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Uppercott: Implication in nephrotoxicity in male albino wistar rats

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Abstract
The extensive use of pesticides in agriculture and for public health purposes, has led to drastic effects in many non-target species including man. In this study, the effect of cypermethrin and dimethoate mixture (uppercott) a popular agricultural pesticide on the kidney was investigated in male wistar albino rats. The rats were placed in five experimental groups with six (6) animals per group. Group 1 & 2 were taken as normal control and oil control respectively, given only standard feed and tap water, groups three (3) to five (5) were given 2.5%, 5% and 7.5% of uppercott LD_{50} for 28 days. Results indicated that higher doses of uppercot (5% and 7.5%) significantly ($p<0.05$) increased serum urea compared to their controls while creatinine concentration was highest in the 7.5% group compared to control and other groups. In serum electrolytes, 2.5% group significantly ($p<0.05$) retained Na^+ (sodium ion) compared to their controls (NCM and OCM), and also compared to 5% and 7.5% experimental groups respectively. Serum total proteins significantly ($p<0.05$) increased in 7.5% compared to controls and other test groups. The histology performed also helped confirm all the results as gotten from the biochemical assays. The study has led to the conclusion that uppercott pesticide may be a potential nephrotoxic agent.

Keywords: creatinine, electrolytes, nephrotoxic agent, uppercott pesticide, urea

Introduction
Uppercott (Cypermethrin (30g/l) and dimethoate (250g/l) mixture is known to be an agricultural pesticide. Generally, the use of pesticides has improved and increased agricultural production but has caused harmful effects to non-target organisms. Thus, this present study was aimed at investigating the effect of the combination of cypermethrin and dimethoate on kidneys of male wistar albino rats.

Cypermethrin is a neurotoxin [1, 2]. It acts by increasing the open time of sodium channels leading to prolonged membrane depolarization, enhanced neurotransmitter release and repetitive neuronal activity; eventually resulting in the depletion of the neurotransmitter and block of excitation in the nerve [3]. Poisoning is always due to accidental spillage during spray operations.

Literature on several herbicides such as propanil has been associated with toxicity in humans and other mammals [4]. The toxic substance greatly reported on propanil is the methemoglobinemia [4]. Reduction of the production of cytokines as well as the number of CD4+CD8+ thymocytes has been reported as a result of propanil exposure.

Deltamethrin (DLM) is another broad spectrum insecticide and has been reported to be associated with neurotoxicity, immunosuppression, allergy, hypertension, decreased testosterone levels, hepatotoxicity, and nephrotoxicity on exposure [5]. These effects of DLM are considered to be as a result of binding to a distinct receptor site on voltage-gated sodium channels and extending the open state by constraining the inactivation and deactivation of channels [6].

Several herbicides have been reported to cause nephrotoxic acute kidney injury on exposure. Their different pathophysiological mechanisms overlap to cause renal injury after ischaemia-reperfusion. Poisoning with pesticides contributes majorly to cases of nephrotoxic acute kidney injury in the African region.

Materials and Methods
Materials and apparatuses
Refrigerator (thermocool, Nigeria), homogenizer (Kinematica, England), spectrophotometer (6320D, Jenway), wooden cages, feeding troughs, weighing balance (AE 260, Mettler), cuvette, water bath (Gallenkamp, England), MSE clinical centrifuge, water bottles, non-heparinized samples, heparinized samples, syringes and needles, canula, test tubes, test tube racks, reagent kits, chloroform and deionized water.

Chemicals
All liver function tests were done with Agappe assay kits, Bilirubin assay was done with Span assay kits and Antioxidant assays were done with Fortress kits.

Procedure
Kidney function test
Urea
Principle

$$\text{Urea} + H_20 \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$2\text{NH} + \text{2-Ketoglutarate} + 2\text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + 2NAD^+ + 2H$$

Procedure

1. 1000µl of working reagent with 100µl of standard and sample was mixed together a test tube, standard and sample respectively
2. Spectrophotometer wavelength was set at 340nm, temperature to 37 ºC
3. Each test tube content was mixed and its optical density (T1) read 30 seconds after the sample and standard addition.
4. T2 was read exactly 60 seconds after the first reading

Creatinine

Principle

Creatinine reacts with picric acid to produce a colored compound, creatinine alkaline picrate. The change in absorbance is proportional to the creatinine concentration.

Procedure

1. 1000µl of working reagent with 100µl of standard and sample was mixed together a test tube, standard and sample respectively
2. Spectrophotometer wavelength was set at 492nm, temperature to 37 ºC
3. Each test tube content was mixed and its optical density (T1) read 60 seconds after the sample and standard addition.
4. T2 was read exactly 60 seconds after the first reading

Albumin

Principle

The reaction between albumin from serum or plasma and the dye bromocresol-green produces a change in colour that is proportional to the albumin concentration.

Procedure

1. 1000µl of working reagent with 10µl of standard, sample, and blank was mixed together a test tube, standard and sample respectively
2. It was mixed and incubated for 1 minute
3. Spectrophotometer wavelength was set at 630nm, temperature to 37 ºC and absorbance of standard and sample were measured against reagent blank.

Potassium

Principle

Na-Tetraphenylborate $+K^{+}$-------> K- Tetraphenylborate + Na^{+}

Potassium is estimated by Turbidometric method. The extent of turbidity is proportional to the potassium concentration and is measured photometrically at 578 nm (570-620 nm).

Procedure

1. 1000ml of potassium reagent with 25ml of standard, sample, and blank was mixed together a test tube, standard and sample respectively
2. It was mixed and incubated at room temperature for 25 minutes
3. Spectrophotometer wavelength was set at 578nm, temperature to 37 ºC and absorbance of standard and sample were measured against reagent blank within 10 minutes

Sodium

Principle

Sodium is estimated by colorimetric method based on modified Maruna and Trinders method. Sodium and proteins are precipitated together by Magnesium uranyl acetate as Uranyl magnesium sodium acetate salt. Excess of uranyl salt reacts with potassium ferrocynide to produce a brownish color. The intensity of the color is inversely proportional to the sodium concentration in the specimen and is measured photometrically at 530 nm (500-546 nm).

Uranyl ions + Mg ion + Na^{+}	Uranyl Mg Na Precipitate
Free Uranyl ions + K_4 Fe $(CN)_6$	Brown colored complex

Procedure

1. 1000µl of sodium R1 with 10µl of standard and sample was mixed together a test tube, standard and sample respectively
2. It was mixed and incubated at room temperature for 25 minutes
3. It was centrifuged at 2000 rpm and supernatant obtained and transferred for standard and test
4. 1000µl of sodium R2 was mixed with 20µl standard and test in separate test tubes
5. 1000µl of sodium R2 and 20µl sodium R1 was mixed in a separate test tube labelled blank
6. Contents of separate test tubes were mixed and allowed to stand for 5 minutes at room temperature
7. Spectrophotometer wavelength was set at 546nm
8. Absorbance of standard and test was measured against reagent blank.

Total Protein

Principle

Colorimetric determination of total protein based on the principle of the Biuret reaction (copper salt in an alkaline medium). Protein in plasma or serum sample forms a blue colored complex when treated with cupric ions in alkaline solution. The intensity of the blue color is proportional to the protein concentration.

Procedure

1. 1000µl of reagent was dispensed into 3 test tubes labelled blank, standard and test.
2. 20µl of standard and test was mixed in the respective labelled test tubes.
3. It was mixed and incubated at 37ºC for 10 minutes
4. Spectrophotometer wavelength was set at 546nm and absorbance of standard and sample were measured against reagent blank.

Chloride

Principle

In an acid medium chloride ions and mercury – II – thiocynate form thiocynate ions. These ions react with HNO3 and iron-III-ions and effect a red color. The intensity of the color is directly proportional to the concentration of chloride ions.

Procedure

1. 1000µl of reagent was dispensed into 3 test tubes labelled

blank, standard and test.
2. 10µl of standard and test was mixed in the respective labelled test tubes.
3. It was mixed and incubated for 1 minute.
4. Spectrophotometer wavelength was set at 505nm and absorbance of standard and sample were measured against reagent blank.

Procurement of Uppercott
Uppercott pesticide was gotten from Agro chemical company in Calabar.

Olive oil
Olive oil was used as the vehicle. It is widely accepted as a vehicle in toxicological studies because it does not induce toxicity and is easily absorbed into the body

LD_{50} determination
The LD_{50} was determined using the method of Lorke, 1983. This method has two phases of phases 1 and 2.
Phase 1: This phase requires nine (9) animals which are divided into three (3) groups each. Each group of animals are administered different doses (5, 10 and 20 mg/kg b.wt) of uppercott. The animals are placed under observation for 24 hours to monitor if mortality will occur.
Phase 2: This phase involves the use of three (3) animals, which are divided into three groups of one animal each. The animals were administered higher doses (20, 40 and 60 mg/kg b.wt) of uppercott and then observed for 24 hours for behavior as well as mortality.
The LD_{50} is then calculated by the formula;

$$LD_{50} = \sqrt{(D_0 \times D_{100})}.$$

Where D_0 = Highest dose that gave no mortality
D_{100} = Lowest dose that produced mortality.
The LD_{50} was determined to be 14.14 mg/kg b.wt.

Experimental animals
Thirty (30) 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. All animals were maintained under standard conditions and were given normal pellet diet *ad libitum*. All the animal experiments were carried out using the guidelines of the Institution's Animal Ethical Committee in accordance with the Principles of Laboratory Animal Care.
They were divided into five (5) groups of nine (9) rats each. The groups are shown below:

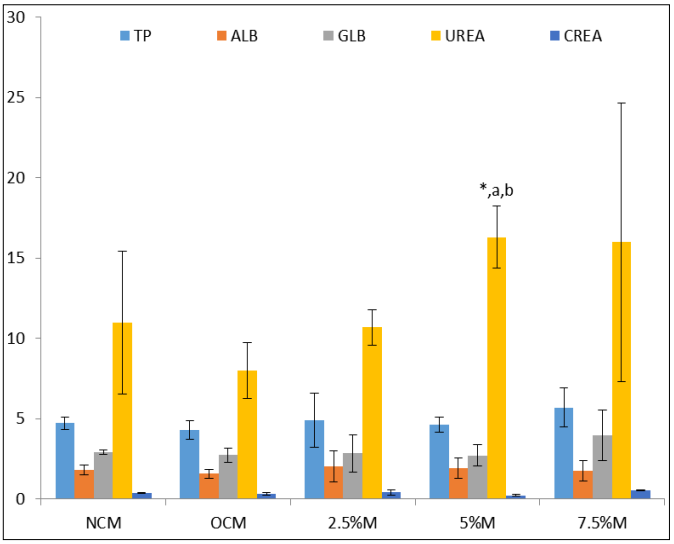
Table 1: Distribution of animals into experimental groups

Groups	Number of animals	Treatments
Group 1	6 males	Control (untreated)
Group 2	6 males	Control administered only oil
Group 3	6 males	2.5% (0.35 mg/kg b.wt) of LD_{50}
Group 4	6 males	5% (0.71 mg/kg b.wt) of LD_{50}
Group 5	6 males	7.5% (1.06 mg/kg b.wt) of LD_{50}

After one week of acclimatization, the rats in groups 3 to 5 were exposed to oral administration of uppercott at the different doses for 28 days. At the end of the exposure, 5 male rats were selected at random from each group and sacrificed and the various biochemical analysis were run to check for toxicity effects as compared to the control group. Blood samples were collected by cardiac puncture into plain screw-cap sample bottles for the total protein (Tp), globulin and albumin tests, and renal function tests. The blood samples collected were allowed to clot, and the serum extracted with Pasteur pipette after spinning with MSE model (England) table-top centrifuge at 2000 rpm for 5 minutes. The serum collected were used for biochemical analyses. All biochemical analyses were carried out within 24 hours of serum separation.

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 $P < 0.05$ was regarded as significant in comparison with appropriate controls.

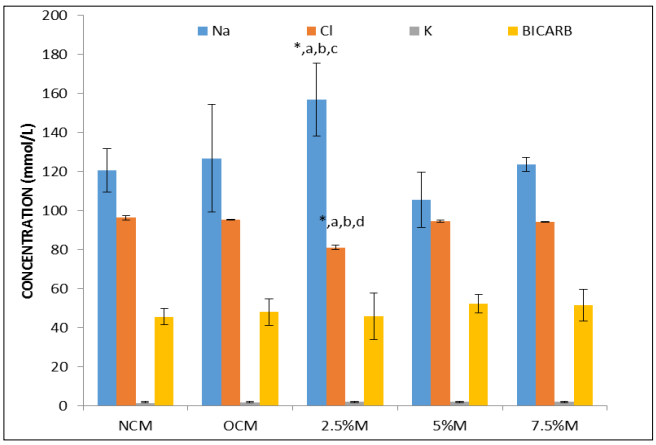
Results
Serum proteins, urea and creatinine concentration
These parameters are shown in figure 1. It was noticed that higher doses of uppercott (5% and 7.5%) significantly ($p<0.05$) increased serum urea compared to their controls while creatinine concentration was not significantly affected in all treatment and control groups.



Values are expressed as mean ± SD, n = 7-8
*p<0.05 vs NCm, [a]P<0.05VS OCM, [b]P<0.05 VS 2.5%M

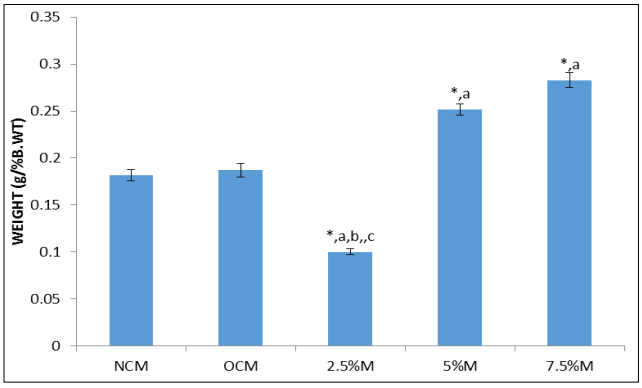
Fig 1: Serum Proteins Concentration, Urea and Creatinine (Males)

Changes in serum electrolytes
The changes in the serum electrolytes are as shown in figure 2. 2.5% treatment group significantly ($p<0.05$) retained Na^+ (sodium ion) compared to their controls (NCM and OCM), and also compared to 5% and 7.5% experimental groups respectively.



Values are expressed as mean ± SD, n = 7-8
*p<0.05 vs NCM, [a]P<0.05VS 2.5%OCM, [d]p<0.05vs 7.5%M.

Fig 2: Serum Electrolyte Concentration in the Experimental Rats (Males)



Values are expressed as mean ± SD, n = 7-8,*p<0.05 vs NCM, [a]P<0.05VS OCM, [b]P<0.05 VS 5%M and [C]p<0.05 vs 7.5%M.

Fig 3: Relative Weight of the Kidneys in the Experimental Animal (Males)

Histology of the kidney

Figure 4a$_{1M}$-8a$_{5M}$ shows the histology of the kidneys of male rats). In normal control groups (NCM) there were normal glomeruli and renal tubules. The glomeruli consist of a cellular mesangium composed of mesangial cells and interwoven arterioles surrounded by a bowman space with normal architecture. In oil control animals, there was a no distortion of the cyto-architecture. However, in the uppercott exposed animals, there was a little distortion of the glomeruli cells, with a higher effect in the 7.5% exposure.

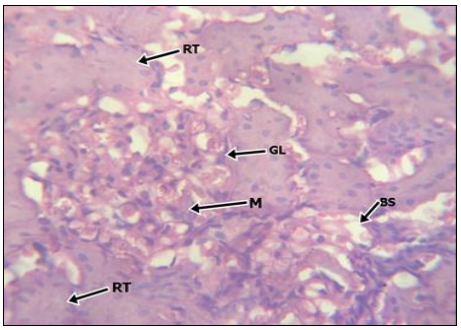


Fig 4a$_{1M}$: Photomicrograph of normal control (NC) rat kidney. (Mag. x 400)

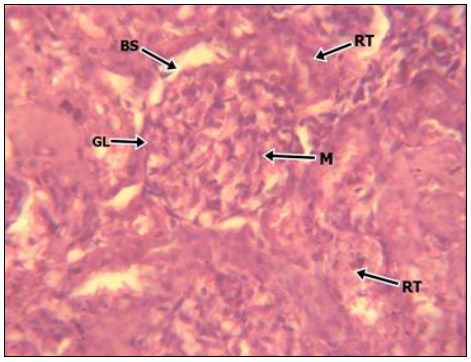


Fig 5a$_{2M}$: Photomicrograph of oil control (OCM) rat kidney. (Mag x 400)

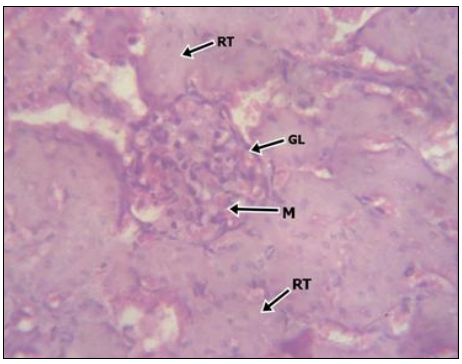


Fig 6a$_{3M}$: Photomicrograph of rat kidney exposed to 2.5% uppercott insecticide (Mag. x 400)

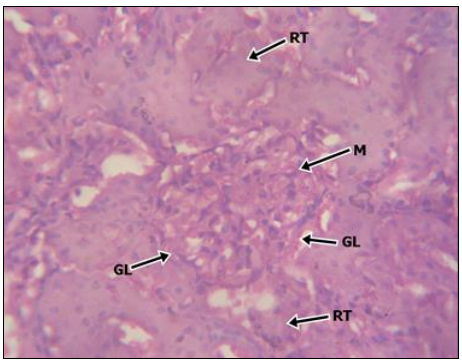
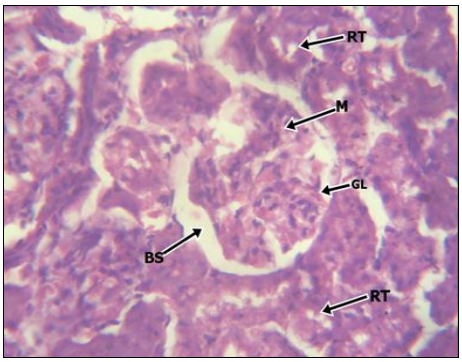


Fig 7a$_{4M}$: Photomicrograph of rats kidney exposed to 5% uppercott insecticide (Mag.x 400)



Key: GL- Glomerulus, M- Mesangium
RT- Renal Tubules, BS- Bowman Space

Fig 8a$_{5M}$: Photomicrograph of rat kidney exposed to 7.5% uppercott insecticide (Mag. x 400)

Discussion and Conclusion

These parameters are shown in figures 1, 2 and 3. It was noticed that higher doses of uppercott (5% and 7.5%) significantly ($p<0.05$) increased serum urea compared to their controls in both sexes of animals while creatinine concentration was very insignificant across all test groups though 7.5% groups were the most raised. This agrees with the findings of [7] where he observed that toxicity from exogenous substances like petrol and diesel could lead to elevated urea and creatinine levels. Serum total proteins were increased in 7.5% compared to controls and other test groups. This was contrary to reports by [8] where he discovered that "sheep exposed to cypermethrin a component of uppercott showed a significant reduction in serum total protein and albumin. The decrease in serum protein was chiefly because of decreased albumin and not the globulin fraction" [9]. Reported that "the reduction in plasma protein, particularly albumin, in animals treated with pesticides could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver". Additionally, having lower levels of protein in the blood was also reported to be chiefly because of massive loss through nephrosis [10]. Furthermore, decreased protein in the blood may be due to either reduction in protein synthesis or an increase in protein breakdown [11]. Also, the observed reduction in plasma proteins could be partly due to the detrimental effect of cypermethrin on hepatic cells.

As indicated in these studies, significant reduction in the levels of total protein, albumin and globulin concentrations were observed in rats treated with dimethoate compared to controls. Similar findings were reported in other studies as a result of oral administration of different doses of dimethoate [12, 13]. According to [14] and [15], "the reduction in serum protein could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver. Also, the protein level suppression may be due to loss of protein either by reduce in protein synthesis or increased proteolytic activity or degradation". Furthermore, the observed reduction in serum protein levels could be partly due to the detrimental effect of dimethoate on hepatocytes, evidenced by elevated activities of serum AST, ALT and ALP [16, 17] reported that "albumin levels are decreased in liver disease" [18]. Stated that "a decrease in globulin is expected as globulin (mostly ã-globulins) may be consumed in the production of antibodies in response to dimethoate administration". Uppercott pesticide has also been implicated in hematotoxicity and reproductive toxicity [19, 20].

Furthermore, the relative weight of the kidneys increased significantly with respect to the control. This could be as a result of vessel congestion and infiltration of the lymphocyte. This is in tandem with the work of [3].

In conclusion, exposure to uppercott pesticide has been implicated in possible nephrotoxicity therefore caution should be employed during usage.

Contributions of Authors

Modo Emmanuel U. – Carried out the work

Agiang, Margaret & Uboh Friday – Designed the work

Orji, Blessing; Okoro, Favour & Oplekwu Rowland – Managed the analyses of the study and wrote the first draft of the work

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