate), and 200 ml serum added. After mixing, these were allowed to stand at 37°C for 10 minute. 1 ml Fehling II (930 mM potassium sodium tartrate, 1.9 M sodium hydroxide) solution was mixed with it. A reagent blank was prepared as above without using sodium nitrite. After 5 minutes, absorbance was recorded against blank at 578 nm.

(b) Direct bilirubin

To 50 µl sodium nitrite solution, 200 µl sulfanilic acid, 2 ml isotonic saline and 200 µl of serum was added, mixed well and allowed to stand at 37°C for 5 minutes. A reagent blank was prepared as above using sodium nitrite. After 5 min, absorbance was recorded against blank at 546 nm.

2.4.2. Determination of serum aspartate aminotransferase (AST) Activity

AST was assayed using Agappe assay kits [9]. 100 µl serum was added to 1 ml of working reagent. The change in absorbance per 20 second during 1 minute was recorded against blank at 340 nm.

2.4.3. Determination of serum alanine aminotransferase (ALT) Activity

ALT was assayed using Agappe assay kits [9]. 100 µl of serum was added to 1 ml working reagent. After mixing tubes were incubated for 1 minute at 37°C. The change in absorbance per minute during 3 minute was recorded against blank at 340 nm.

2.4.4. Determination of serum alkaline phosphatase (ALP) Activity

Alkaline Phosphatase activity in serum was assayed using Agappe assay kits [10]. 20 µl serum was added to 1 ml of working reagent. The tubes were incubated for 1 minute at 37°C. The change in absorbance per minute during 3 minute was recorded against blank at 405 nm.

2.4.5. Superoxide dismutase (SOD) activity assay

The enzyme activity was assayed using Fortress assay kits by measuring the oxidation of NADH [11]. 0.2 ml ethanol-chloroform mixture (1 ml ethanol and 0.6 ml chloroform) was added to 0.5 ml hemolysate to remove hemoglobin. After shaking, 0.4 ml distilled water was added. It was mixed thoroughly and incubated for 15 minutes and centrifuged. The supernatant was used for determining superoxide dismutase activity. The assay system contained 0.8 ml 0.1 M triethanolamine-diethanolamine-HCl (TDB) buffer, pH 7.4; 0.04 ml 7.5 mM NADH, 0.025 ml (100 mM / 50 mM) EDTA – MnCl₂, pH 7.0 and 0.1 ml sample (or solvent for the control). After thorough mixing, the absorption was recorded at 340 nm, against air for a stable baseline recorded over a 5 minute period. Then 0.1ml 10 mM B-mercaptoethanol was added and the final volume in the cuvette was 1.065 ml. After mixing, the decrease in the absorbance was monitored for about 20 minutes to allow full expression of the chain length leading to NADH oxidation.

2.4.6. Statistical analysis

Data obtained was expressed as Mean ± Standard Deviation and analyzed using the SPSS package 19.0. One-way Analysis of Variance (ANOVA) was used. Values at P < 0.05 were regarded as statistically significant.

3. Results

3.1 Effect of uppercott pesticide on serum liver enzyme activities

Results obtained revealed that AST, ALT and ALP activities were all significantly (p<0.05) increased across all test groups compared to the control as seen in figure 1 below.

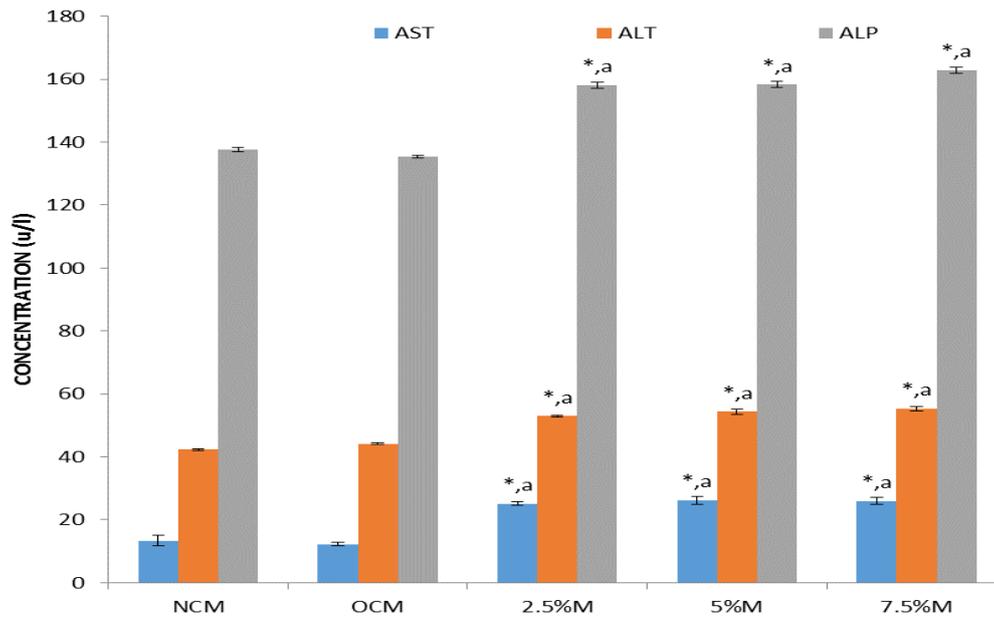


Fig. 1: Serumliver Enzymes Concentration in the Male Experimental Rats.

Values are expressed as mean \pm SD, n = 5. * = significantly different ($p < 0.05$) compared with normal control (NCM), ^ = significantly different ($p < 0.05$) compared with oil control (OCM)

3.2. Effect of uppercott pesticide on serum bilirubin concentrations

both the total and conjugated bilirubin levels across all test groups compared with the control.

Both total and conjugated bilirubin concentrations are shown in figure 2 below. There was no significant difference observed in

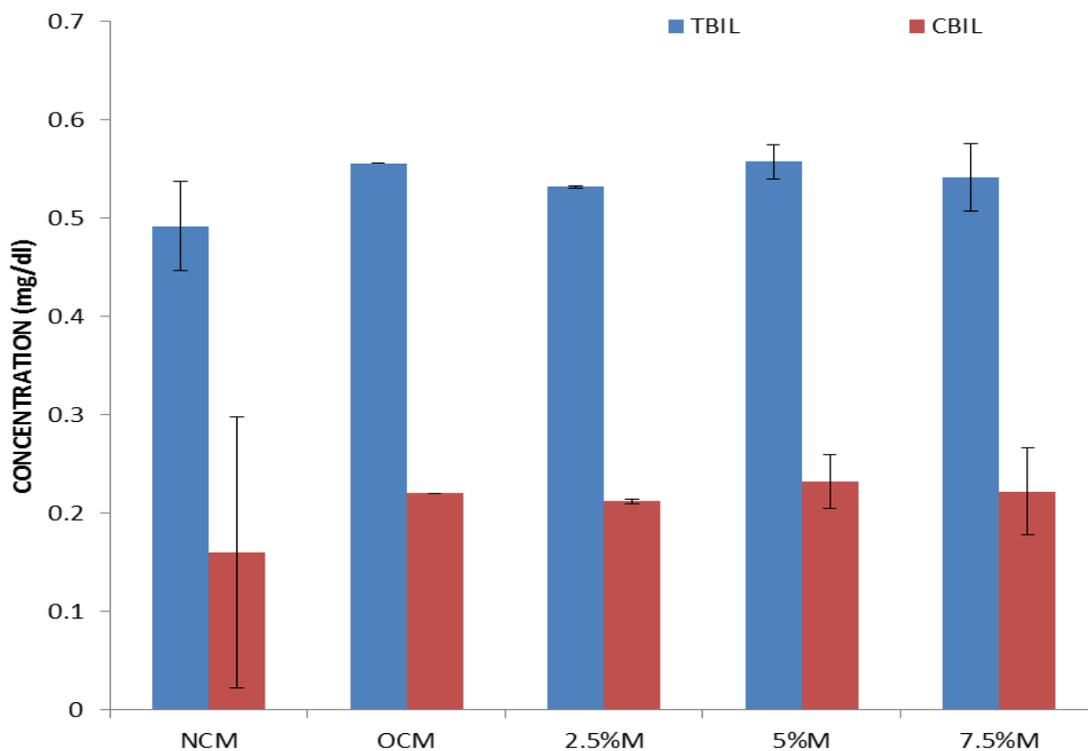


Fig. 2: Bilirubin Concentration the Male Experimental Rats.

Values are expressed as mean \pm SD, n = 5. $P > 0.05$

3.3. Effect of uppercott pesticide on serum superoxide dismutase activity

As shown in figure 3 below, serum SOD activities were significantly ($p < 0.05$) decreased when all other groups were compared with the normal control group and when all uppercott treated

groups were compared with the oil control in a dose-dependent manner. Also, comparison of groups 4 and 5 (5% and 7.5% uppercott) with group 3 (2.5% uppercott) revealed significant ($p < 0.05$) decreases in SOD activities.

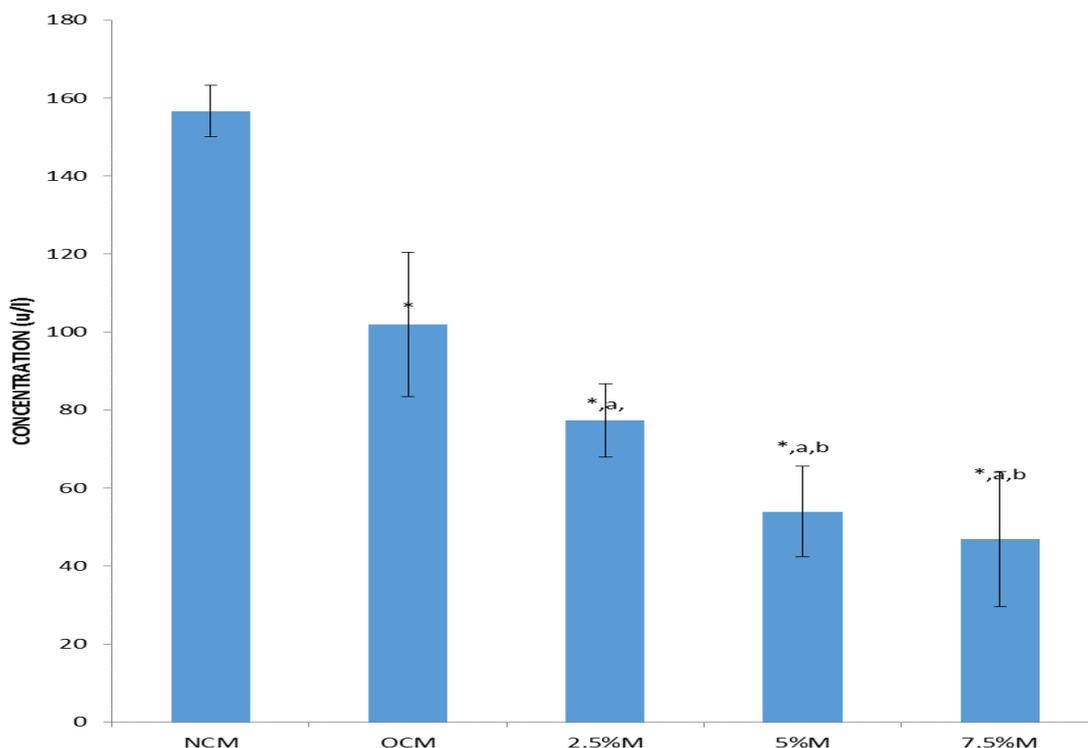


Fig. 3: Superoxide Dismutase Concentration in the Male Experimental Rats.

Values are expressed as mean \pm SD; n = 5; * = significantly different (p<0.05) compared with normal control (NCM); ^a = significantly different (p<0.05) compared with oil control (OCM); ^b = significantly different (p<0.05) compared with group 3 (2.5% uppercott).

3.4 Effect of uppercott pesticide on liver histology

Plates 1a1M-1a5M show the histological sections of the liver tissues. There was preserved histo-architecture of the liver cells having central veins and radially displaced hepatocytes in normal control (NCM). The central veins were slightly congested and the sinusoidal spaces were prominent. There were patchy necrotic sessions in the liver tissues of the oil control and other experimental groups (OCM, and 2.5%, 5% and 7.5%). These were shown in figure 4

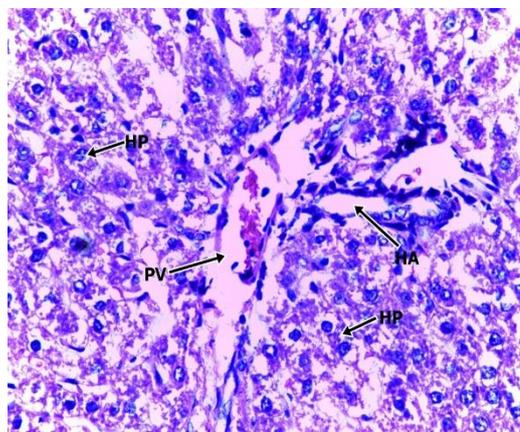


Plate1a1M: Photomicrograph of Normal Control (NC) Rat Liver. (Mag. X 400).

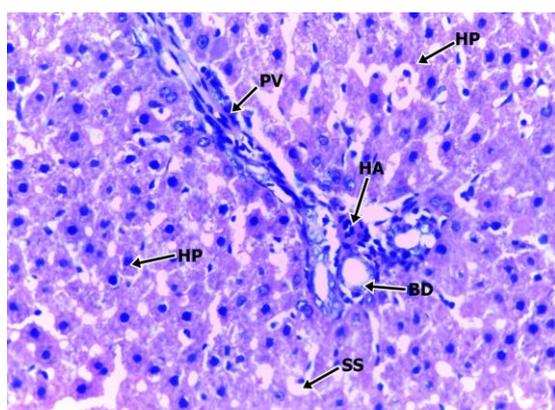


Plate1a2M: Photomicrograph of Oil Control (OCM) Rat Liver. (Mag X 400).

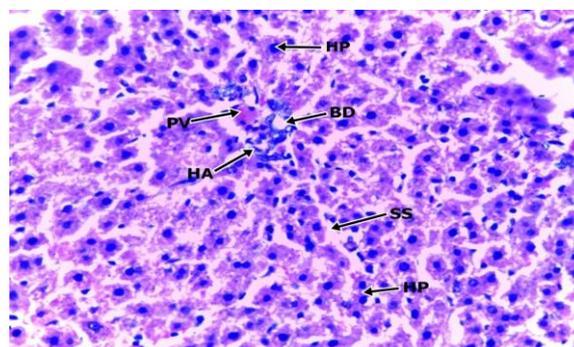


Plate1a3M: Photomicrograph of Rat Liver Exposed To 2.5% Uppercott Insecticide (Mag. X 400).

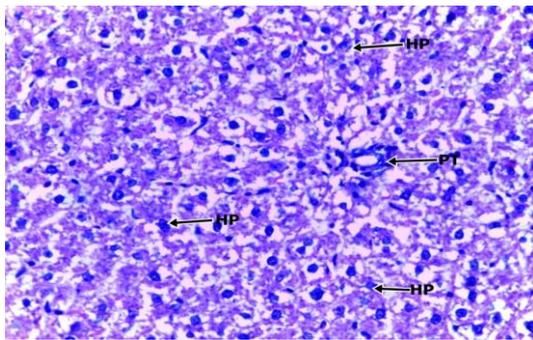


Plate 1a4m: Photomicrograph of Rats Liver Exposed To 5% Uppercott Insecticide (Mag. X 400).

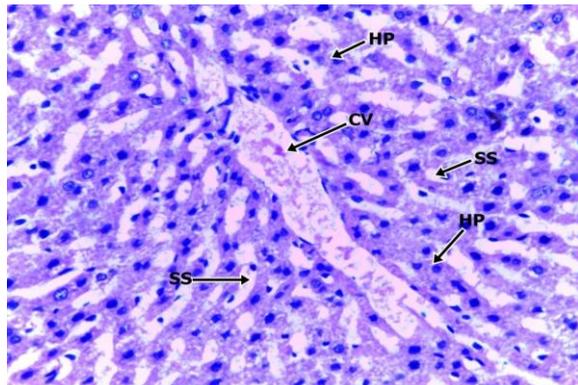


Plate 1a5M: Photomicrograph of Rat Liver Exposed To 7.5% Uppercott Insecticide (Mag. X 400).

HP- hepatocytes, SS- sinusoidal space, CV- central vein, HA- hepatic artery

Fig.4: Effect of Uppercott Pesticide on Histology of Liver Tissues in Experimental Rats.

4. Discussion

Most orally ingested xenobiotics and drugs are metabolized in the liver into toxic intermediates, making the liver a major target organ for xenobiotic and drug toxicity [12]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are transaminases that catalyse the transfer of amino groups for biochemical purposes. They are majorly found in the liver. Liver injury stemming from its exposure to toxic chemicals leads to elevated levels of these enzymes in the blood. Thus, the degree of liver damage relates directly to the level of AST and ALT in the blood [13] [14] [15]. Alkaline phosphatases (ALP) are enzymes involved in catalyzing the hydrolysis of organic phosphate at alkaline pH. They are very high in concentration in hepatobiliary tract [16]. Serum ALP activity may be elevated due to xenobiotic-induced hepatotoxicity [17].

In the present study, significantly elevated levels of AST, ALT and ALP in the test groups as compared with the control (Fig. 1.) suggests hepatotoxicity of uppercott pesticide leading to the compromise of liver integrity. This is supported by the results of the liver histology in this study. This also corroborates the findings from other studies on the hepatotoxic effects of toxic chemicals in experimental rats [18][19]. It was also observed that there was an insignificant rise in the levels of bilirubin in the test animals as compared with the controls. This might be attributed to prolonged exposure to pesticides which interrupted the normal red blood cell metabolism, affecting the hepatic dysfunction and increased the level of bilirubin in the blood thereby causing hyperbilirubinemia which might be due to production of excess bilirubin than the normal liver can excrete [20] [21] [22].

Most toxic chemicals are known to generate free radicals and other reactive oxygen species as a way of eliciting their toxicities. Superoxide dismutase (SOD) is an enzyme that inhibits the action of superoxide free radical which can cause oxidative stress. It catalyses the dismutation of superoxide radical to hydrogen peroxide which is then eliminated by another endogenous enzyme, catalase [23]. SOD can therefore function in primary defense and inhibits further production of free radicals. In the present study, exposure of experimental rats to different doses of uppercott pesticide resulted in significant decreases in SOD activities compared with the control (Fig. 3). This may be due to induction of oxidative stress by the pesticide since there is perturbation of antioxidant/oxidant biological status in favour of oxidant status in oxidative stress.

5. Conclusion

The present study revealed that Cypermethrin and dimethoate mixture (uppercott) exposure is a potential threat to normal functioning of the liver. Uppercott pesticide is therefore a potential health threat and hence, a need for caution in the agricultural and household usage of this chemical is greatly advised.

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