

Responses of Male Albino Rats under Stress of Mixed Pyrethroids

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Abstract: The toxicity of mixed pyrethroids insecticides to mammalian animals has received much attention in recent years because animals exposed to these insecticides exhibited changes in their physiological, haematological and biochemical activities beside other pathological features. The study aimed to investigate the responses of male albino rats under stress of inhalational exposure to mixed pyrethroids on the haemogram, lipids, electrolytes, liver and kidney functional indices. Twenty male rats were maintained on standard healthy laboratory conditions and had free access to food and drinking water *ad libitum*. The rats were divided into four equal groups A-D. The group A represented the healthy control rats, while groups B, C and D were inhalationally exposed to mixed pyrethroids at doses of 30, 60 and 90 mg kg⁻¹ respectively for 2 weeks. At the end of the exposure, blood sample was collected for haematological studies while serum was used for biochemical analysis. There was significant reduction in PCV, MCV, MCH and MCHC compared to the healthy control group while total leucocytes counts was significantly elevated. There was also significant increase in the activities of ALT, AST as well as marked hypoproteinaemia, hypoalbuminaemia and azotaemia indicating liver and kidney dysfunction. However, the lipids and the electrolytes were equally perturbed. It was concluded that exposure to mixed pyrethroids predisposes the male rats to stress, altered haemogram with varying disturbances of various biochemical parameters resulting in death of rats exposed to highest dose of the mixed pyrethroids.

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INTRODUCTION

Pyrethroids are synthetic chemical analogues of pyrethrins which are naturally occurring insecticidal compounds produced in the flowers of *Cyrysanthemum cinerariae folum*^[1, 2].

Insecticides are the major sources of potential environmental hazards not only to birds, fish and other animals but also to humans when they become part of food chains^[3]. Long term exposure to these products causes countless abnormalities and reduces the life span of organisms^[4, 5].

Pyrethroids has been documented to present clinical manifestation such as ataxia, incoordination, licking of limbs, salivation and nasal discharges in male wistar rats as reported in our earlier study^[6], also it has been reported that pesticides when used in excessive dosage, it can become part of food chain, triggering series of hematological^[4, 7, 8] biochemical^[4, 10], reproductive^[9, 10], pathological changes^[11, 12] and lead to abnormalities in respiratory, nervous, immune and endocrine systems^[5].

Our earlier studies reported that exposure to pyrethroid via inhalation result in microcytic hypochromic anaemia, azotaemia and reduced transaminase activities when male wistar rats were exposed to graded doses of pyrethroids over a period time^[6].

Lipids and electrolytes are major components of biological membranes, they stabilized biological membranes and membrane bound enzymes. These electrolytes serve as cofactor for the enzymes and form a complex with the lipids. These lipids and electrolytes play a major role in cell metabolism and promote membrane integrity^[13]. Pyrethroids are considered as comparatively safe pesticides but their increased utility due to enhanced toxic potential and easy biodegradability necessitate non-target toxicity assessment.

Exposure to pyrethroid had already been reported to cause various haematological and biochemical alterations, this study is to unravel the lipids and electrolytes in addition to haematobiochemical changes that might be altered upon inhalation exposure to mixed pyrethroids in male wistar.

MATERIALS AND METHODS

Experimental animals: A total of twenty male albino rats were used for this investigation. The average weight of the rats was 152 ± 3.50 g. They were provided with laboratory animal feed (Fat/oil 6%, crude fibre 5%, calcium 1%, Available phosphorus 0.4%, Lysine 0.85%, Methionine 0.35%, Salt 0.3%, Crude protein 18%, Metabolis-able Energy 2900 Kcal kg^{-1} ,

manufactured by TOPFEEDS® (Lagos, Nigeria) and water provided. Experimental animals were acclimatized to their environment before the start of the experiment.

Animal ethics: All experimental protocols carried out on the animals were in accordance with the international accepted principles for laboratory animal use and were approved by the Ethics Committee (UIL/FVERC/001/2018) on Laboratory animal use of the Faculty of Veterinary Medicine, University of Ilorin, Nigeria.

Experimental chemical: The pyrethroid used for this investigation contains 0.05% imiprothrin, 0.05% prallethrin and 0.015% cyfluthrin (all synthetic pyrethrin) in a 300 mL pressurized liquid canister.

Chemical exposure: The animals were randomly divided into four Groups (A-D) of five animals per group. Group A was the control non-exposed group to the pyrethroid while Groups (B-D) were exposed to 30.00 mg kg^{-1} body weight, 60.00 mg kg^{-1} body weight and 90.00 mg kg^{-1} body weight of pyrethroid insecticide via. inhalation in separate desiccators. The duration of exposure was five minutes daily per group for 14 days.

Haematology: Aliquot of blood sample was collected into EDTA bottle for determination of packed cell volume, red blood cell and white blood cell count were determined as described by Reitman and Frankel^[17].

Serum preparation: Aliquot of blood samples were collected into plane tubes and centrifuged at 4000 rpm for 10 min to separate the sera from the cellular components. The sera were then decanted and stored in Eppendorf tubes for further analysis as described by Schalm *et al.*^[14].

Biochemical analysis: Total serum protein and albumin concentrations were estimated according to the methods by Tietz *et al.*^[15], respectively while blood urea nitrogen and creatinine concentrations were estimated as described by Henry^[16] using commercial kits (Randox®, Spain). The aspartate and alanine aminotransferases activities were assayed as described by Reitman and Frankel^[17]. Serum triglycerides^[18], phospholipids^[19], cholesterol^[20], HDL-cholesterol^[21], LDL-cholesterol^[21], total bilirubin^[22], Alkaline phosphatase^[23] were determined. Sodium, potassium, chloride and bicarbonate were analysed using Genius Electrolytes Analyzer Model: 200.

Statistical analysis: Results were expressed as mean±standard error of mean. Analysis of the data was done using one-way analysis of variance followed by the Duncan multiple range test. A $p<0.05$ was considered significant. All analyses were done using Graph Pad Prism Version 5.

Clinical observations: Exposure of rats to the pyrethroids produced varying degrees of clinical observations and these were in a dose dependent manner. There were both nasal and ocular discharges, chemotactic response to the source of the pyrethroids, incoordination and staggering, ruffled body coat, scratching of the nostril and the irritation of the eyes along with frequent mild watery stool.

These animals were docile throughout the period of exposure to the mixed pyrethroid. Mortality was observed in Group D exposed to highest concentration of the pyrethroids. The mortality occurred on seventh day of exposure within the desiccator and on the eleventh and twelfth day shortly after exposure when returned into the cage.

RESULTS AND DISCUSSION

Table 1 shows the effect of mixed pyrethroids on red blood cells, packed cell volume, haemoglobin concentration and red blood cells indices. There was no significant change in the red cells count between Group B and the control Group A; however there was significant decrease in the red cell count, packed cell volume and haemoglobin concentrations between the control group when compared with Groups C and D.

There was a significant decrease in the Mean Corpuscular Volume (MCV) in Groups C and D when compared with the control group A, same goes for the mean corpuscular haemoglobin concentration. Table 2

shows the effect of mixed pyrethroids on the white blood cells and differential white blood cells. There was a significant increase in total white blood cells and neutrophils of all the exposed groups compared to the control group while a significant decrease was observed in the lymphocytes counts most especially in Group D that was exposed to higher concentration of the mixed pyrethroid.

Table 3 shows the effects of mixed pyrethroids on some biochemical parameters in male rats. There was significant increase ($p<0.05$) in serum total protein and albumin of the exposed groups compared to the control group. The highest serum protein concentration was obtained in Group D.

There was significant increase in both the blood urea nitrogen and creatinine concentration of the exposed groups compared to the control group.

The ALT, AST, ALP and bilirubin showed significant increase in the exposed groups compared to the control most especially in Groups C and D.

Triglyceride, cholesterol and phospholipid concentrations significantly increased in the exposed group compared to the control group. The increase was more prominent in Group D. The HDL-cholesterol were significantly decreased while LDL-cholesterol were significantly increased compared to the control group. The sodium, potassium, chloride and bicarbonate were significantly higher compared to the control group.

A wide range of synthetic pyrethroids whether as a mono constituent or mixed constituents is in circulation to control insect pests, plant pathogens and weeds in both developed and developing countries. These pyrethroids when used on these pests equally gained entrance into the body systems of the applicators either through inhalation, dermally or through the food chain, hence there are needs to examine the blood and its constituents to ascertain some of the changes that might be induced as a result of exposure to these pyrethroids.

Table 1: Effect of mixed pyrethroids on red blood cells, packed cell volume, haemoglobin concentration and red blood cells indices

Parameters	Group A	Group B	Group C	Group D
RBC ($\times 10^{12}/L$)	8.38±0.30 ^a	9.74±1.31 ^a	7.14±1.19 ^b	6.81±1.17 ^b
PCV (%)	41.15±0.85 ^a	42.85±5.55 ^a	37.60±5.23 ^b	33.80±3.66 ^b
Hb (g dL ⁻¹)	14.95±0.15 ^a	13.25±2.25 ^a	15.50±2.21 ^a	16.40±1.61 ^b
MCV (fL)	51.85±2.05 ^a	50.50±2.26 ^a	48.10±1.74 ^b	42.47±2.19 ^b
MCH (Pg)	18.25±1.25 ^a	18.20±0.20 ^a	17.80±0.29 ^a	15.50±1.05 ^b
MCHC (g dL ⁻¹)	38.25±1.45 ^a	35.95±0.75 ^a	34.97±0.49 ^b	30.10±1.21 ^b

Values within the same rows with different superscripts are significantly different at $p<0.05$

Table 2: Effect of mixed pyrethroids on white blood cells and differentials

Parameters	Group A	Group B	Group C	Group D
TWBC ($\times 10^9 L^{-1}$)	7.35±2.11 ^a	13.55±2.70 ^b	10.77±2.70 ^b	10.01±2.40 ^b
Neutrophil (%)	6.11±2.01 ^a	9.50±4.50 ^b	19.33±6.17 ^b	22.01±4.10 ^b
Eosinophils (%)	1.22±0.50 ^a	2.55±0.30 ^a	2.01±0.32 ^a	2.52±0.13 ^a
Monocytes (%)	5.03±1.01 ^a	5.99±0.03 ^a	6.67±1.86 ^a	8.00±0.28 ^b
Lymphocytes (%)	86.50±4.50 ^a	82.00±6.00 ^a	70.67±8.17 ^b	68.67±6.96 ^b

Values within the same rows with different superscripts are significantly different at $p<0.05$

Table 3 : Effect of mixed pyrethroids on some biochemical parameters

Parameters	Group A	Group B	Group C	Group D
Total Protein (mg/dl)	6.80±0.10 ^a	7.30±0.80 ^a	8.80±1.00 ^b	10.70±0.80 ^b
Albumin (mg/dl)	4.14±0.04 ^a	3.78±0.09 ^b	3.62±0.14 ^b	3.94±0.02 ^b
BUN (mg/dl)	3.25±0.11 ^a	3.98±0.09 ^b	4.09±0.10 ^b	5.20±0.13 ^c
Creatinine (mg/dl)	1.53±0.01 ^a	1.67±0.02 ^b	1.72±0.02 ^b	1.76±0.03 ^b
ALT (UL ⁻¹)	34.60±0.80 ^a	38.00±1.80 ^a	46.80±2.20 ^b	52.40±2.60 ^b
AST (UL ⁻¹)	120.80±0.90 ^a	124.30±1.40 ^a	127.60±1.70 ^b	136.00±1.10 ^b
ALP (UL ⁻¹)	2.51±0.03 ^a	2.61±0.02 ^a	2.71±0.02 ^b	2.87±0.01 ^b
Bilirubin (mg/dl)	0.40±0.06 ^a	0.50±0.07 ^a	0.70±0.14 ^b	0.90±0.06 ^b
Triglyceride (mg/dl)	67.0±1.54 ^a	69.40±1.96 ^b	72.00±1.98 ^b	74.20±1.82 ^b
Cholesterol (mg/dl)	120.20±0.80 ^a	124.70±1.60 ^a	140.11±1.00 ^b	143.40±2.50 ^b
Phospholipid (mg/dl)	120.20±0.80 ^a	124.70±1.60 ^a	140.11±1.00 ^b	143.40±2.50 ^b
HDL-Cholesterol (mmol/L)	0.36±0.10 ^a	0.28±0.07 ^b	0.18±0.06 ^a	0.15±0.00
LDL-Cholesterol (mmol/L)	0.18±0.06 ^a	0.26±0.06 ^b	0.26±0.06 ^b	0.60±0.00 ^c
Sodium (mmol/L)	145.00±1.70 ^a	148.00±4.80 ^a	134.00±5.10 ^b	155.00±0.00 ^c
Chloride (mmol/L)	69.00±2.00 ^a	73.00±3.20 ^b	77.00±6.00 ^b	106.00±0.00 ^c
Potassium (mmol/L)	4.40±0.24 ^a	4.90±0.33 ^a	5.20±0.99 ^b	4.50±0.00 ^a
Bicarbonate (mmol/L)	18.20±0.73 ^a	19.40±0.60 ^a	22.80±1.65 ^b	20.00±0.00 ^b

Values within the same rows with different superscripts are significantly different at p<0.05

The available fluid that can be used as an important diagnostic tool to assess toxicity of any xenobiotic, including pyrethroid pesticides is blood. Almost every living tissue is exposed to this fluid for exchange of material. Blood is essential for survival, therefore, alteration of any component of blood can be assessed by evaluating the haemogram and serum biochemistry, provided the appropriate parameters are considered^[24].

The result of the study showed that anaemia was observed in the groups exposed to mixed pyrethroids. The anaemia is microcytic hypochromic which is non-regenerative. However in group B there was relative mild polycythemia which could be as a result splenic contraction pushing stored red cells into circulation. The relative polycythemia is not consistent in other groups except the prominent anaemia.

This result is very consistent with our earlier report Okediran *et al.*^[6] and others researchers Atamanalp and Yanik^[7], Fetoui *et al.*^[24]. It was reported that exposure to pyrethroids predisposes to series of haematological abnormalities as evidenced in reduced packed cell volume and other erythrocytes indices such as reduced mean corpuscular volume and mean corpuscular haemoglobin concentration. Further, pyrethroids are also known to cause haemolytic anaemia resulting in lysis of the red blood cell membrane, thereby enhancing lactate dehydrogenase levels in serum of pyrethroids intoxicated rats^[25-27].

There was a mild neutrophilia with concomitant lymphopenia. This neutrophilia and lymphopenia could be attributed to response to stress induced by the mixed pyrethroids exposure which could be mediated by the cortisol released by the adrenal cortex. This result could also indicate stimulation of the neutrophil cell line for it increases while the lymphocytes germinal cell line are deactivated or destroyed. The result is similar with other workers that exposure to toxicants or chemicals induces

wide alterations in the leucocytes responses^[28, 29]. Protein constitutes the building block and the basic molecule for any biochemical reaction.

They are intimately related with almost physiological processes which maintain a simple biochemical system in living condition. Exposure to pyrethroids produces varying alterations in the protein, albumin and globulin concentrations. Some reported a decline in protein concentration^[6, 30] while^[31-34] reported globulin concentration increases under stress caused by xenobiotic substances. Pyrethroids have a profound influence on proteins that play an important role in fluid homeostasis necessary for cellular activity. The toxicity of pyrethroids is possibly due to its stress-causing effect^[35]. Stress conditions cause release of adrenocorticotrophic hormone, triggering consequent secretion of cortisol by the adrenal cortex^[36] which reduces cellular protein stores, excepting in liver. Further, plasma proteins produced by liver become released into blood, raising the protein level.

Creatinine is a metabolite of creatine and is excreted completely in urine via glomerular filtration. An elevation of its level in the blood is thus an indication of impaired kidney function^[37]. Yousef *et al.*^[38] found an increase in serum creatinine in rats-treated with cypermethrin. We observed a mild to moderate azotaemia in the group exposed to the mixed pyrethroids. This is an indication that the pyrethroid has a nephrogenic effect because of its inability to excrete the urea and creatinine resulting in its accumulation in the serum. Okediran *et al.*^[6] Ahmad *et al.*^[39] and Ravel^[40] earlier reported azotaemia which is due to inability of the kidney to clear these nitrogenous wastes from the plasma as a result of kidney defect due to exposure to the pyrethroids.

Mixed pyrethroid toxicity caused elevated activity of enzymes AST, ALT, ALP and LDH along with those of other molecules in serum of pyrethroids intoxicated rats. ALT activity is related to general hepatocellular and AST to mitochondrial damage. Increased aminotransferase

(AST and ALT) activity in serum in this study is a reflection of hepatocellular damage under the influence of mixed pyrethroids stress, leading to leakage of these enzymes into general circulation. This is similar to other observations by several workers Manna *et al.*^[25] Bhushan *et al.*^[41] who reported increase activities of aminotransferases in rats exposed to different xenobiotics, since, liver is a prime organ associated with xenobiotic metabolism, production of metabolically toxic intermediates capable of causing hepatocellular damage may occur during metabolism of pyrethroids in liver, causing respective leakage of these enzymes in blood^[41].

Increase in Activity of serum alkaline Phosphatase (ALP) can be attributed initially to patho-physiological changes in the liver as a consequence of pyrethroid toxicity, due to damage in membrane permeability of hepatocytes, resulting in leakage of this enzyme into the blood stream, thereby altering normal hepatocellular architecture^[41]. Also, alkaline Phosphatase (ALP) is excreted through liver via bile juice; any obstruction in biliary tract (cholestasis) impedes its excretion. Cholestasis leads to regurgitation of enzymes due to obstruction of its passage through the bile duct. The back pressure results in leaching of the enzyme into blood, raising its concentration.

Bilirubin is formed from haemoglobin metabolism in the reticuloendothelial system, and then circulates binded to plasma albumin. Approximately 80% of circulating bilirubin is derived from red blood cells; the remaining 20% bilirubin is formed from ineffective erythropoiesis resulting from destruction of erythroid cells in bone marrow^[42]. Excessive destruction of red blood cells releases increased amounts of haemoglobin and then predisposes to hyperbilirubinemia. Water insoluble bilirubin attached to plasma albumin that is later taken up by hepatocytes requires dissociation of bilirubin-albumin complex for transport across the hepatocytes and binding to ligand in the hepatocytes. Pyrethroids-induced disruption of this phase of bilirubin along with disturbed membrane integrity of hepatocytes following pyrethroid toxicity might also affect transport and binding of bilirubin. Further, bilirubin within the hepatocytes is conjugated with glucuronic acid via the enzyme glucuronyl transferase. Inhibition of this enzyme by the mixed pyrethroid prevents conjugation reaction leading to accumulation of bilirubin and thus hyperbilirubinaemia ensued^[43].

Serum lipids include triglycerides, cholesterol and it lipoprotein fractions such as HDL and LDL-cholesterol, phospholipids and free fatty acids^[42]. The lipid constituents of lipoproteins are mostly triglycerides, free and esterified cholesterol and phospholipids. The non-polar triglycerides and cholesterol esters are usually present in the core of lipoproteins whereas polar

phospholipids along with apoproteins forms outer coat. However, the proportion of triglycerides, cholesterol and phospholipids differs in various lipoproteins. Lipoproteins are involved in the transportation of lipids in the body, e.g., Chylomicrons involved in the transport of dietary or exogenous triglycerides from intestine to liver; Very Low Density Lipoproteins (VLDL) involved in the transport of endogenous triglycerides from liver to extra hepatic tissues; Low Density Lipoproteins (LDL) is the major vehicle for the transport of cholesterol from liver to extra hepatic tissues while High Density Lipoproteins (HDL) is the major vehicle for the transport of cholesterol from extra hepatic tissues to the liver. Any increase in the level of these forms will lead to an increase of total lipid concentration in serum. Altered HDL-cholesterol and LDL-cholesterol was observed which could be due to alterations in the transport function of this cholesterol. It could be that since the transportation of this cholesterol is altered there is retention of cholesterol in the extra hepatic tissue bringing about reduction in HDL-cholesterol concentration and an increase in LDL-cholesterol thus enhancing deposition of cholesterol in the extra hepatic tissue. These alterations in the cholesterol metabolism and it lipoprotein fraction could predispose to atherosclerosis. One of the causes of increased total lipid concentration appears to be disturbance of carbohydrate metabolism, due to probable cytotoxic effect of pyrethroids on cells of the pancreas leading to relative deficiency of insulin^[44]. In such conditions, carbohydrates are not available to body tissues as insulin is not available to facilitate glucose transport in cell. In insulin deficiency, carbohydrates are not used to meet energy demands of body and most of the energy is derived from fats. The fat stored in adipose tissue is then hydrolysed and thus the amount of free fatty acids in blood is increased resulting in increased serum total lipid concentration^[45,46]. Lipids concentrations could also increase because xenobiotic substances could activate the sympathetic nervous system, resulting in release of epinephrine and norepinephrine by adrenal medulla^[47] which activates hormone-sensitive triglyceride lipase in tissue, resulting in hydrolysis of stored triglycerides from fat stores and mobilization of free fatty acids in the blood stream causing raised serum total lipid concentration^[45].

Phospholipids have both metabolic and structural function in mammals and are the main precursors of lipoproteins, the carriers for triglyceride transport^[48]. Pyrethroids have been found to decrease serum cholinesterase activity^[49] which is also responsible for enhanced serum phospholipid concentration.

The electrolytes were altered in the mixed pyrethroids exposure due to it retention in the plasma. There is tendency that the transports processes were inhibited possibly due to inhibition of ATPases that are involve in transport processes across the membrane.

Pyrethroids are potent neurotoxicants that interfere with nerve cell function by interacting with voltage-dependent sodium channel and chloride channel resulting in the repetitive firing of neurons and eventually causing paralysis^[50]. The lipids that were altered could also triggered the alteration of these electrolytes because the enzymes which are involve in the transport are protein in nature and once configuration is altered it result in malfunction of these electrolytes across the membrane and subsequent accumulation in the serum.

In conclusion, mixed pyrethroids have strong potential to disturb normal haemogram and blood biochemistry of mammals. In this study, all the considered parameters were significantly altered in the serum of albino rats following mixed pyrethroids exposure. This strongly indicates that proper precautions need to be taken in the use of these pyrethroids.

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