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Biochemical Effects of Pesticides on Serum and Urinological System of Rats

¹O.Y. Adeniran, ²M.A. Fafunso, ¹O. Adeyemi, ¹A.O. Lawal, ¹A. Ologundudu and ¹A.A. Omonkhua
¹Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria
²Department of Biochemistry, University of Ibadan, Ibadan, Nigeria

Abstract: Biochemical effects of sub-acute doses of luxan lindane and sherpa plus 280 pesticides on the blood and urine of albino rats were investigated. Single acute dose of 100 mg kg⁻¹ body weight of both pesticides were administered orally to rats. Blood samples from the rats were subjected to biochemical and haematological analyses while the urine was subjected to urinalysis. The pesticides were observed to elevate, not significantly, the levels of ALT and AST in the serum of rats relative to control (p<0.05). Significant reduction in the levels of plasma proteins, albumins, glucose and calcium were noticed in test rats relative to control (p<0.05). PCV, Hb, RBC and WBC concentrations were significantly lowered in rats treated with the pesticides relative to control (p<0.05). On the whole, it may be inferred that exposure to these pesticides may have severe health implications.

Key words: Luxan Lindane, sherpa plus 280, haemoglobin, packed cell volume, alanine transaminase, aspartate transaminase

INTRODUCTION

Chemical used for the control of insects, weeds, rodents and plants diseases are biologically active compounds^[1]. A wide variety of these chemical compounds generally known as pesticides is available and a clear understanding of the distinction between them is essential to proper management.

The goal of pesticide use is to apply products that will remain in the target area long enough to control the specific pest(s) and then degrade into harmless compounds in the soil, air or water without contaminating the environment. Unfortunately, pest control strategies, especially those relying heavily on pesticides have a detrimental impact on the environment.

Once applied, many pesticides are mobile in the environment. This movement can be beneficial if the pesticide is carried to a specific target area, like a plant's root zone or if it helps to ensure that degradation occurs at the proper time and place^[2]. When large quantities are applied especially by air craft, only 40 to 50% of the pesticide applied in this way lands in the target areas, less than one percent hitting the target pest themselves^[3]. Sometimes, however, non-targets insects, plants and other organisms come into contact with the pesticide. Although they remain in small quantities, their variety, toxicity and persistence are affecting biological systems in nature and eventually affect human health.

Lindane is an organochlorine insecticide and is the gamma isomer of hexachlorocyclohexane (HCH). Of the eight stereoisomers of HCH, only the gamma isomer, lindane is insecticidal^[4]. The uptake of lindane by rats or mice has been studied after oral administration. Lindane taken up from the intestine is transferred almost exclusively to the blood^[5]. Organophosphorus and carbamate pesticides are not stored in body fat^[6]. It is generally believed that asphyxiation is the ultimate cause of death in mammals poisoned by organophosphate and artificial respiration is known to enable the animal survive other fatal doses^[7].

However, effects of organochlorine and organophosphate pesticides on body fluids have only been sparsely studied. The paucity of biochemical investigation on such pesticides spurred the interest of the present study to delve into the biochemical effects of Luxan lindane (organochlorine pesticide) and sherpa plus 280 (organophosphate pesticide) on the blood and urine of albino rats.

MATERIALS AND METHODS

Time of experiment: The present study was carried out between February and October, 2001 in Nigeria.

Reagents and chemicals: Luxan Lindane 20% EC (organochlorine) and sherpa plus 280 (organophosphate)

pesticides were purchased from Chemical and Allied Products Limited (CAPL), Ring Road Ibadan, Nigeria.

Other chemicals and reagents used in this study were of analytical (Analar) grade and were products of British Drug House (BDH), Poole, England.

Experimental animals: Twenty six albino rats (male and female) weighing between 120-180 g were used for this study. The rats were purchased from the Pre-Clinical Animal House, University of Ibadan, Nigeria.

Animal treatment and pesticide administration: Group 1 and 4 contains five female animals each while Group 2 and 3 contains five male animals each. Group 5 (Control M) contain 3 male and Group 6 (Control F) contains 3 female animals.

A dose of 100 mg kg⁻¹ body weight of sherpa plus 280 and 100 mg kg⁻¹ body weight of Luxan lindane 20% EC were administered orally to the animals as follows:

Group 1: 100 mg kg⁻¹ BW sherpa-plus 280

Group 2: 100 mg kg⁻¹ BW sherpa-plus 280

Group 3: 100 mg kg⁻¹ BW Luxan Lindane 20% EC

Group 4: 100 mg kg⁻¹ BW Luxan Lindane 20% EC

Group 5: (Control M): Control male rats treated with no pesticide

Group 6: (Control F): Control female rats treated with no pesticide

(BW = Body Weight)

The administration was done by means of oral intubator. Animals were kept in plastic cages and fed with rat chow for four weeks after the administration.

Collection of biological samples: Urine samples were collected from the animals in a standard technoplast metabolic cage according to groups and these were kept at 4°C until needed for use.

At the end of feeding exercise, the animals were slightly anesthesized with chloroform in a desiccator. Blood samples were collected from each animal by cardiac puncture, using a syringe with needle. Blood samples were immediately transferred into anti-coagulant coated tubes as described thus:

- EDTA tubes for haematological test
- Fluoride-oxalate tubes for glucose estimation
- Heparinized tubes for chemical and biochemical parameters

Part of the blood samples were transferred into micro test tubes and allowed to clot. This was then centrifuged at 3000 rpm for 15 min and the serum was separated and stored at 4°C until required for use.

Haematological analysis: Haematological parameters such as Haemoglobin (HB), Packed Cell Volume (PCV),

Red Blood Cell (RBC) count, White Blood Cells (WBC) count and differential white blood cell count was determined using standard methods described by Canan^[8].

Chemical pathology: Blood glucose was determined by the glucose oxidase method described by Tinder^[9]. In this method glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. Peroxidase enzyme oxidizes the hydrogen peroxide to a chromogenic oxidation product, the intensity of which is measured at 515 nm. Total plasma albumins were determined using the dye binding method described by Doumas *et al.*^[10]. Here, bromocresol green at a pH below the isoelectric point of albumins reacts with albumins to produce a colour which is measured spectrophotometrically at 615 nm. The biuret method described by Gornall *et al.*^[11] was employed in the determination of total plasma protein. In this method the peptide linkage in the amino acid chain make up a protein molecule complexed with copper ions in an alkaline medium to produce a violet clour that is read spectrophotometrically at 540 nm. Plasma calcium was estimated using the method described by Tinder^[12]. Calcium was precipitated from plasma by nephthal hydroxamic acid. This then forms a red colour with ferric nitrate and measured spectrophotometrically at 450 nm. The method described by Gottfried^[13] was employed in the determination of total plasma triglyceride. Triglyceride is hydrolysed to glycerol which is oxidized to formaldehyde. Formaldehyde condenses with acetylacetone to form yellow dehydrolutidine derivatives which are then measured spectrophotometrically at 425 nm.

Enzyme assay: Serum ALT and AST activities were measured as described by Reitman and Frankel^[14]. Monitoring the concentration of pyruvate hydrazone (in the case of ALT) and oxaloacetate hydrazone (in the case of AST) formed with 2,4-dinitrophenyl hydrazine at 546 nm. ALP activity was determined by the method described by Bessey *et al.*^[15] modified by Wright *et al.*^[16]. Here, ALP hydrolyzed P-Nitrophenylphosphate (PNPP) to P-Nitrophenol, a yellow compound and phosphoric acid. The intensity of the yellow colour is measured spectrophotometrically at 400 nm.

Urinalysis: Urinalysis was done using the N-multistix paper strips; combi-screen 9 produced by Analytician Biotechnologies, AG Linchtenfels, Germany. One strip each was dipped into urine of the animals in each group. Presence or absence of selected biochemical indices was determined based on colour change on the paper strip. Some of the indices determined were pH, glucose, total protein, bilirubin, ketones and blood.

RESULTS AND DISCUSSION

Table 1 presents Hb concentration, RBC and Packed Cell Volume (PCV) between the female rats treated with 100 mg kg⁻¹ b.wt sherpa-plus 280 (Group 1) and the control female rats (Group 6) shared no significant difference ($p < 0.05$).

Conversely, Hb, RBC and PCV of male rats treated with 100 mg kg⁻¹ b.wt sherpa-plus 280 (Group 2) are significantly lower than those of the control male rats (Group 5). This observation suggests that the pesticide may exhibit sex selectivity in action. The reason for the sex dependent haematological effect of sherpa-plus 280 is not clear, but it could be suggested that male sex hormone potentiates the metabolites of the pesticide towards such effect on the blood. It has earlier been reported that organophosphorus pesticides causes low blood pressure, dizziness, *Inter alia* in albino rats^[6]. WBC count and leucocytes count showed significant differences between rats in Group 1 and 6 as well as rats in Group 2 and 5 ($p < 0.05$). This observation revealed that the pesticide actually elicit immune responses which is depicted by the secretion of phagocytic cells. Again, the effect is more significant on male rats than female rats relative to control ($p < 0.05$). Wharton *et al.*^[17] and Wyrobek *et al.*^[18] in separate studies reported changes in sperm morphology but no adverse effects on reproduction upon exposure to organophosphorus pesticides.

Luxan Lindane administration did not show sex selectivity in its effect on blood of albino rats, Hb, RBC, PCV count reduced significantly in treated rats relative to control rats ($p < 0.05$). In contrast, WBC and leucocyte count increased significantly in all the test rats relative to the control rats ($p < 0.05$). This observation suggested that Lindane may act by lysing red blood cells. In addition, the pesticide commands the secretion of cells that fights against foreign invaders confirming that Lindane is toxic to biological system. The present observation is supported by previous study^[4] that up take of Lindane by rats or mice are transferred from intestine almost exclusively to the blood. The reported LD₅₀ values for lindane given by different routes of administration are of the same order of magnitude in the various species of laboratory animals and no sex-dependent difference was seen^[19].

Table 2 shows plasma protein and albumins, reduced significantly in all the test animals relative to the control ($p < 0.05$). Both pesticides did not exhibit sex-dependent differences. This observation may be that these pesticides bind to free amino acids in the blood and transforming them to other biomolecules that are perhaps toxic to the system. This submission derived merit from the report by

Thomas^[20] that some pesticides react with serum albumin and binds blood free amino acids thereby altering protein metabolism. Alternatively, these pesticides may increase the rate of protein-excretion in the test rats. This suggestion is supported by previous study by Quaidie^[7] that exposure to organophosphorus pesticide caused polyuria and dyspepsia.

Conversely, plasma calcium and triglycerides are not significantly different in the entire groups relative to the control ($p < 0.05$). However, the result should be interpreted with caution as it does not mean that the pesticides have no effect on lipid metabolism. Lindane has been reported to concentrate in fatty tissues^[21].

Serum enzymes are useful tools in diagnosis as they pick any disturbances to the system early enough to allow for projection and possible remedies. In the light of this, selected serum enzymes were assayed for following oral administration of 100 mg kg⁻¹ b.wt of sherpa-plus 280 and Luxan Lindane (Table 3). Serum ALT and AST are aminotransferases usually employed in assessing the liver function^[22]. They are found in the cytoplasm and mitochondria of most cells. In the present study, serum ALT and AST of test rats are not significantly different from those of control ($p < 0.05$). This observation suggests that these pesticides may not transverse the hepatocyte permeability barrier into cytoplasm or mitochondria where the transaminases are localized within the period of experimentation. Although, not significant, serum ALT and AST are slightly higher in test rats than in the control rats. The results lent credence to study carried out by Westlake *et al.*^[23] who reported increased levels of those two enzymes in Japanese quails treated with organophosphate and carbamate pesticides. Sharma^[24] reported increased ALT and AST in the serum of fish treated with carbamate pesticides.

Moreover, the effects of Luxan Lindan and sherpa-plus 280 on serum ALP was also investigated (Table 3). ALP is a membrane bound enzyme which has found application in diagnosis of liver damage. Two abnormal levels of this enzyme is known, abnormally high level which usually suggests leakage from tissues and abnormally low level which may imply enzyme repression or inhibition. In the present study significantly high serum ALP was observed in all the test rats relative to the control ($p < 0.05$).

The observation revealed that the pesticides may have been metabolized to harmful metabolites which may have been responsible for leakage of the enzyme (ALP) into the serum. The enzyme activity in the test rats is about 3 folds than in the control. This observation conformed to the study carried out by Oloyede *et al.*^[25] who reported high serum ALP in serum of rats treated with chemically polluted water.

Table 1: Effect of sub-acute doses of luxan lindane and sherpa-plus 280 pesticides on haematological parameters of rats

Groups	Haemoglobin concentration (mg dL ⁻¹)	Red blood cell count (L ⁻¹)	White blood cell count (μL ⁻¹)	Packed cell volume (%)	Total and differential leucocytes count				
					WBC	Lymphocytes	Neutrophils	Eosinophils	Monocytes
1	8.13±1.29 ^a	4.28±0.95 ^a	1900±13.48 ^{**}	25.00±1.0 ^a	3450±12.5 ^{**}	1725±4.8 ^{**}	1621±6.25 ^{**}	69±1.69 ^{**}	4.5±1.25 ^{**}
2	6.75±2.47 ^{b*}	3.61±1.27 ^b	6537±17.23 ^{b**}	20.25±1.85 ^{b*}	6350±16.00 ^{b**}	3238±8.48 ^{b**}	3048±7.25 ^{b**}	63.5±1.20 ^{b**}	132±4.7 ^{b**}
3	5.47±2.92 ^{b*}	2.97±0.96 ^{b*}	4750±16.78 ^{b*}	18.33±2.64 ^{b*}	5150±14.20 ^{b*}	2626±11.5 ^{b*}	2369±4.26 ^{b*}	154.5±2.35 ^{b*}	170±4.71 ^{b*}
4	5.48±3.69 ^{a*}	3.41±1.63 ^a	4315±5.44 ^{a*}	19.25±2.35 ^a	4250±13.5 ^{a*}	2125±11.5 ^{a*}	2040±4.85 ^{a*}	42.5±2.31 ^{a*}	2.5±3.50 ^{a*}
Control M	8.40±0.85 ^b	4.53±0.22 ^b	6296±17.9 ^b	28.00±2.83 ^b	6050±14.2 ^b	3146±13.46 ^b	2722±9.33 ^b	60.5±1.21 ^b	121±4.21 ^b
Control F	8.25±1.63 ^a	4.25±1.03 ^a	4700±15.35 ^a	25.00±3.07 ^a	4950±12.4 ^a	2180±12.8 ^a	2326±6.36 ^a	148.5±2.89 ^a	49.5±4.45 ^a

Tabulated results are means of five determinations ± SEM. Values carrying a, a* and a** are significantly different and value carrying b, b* and b** are also significantly different (p<0.05)

Table 2: Effect of sub-acute doses of luxan lindane and sherpa-plus 280 pesticides on some biochemical indices in the plasma of rats

Groups	Total plasma protein (g/100 mL)	Total plasma albumins (g/100 mL)	Total plasma calcium (g/100 mL)	Plasma triglycerides (mg/100 mL)
1	6.90±0.01 ^{a*}	3.90±0.01 ^{a**}	4.19±0.01 ^a	80.00±7.44 ^a
2	6.63±0.07 ^{b**}	3.63±0.07 ^{b**}	6.25±0.08 ^{b**}	66.00±4.53 ^{b**}
3	6.65±0.07 ^{b*}	3.88±0.04 ^{b*}	4.08±0.02 ^{b*}	73.50±2.12 ^{b*}
4	6.95±0.19 ^{a*}	4.15±0.05 ^{a*}	4.46±0.06 ^{a*}	74.75±2.09 ^{a*}
Control M	7.20±0.04 ^b	4.15±0.01 ^b	5.54±0.04 ^b	85.50±4.95 ^b
Control F	7.20±0.01 ^a	3.85±0.07 ^a	4.18±0.05 ^a	75.00±0.01 ^a

Tabulated results are means of five determinations ± SEM. Values carrying a, a* and a** are significantly different, and values carrying b, b* and b** are also significantly different (p<0.05)

Table 3: Effect of sub-acute doses of luxan lindane and sherpa-plus 280 pesticides on specific activities (nmol/min/mg protein) of some serum enzymes of rats

Groups	Alanine transaminase	Aspartate transaminase	Alkaline phosphatase
1	39.67±1.53 ^a	31.00±1.00 ^a	121.33±3.71 ^{a*}
2	26.00±8.72 ^b	20.00±10.00 ^b	145.33±7.76 ^{b**}
3	46.00±8.49 ^b	38.50±9.19 ^b	121.50±0.71 ^{b*}
4	37.00±5.95 ^a	29.00±9.02 ^a	113.00±22.72 ^{a*}
Control M	39.00±4.24 ^b	34.50±9.19 ^b	39.00±9.90 ^b
Control F	36.00±5.66 ^a	27.00±1.41 ^a	36.00±15.56 ^a

Tabulated results are means of five determinations ± SEM. Values carrying a, a* and a** are significantly different and values carrying b, b* and b** are also significantly different (p<0.05)

Table 4: Effect of sub-acute doses of luxan lindane and sherpa-plus 280 pesticides on some biochemical indices in the urine of rats

Groups	Glucose	Bilirubin	Ketone	Blood	Protein	pH
1	-	-	-	-	+	8.5
2	-	+	-	-	+	8.0
3	-	-	-	-	+	8.5
4	-	-	-	-	+	8.0
Control M	-	-	-	-	-	8.0
Control F	-	-	-	-	-	8.0

- = Absent, + = Present

Urinalysis, a routine test in the evaluation of the state of the kidney revealed traces of protein in the urine of all test rats (Table 4). This may be an indication of kidney lesion as a result of administration of pesticides. This submission lent credence to previous study^[26] in which higher incidence of protein was found in the urine of pesticide treated rats. It is interesting that no glucose, ketone or blood cells were found in all the urine samples analyzed.

CONCLUSIONS

The present study has attempted to evaluate the toxicity of two newly introduced pesticides into the Nigeria market. Fall out from the study revealed that

sherpa-plus 280 may be exhibiting a sex-dependent difference in its haematological effect as it reduced Hb of male rats but not the female rats treated with 100 mg kg⁻¹ b.wt of it. The two pesticides also showed severe effect on ALP as demonstrated by the significantly high serum ALP of test rats relative to control (p<0.05). We therefore, enjoin the users of these pesticides to demonstrate great care during application and that crops should not be overexposed to them so that it will not pose any threats to public health.

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