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Original Research Article

The Effects of Flauzifop-p-butyl on Behavioural Changes, Acetylcholinesterase, Serum Biochemical Parameters, and Haematological Indices in Albino Rats

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ABSTRACT

Fluazifop-p-butyl (FPB) is an herbicide that inhibits the enzyme acetyl-CoA carboxylase (ACCase), which is active in lipid biosynthesis in plants. This study evaluates the effects of FPB on the behaviour, brain acetylcholinesterase, biochemical parameters, and hematological indices in orally exposed rats. Thirty-two (32) male albino rats (*Rattus norvegicus*) (80 – 188 g) were randomly grouped into A to D. Group-D served as control, while animals in groups A, B, and C received FPB at 75 mg/kg, 37.5 mg/kg, and 18.75 mg/kg, respectively, for 14 days. FPB caused significant changes in behaviour, acetylcholinesterase, some biochemical parameters, and some hematological indices at $p < 0.05$. When compared to the control group, the treatment group exhibited a generally torpid state, a lower reactivity to touch, and decreased food consumption. These responses abated during the 7 days of withdrawal. It was found that the white blood cell (WBC) total count decreased in treatment groups compared to control. This decrease was duration- and dose -dependent even after the 7 days of withdrawal. Neutrophil count followed the pattern of WBC total count, while lymphocyte count increased significantly. Urea, creatinine, alanine transaminase, aspartate transaminase, alkaline phosphatase, acid phosphatase, and acetylcholinesterase significantly increased ($p < 0.05$), while total protein and glucose significantly decreased following the administration of FPB. Hepatosomatic indices were not affected in the FPB-treated groups. Overall, the results suggest that FPB modulates the behaviour, acetylcholinesterase, and haematological and biochemical parameters in rats.

Keywords: Fluazifop-p-butyl, Behaviour, Acetylcholinesterase, Haematology, Biochemical, Albino rat

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Introduction

The use of chemicals in weed management has risen dramatically, and it is now widely used even in local regions. There are varieties of chemicals used for weed control. Fluazifop-p-butyl is one of such chemicals. It was developed in the 1970s as a selective herbicide under the family aryloxyphenoxypropionates.[1] Initially, fluazifop-butyl was developed, with both the S- and R-enantiomers combined. The R-enantiomer was later shown to be responsible for herbicidal action. Although this study focused on fluazifop-P-butyl, the present commercial products, according to studies, contain fluazifop-butyl, fluazifop acid, and its primary metabolite and have also been taken into account where appropriate [Figure 1].[2]

Laboratory rats are routinely examined in a wide range of scientific studies, from ethological observations on the development and progression of various behavioural patterns to experimental investigations that entail manipulation of the animal or its surroundings.

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These laboratory studies include enquiries into motivation, the effects of drugs or specific treatments, and the animals' responses to stress. Thus, behaviour narrows down to the molecular and physiological activity in animals; in fact, animal behaviour serves as a connection between molecular, physiological, and ecological components of biology.[3] Behaviour serves as an interface between organisms and their surroundings, as well as between the nervous system and the ecology. The behaviour of animals regularly offers the first hints or early warning signs of environmental hazards and degradation.[4] Hence, its use for the determination of the ecotoxicity of fluazifop-p-butyl.

Fluazifop-p-butyl targets Acetyl-CoA carboxylase (ACCase) in plants. Acetyl-CoA is in direct relationship with acetylcholine (ACh) and acetylcholinesterase (AChE) in humans. Hence, there is the possibility that fluazifop-p-butyl may affect the actions of ACh and AChE. Acetylcholinesterase is the primary cholinesterase in many animals and in humans.[5,6] It is the enzyme that breaks down acetylcholine (a neurotransmitter) at the neuromuscular junction. AChE is thus a major enzyme in the neuromuscular physiology of humans and animals; hence, the physiology of AChE is linked to the behaviour of humans and animals.

It has been established by many studies that the activity of acetylcholinesterase is altered by many herbicides. There are two general types of AChE inhibitors: the cholinesterase inhibitors and the butyryl-cholinesterase inhibitors.[7] The class of herbicides known as organophosphates are examples of the cholinesterase inhibitors. The inhibition or increase of AChE activity by the aryloxyphenoxypropionate class of herbicides is still unclear. Hence, the current study seeks to evaluate the effect of an herbicide in that class (fluazifop-p-butyl) on the AChE activities. The resulting effect of fluazifop-p-butyl on the AChE activity is directly linked to behaviour because AChE has neurological and muscular functions. The purpose of this study is to investigate how fluazifop-p-butyl affects albino rats' behaviour, acetylcholinesterase levels, haematological indices, and biochemical parameters.

Materials and Methods

Experimental animal and test chemical
Thirty-two (32) adult male albino rats were purchased from the Animal Breeding and Genetics Unit, Department of Zoology and Environmental Biology (ZEB), University of Nigeria, Nsukka. They were kept in four separate cages, fed with Chikun feed (finisher), and were allowed to acclimatise for seven (7) days prior to the experiment. The Faculty of Biological Science, University of Nigeria Nsukka, Animal Care Committee (UNN-ACC-000302-2023) provided ethical approval, which was strictly adhered to.
Fluazifop-p-butyl was purchased as Fusilade Forte (active ingredient being fluazifop-p-butyl, 150 g/L) from Agromint, Ibadan, Nigeria.

Experimental design
Thirty-two (32) albino rats (weighing 80g to 188g) were randomly assigned to four groups (A, B, C, and D) and (n = 8) per group. D served as the control. Each group was separated into two (2) replicates (r = 2), each containing four (4) albino rats. Each group was maintained in its own cage and given free access to clean drinking water and a normal meal. Groups A, B, and C received oral doses of 75, 37.5, and 18.75 mg/kg, respectively, for 14 days, followed by a 7-day withdrawal period.[12]. Blood samples were obtained using retro-orbital puncture, and haematological and biochemical were parameters analysed in the research laboratory of the Department of Zoology and Environmental Biology (ZEB), UNN. The studied animals were sacrificed by cervical dislocation, and brain tissue was collected for an acetylcholinesterase enzyme study. Whole livers were also extracted to determine the hepatosomatic index.

Fluazifop-p-butyl administration formula
The Fluazifop-p-butyl administration was calculated using the following formula, as described by Erhirhie *et al*.[8]

Quantity administered (in µL) = $x/1000\ (kg)$ X y(mg/kg) X $1/(150\ X\ 1000\ (mg/L))$ X 10^6

Where, x = body weight and y = dosage (in mg/kg of body weight which are 75 mg/kg, 37.5 mg/kg, and 18.75 mg/kg respectively, for the three treatment groups)

Obtaining test samples
Collection of blood samples
Blood samples were collected by random prick at the tail vein of the animal and following the method as of Herdt and Hoff[9]. Following a retro-orbital puncture, a pasteur pipette was inserted to collect blood from the capillary at the medial canthus into a container containing EDTA anticoagulant and the blood was analysed for haematological parameters such as pack cell volume (PCV), red blood cell count (RBC count), white blood cell total count (tWBC), WBC differential count (neutrophils, lymphocytes, eosinophils, monocytes, and basophils), platelet count, haemoglobin level, and RBC indices. After collecting 5 ml of whole blood into a plain tube, it was allowed to clot for 15 to 30 minutes at room temperature without agitating it. The clot was centrifuged at 1,000-2,000 × g for 10 minutes in a chilled centrifuge. The resultant supernatant, serum, was used for the analyses of the biochemical parameters.

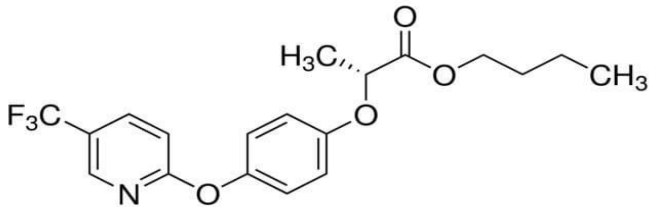

Figure 1: Active compound in Fusilade® Forte herbicide, Fluazifop-p-butyl, $C_{19}H_{20}F_3NO_4$

Collection of brain tissues
Ketamine and xylazine (50-100 mg/kg + 5-10 mg/kg) were safely combined and administered as a single intramuscular injection for anaesthesia. An incision was made on the head, and the entire brain was collected and transferred in an EDTA container devoid of anti-coagulants. Collected brain tissue was properly labelled according to groups and taken to the laboratory for analyses.

Tail suspension test
To perform this test, the rat was suspended upside down by the tail for 6 minutes in a single session, with the first 2 minutes serving as a habituation period (training) and the final 4 minutes measuring immobility.[10]

Observation for behavioural changes
The treatment and control groups' behavioural patterns and ethological changes were closely monitored, as previously described by Ezenwanne and Abuda[11]. The groups were interrupted at times to observe their responding behaviour. There was a feeding pattern detected. The rats' curling habit was examined by holding their tails for a few seconds. Responses to fluazifop-p-butyl chemicals and touch were also detected.

Determination of haematological parameters
The methods described by Dacie and Lewis[12] were accordingly used in the determination of pack cell volume (PCV), red blood cell count (RBC count), white blood cell total count (tWBC), WBC differential count (neutrophils, lymphocytes, eosinophils, monocytes and basophils), platelet count, haemoglobin level and RBC indices.

Biochemical analyses
Total protein, glucose, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and others were analysed using RANDOX® essay kits following the manufacturer's procedure. Brain AChE assay was carried out as described by Silva et al.[13]

Hepatosomatic index (HSI)
Hepatosomatic index was evaluated by measuring the body weight of the animal and obtaining the weight of the liver. Then was calculated with the equation.[14]

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HSI = X/Y where X = weight of the liver and Y= weight of the body.

Statistical analysis

The data was analysed using R version 4.2.0 (R Core Team, 2022). A generalized linear model was fitted accompanied by Analysis of Variance (ANOVA) with treatment dose and exposure duration as fixed factors and univariable haematological parameter as dependent variable. The ANOVA was followed by Tukey HSD *post-hoc* test and the adjusted p-value reported. Results are present as mean ± standard deviation (SD) and the 95% probability level considered the level of significance ($p \leq 0.05$ significant).

Results and Discussion

Behaviour

The behavioural responses are summarized in table 1 below. In the first week, there was no significant difference in the behaviour of the treatment groups and the control group. Some behavioural differences were observed in the second week. These behavioural changes include(s): stereotypic behaviour following the administration of the chemical, the treatment groups were generally calmer than the control, the treatment groups were less responsive to touch than the control groups. These responses were dose dependent and abated during the 7 days withdrawal. All the differences observed in the second week were also observed in the third week with additional behavioural differences amongst the treatment grouped which are explained as thus: When held at the tail, the treatment groups found it difficult curling up against gravity. Every morning, all the animals in the groups received an equal amount of grain (500 g) and water. In terms of left over food, group A had the highest quantity of food uneaten in 24 hours. This shows that group A consumed less quantity of food than others in 24 hours. This followed progressively with reduction in chemical administered. Group A consumed less than B which consumed less than C and group D (finishes all the food in the plate in 24 hours). During withdrawal (Week 4), the behaviour of the treatment groups returned to normal, with little of the seen changes in the second week exhibited.

Table 1: Some observed behavioural responses in rats administered fluazifop-p-butyl for 14 days

Behaviour	Dose(mg/kg)	Duration (Day)			
		1	7	14	7-d Withdrawal
Aggressive sniffing of mouth around the container after chemical administration	75.0	-	+++	+++	-
	37.5	-	+	++	-
	18.8	-	+	++	-
	0.0	-	-	-	-
Calmness	75.0	+	++	+++	++
	37.5	+	++	+++	+
	18.8	+	++	++	++
	0.0	+	+	+	+
Less Responsive to touch	75.0	-	+	++	+
	37.5	-	+	++	+
	18.8	-	+	+	-
	0.0	-	-	-	-
Difficulty in curling against gravity	75.0	-	-	++	+
	37.5	-	-	+	-
	18.8	-	-	+	-
	0.0	-	-	-	-
Feeding depression	75.0	-	-	+++	+
	37.5	-	-	++	-
	18.8	-	-	+	-
	0.0	-	-	-	-

Key: - = absent, + = mild response, ++ = moderate response, +++ = high response.

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Akin to feeding depression, the group that received the highest dose of FPB had the least appetite. When fed 250g of feed, they left more than half uneaten, resulting in a rating of +++. The least concentrate of FBP caused a minor decrease of appetite, resulting in a grade of +. This was also applied to other observed responses.

Haematological assays

As showed in Table 2, administration of fluazifop-p-butyl (FPB) affected some haematological parameters. It resulted in a decrease in packed cell volume (PCV). The decline in PCV was minimal, and was only significant on day-14 at 37.5 mg/kg FBP compared to the control. Haemoglobin concentration (Hb) of the rat was not impacted by 14-day administration of FPB. The Hb concentration was not different between the control and each of the treatment groups for the exposure duration. Similarly, the Red Blood Cell (RBC) remained at a similar level on days 7, 14 and 7-day withdrawal as it was on day 1. The treatment had no effect on RBC for the duration of administration and withdrawal. The platelet count decreased significantly on day-7 at 18.8 mg/kg FPB compared to the control, otherwise, there were no meaningful changes in platelet counts. Platelet counts were similar between the treatment groups and the controls for the administration duration except on day 7 as mentioned above. There were reductions in white blood cells (WBC) in rats administered FPB. On day 1, WBC was similar between the groups, but on day 7, day 14, and even on 7-day withdrawal, WBC decreased significantly in those exposed to FPB ($p < 0.05$). The decline in WBC was duration and concentration-dependent, being most pronounced on day-14 at the highest FPB dose, and on 7-day withdrawal at 75.0 and 37.5 mg/kg FPB.

Table 2: Changes in haematological parameters in rats administered fluazifop-p-butyl for 14 days

	Dose	Duration (day)			
Parameter	(mg/kg)	1	7	14	7-d withdrawal
PCV (%)	75.0	36.67 ± 1.53^{a1}	37.33 ± 6.43^{a1}	43.67 ± 3.21^{ab1}	40.67 ± 2.52^{a1}
	37.5	39.00 ± 1.73^{a1}	38.00 ± 2.65^{a1}	39.33 ± 2.08^{b1}	38.67 ± 1.53^{a1}
	18.8	39.67 ± 0.58^{a1}	37.67 ± 2.08^{a1}	40.67 ± 2.52^{ab1}	42.00 ± 3.61^{a1}
	0.0	36.67 ± 0.58^{a1}	43.00 ± 2.65^{a12}	48.33 ± 1.53^{a2}	42.00 ± 2.65^{a12}
Hb (g/dL)	75.0	12.17 ± 0.50^{a1}	13.70 ± 0.36^{a1}	14.20 ± 1.01^{a1}	13.47 ± 0.80^{a1}
	37.5	12.30 ± 0.69^{a1}	12.33 ± 0.85^{a1}	12.70 ± 0.53^{a1}	13.03 ± 0.25^{a1}
	18.8	13.17 ± 0.15^{a1}	12.70 ± 0.66^{a1}	13.37 ± 0.83^{a1}	13.73 ± 1.17^{a1}
	0.0	13.83 ± 4.07^{a1}	14.27 ± 0.87^{a1}	16.13 ± 0.51^{a1}	14.23 ± 0.86^{a1}
RBC (x 10^{12}/L)	75.0	7.77 ± 0.64^{a1}	7.36 ± 0.15^{a1}	7.29 ± 0.26^{a1}	7.47 ± 0.31^{a1}
	37.5	8.49 ± 0.60^{a1}	7.19 ± 0.22^{a1}	7.63 ± 0.36^{a1}	8.00 ± 0.27^{a1}
	18.8	8.07 ± 0.82^{a1}	7.38 ± 0.26^{a1}	7.78 ± 0.30^{a1}	7.54 ± 0.35^{a1}
	0.0	8.17 ± 0.51^{a1}	7.98 ± 0.23^{a1}	8.06 ± 0.31^{a1}	7.79 ± 0.14^{a1}
WBC (x 10^{9}/L)	75.0	3.85 ± 0.39^{a3}	2.35 ± 0.29^{b2}	1.53 ± 0.09^{c1}	2.49 ± 0.20^{c2}
	37.5	3.89 ± 0.39^{a2}	2.19 ± 0.17^{b1}	2.33 ± 0.11^{b1}	2.45 ± 0.29^{c1}
	18.8	3.50 ± 0.26^{a2}	2.40 ± 0.09^{b1}	2.41 ± 0.09^{b1}	3.18 ± 0.16^{b2}
	0.0	3.82 ± 0.08^{a1}	3.87 ± 0.14^{a1}	3.88 ± 0.13^{a1}	4.09 ± 0.09^{a1}
Platelet (x 10^{9}/L)	75.0	780 ± 65^{a1}	815 ± 28^{ab1}	891 ± 42^{a1}	889 ± 17^{a1}
	37.5	690 ± 79^{a1}	912 ± 91^{a23}	880 ± 41^{a2}	771 ± 41^{a12}
	18.8	730 ± 69^{a1}	734 ± 17^{b1}	778 ± 44^{a1}	846 ± 17^{a1}
	0.0	733 ± 54^{a1}	900 ± 10^{a2}	892 ± 23^{a2}	798 ± 14^{a12}

Significant differences were observed between mean ± SD values with different alphabet superscripts along a column for each parameter and values with different numeric superscripts across a row ($p < 0.05$).

The white blood cell differential following exposure of rats to FPB is shown in Table 3. The neutrophil count followed a similar pattern as WBC. Neutrophil counts decreased significantly in rats exposed to FPB on days 7 and 14, and on 7-day withdrawal. The decrease in neutrophil counts was concentration and duration dependent. Neutrophil counts appeared to return to control level following the 7-day withdrawal. Lymphocyte counts increased in those exposed to FPB. The increase in lymphocyte counts at all three doses of FPB was significant on days 7 and 14 ($p < 0.05$). Neutrophil counts returned to a similar level as the control in rats administered FPB. FPB administration in rats had no obvious effect on monocyte and eosinophil counts. However, the day-1 eosinophil count was not similar across the groups. Basophil was not detected. As shown in Figure 2, the mean corpuscular volume (MCV) was not affected by the administration of FPB to rats. MCV was similar across the groups for the duration of administration and withdrawal. Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were similarly not affected by the administration of FPB to rats.

Biochemical assays

As shown in Table 4, Total protein (TP) on day 1 was not different among the four groups. On day 7 and day 14, TP was reduced significantly in rat administered FPB. On 7-day withdrawal TP in 18.8 mg/kg FPB returned to the level as the control group, unlike the 37.5 and 75.0 mg/kg which remained significantly different from the control. The glucose concentration in rat-administered FPB decreased significantly on days 7 and 14 but returned to similar level as the control on 7-day withdrawal. Urea concentration increased on administration FPB to rat. Urea increased significantly on day 7 and day 14 of administration of FPB. The increase in urea remained significantly high on 7-day withdrawal in groups previously administered FPB. Similarly, creatinine increased significantly on 7 and 14 days of administration of FPB. However, the effects of FPB on creatinine was abrogated on 7-day withdrawal.

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As showed in Figure 3, Aspartate transaminase (AST) activity increased significantly in rat administered FPB. There was a significant increase of AST in all three FPB concentrations on days 7 and 14. On 7-day withdrawal, all AST activity remained higher in all groups previously administered FPB than the control, but only the 75.0 mg/kg remained significantly so. Alanine transaminase (ALT) activities in rat-administered FPB increased significantly on day-7 and day-14.

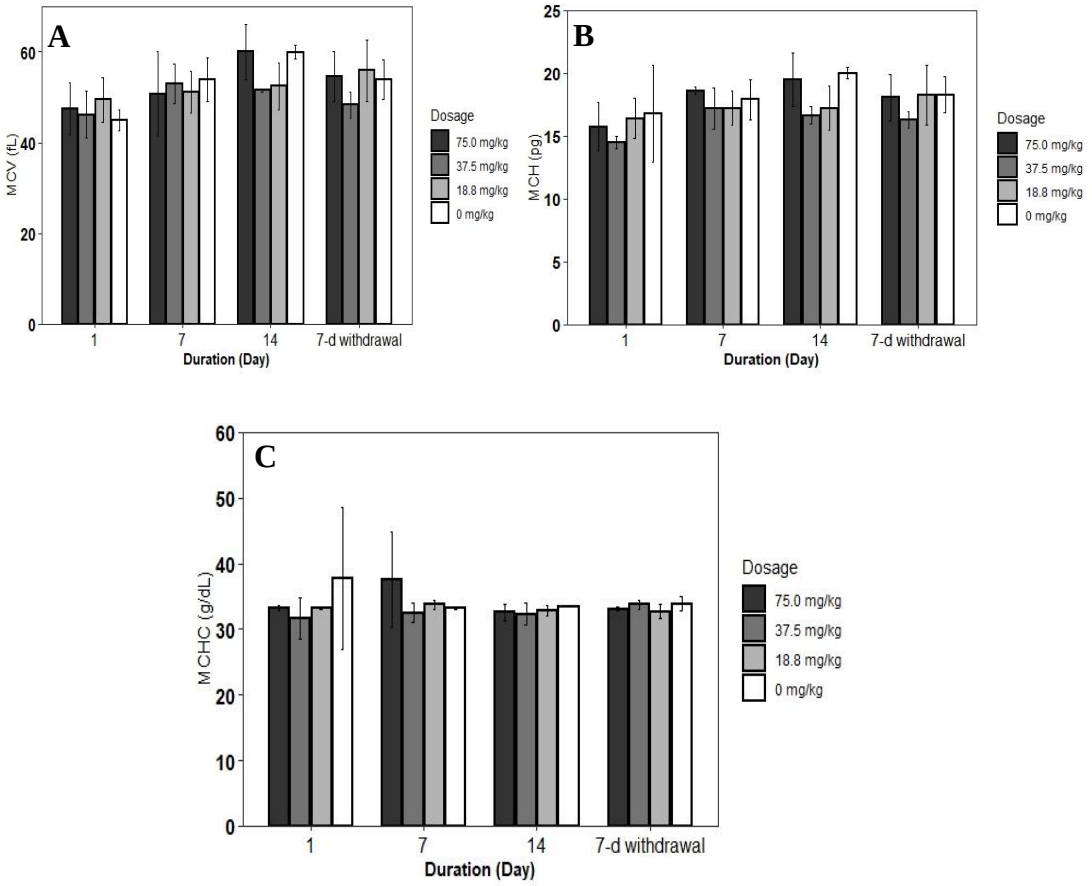

Figure 2: **A**: Mean Corpuscular Volume (MCV); **B**: Mean Corpuscular Haemoglobin (MCH), AND **C**: Mean Corpuscular Haemoglobin Concentration (MCHC) in rats administered fluazifop-p-butyl for 14 Days.

However, the ALT activity returned to the same level as the control on 7-day withdrawal of FPB. Alkaline phosphatase (ALP) activity increased at all three FPB concentrations on day 7 and day 14. On 7-day withdrawal, ALP activities at 37.5 mg/kg and 75.0 mg/kg remained significantly higher than control. The Acid phosphatase (ACP) activity increased in rat administered FPB. In rats administered three concentrations of FPB, ACP increased significantly on day 7 and day 14. On 7-day withdrawal, the 75.0 mg/kg FPB group remained significantly high compared to the control. Acetylcholinesterase activity was affected by exposure to rat to the herbicide. Acetylcholinesterase activity increased significantly at all doses of the herbicide used on day 7 and day 17 ($p < 0.0001$). However, the activity of acetylcholinesterase returned to a similar level as the control on the 7-day withdrawal of the rat from the herbicide.

The hepatosomatic index is presented in Figure 2. It was not affected by exposure of the rat to FPB at the dosage used. The study showed that fluazifop-p-butyl had adverse effects on the behaviour, acetylcholinesterase activities, haematological indices, and biochemical parameters. It is significant to take into account that certain toxicological research on fluazifop-p-butyl indicates that the substance may be less harmful.[15] In rats, the acute lethal dosage 50 (LD_{50}) of fluazifop-p-butyl is 4096 mg/kg when administered orally.[16] This implies that the substance is not very harmful. However, recent studies on fluazifop-p-butyl's toxicity have stated otherwise. Azgin and Goksu reported that the fluazifop-p-butyl had 24-hour *in vitro* acute lethal concentration 50 (LC_{50}) of 1.94 ± 0.02 mg/L in larvae.[16] This suggests that even a small amount of fluazifop-p-butyl in the aquatic environment may have a large toxicological effect. Furthermore, fluazifop-p-butyl has been shown by Ore and Olayinka[17] to cause oxidative stress, nephrotoxicity, and hepatotoxicity in rats.[17] There were notable adverse changes in the behavioural pattern of the test groups which were very obvious in the third week. Aggressive sniffing of the mouth around the cage container after administration of the chemical observed in the second and third week is by the report given by Tu *et al.* [18] According to their report, fluazifop-p-butyl causes skin irritation and may cause eye damage in rats and rabbits. The treatment groups were generally calmer than the control and were less responsive to touch than the control groups.

Acetylcholinesterase acts at the neuromuscular junction. Tail suspension test was done to see if there was any observable muscular weakness in the animals and clearly, there was. Thus, when held at the tail, the treatment groups found it difficult to curl up against gravity. This observation may have occurred due to the same effect inducing calmer behaviour. Reduced food intake was observed in the third week.

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Table 3: White blood cell differential (%) in rats administered fluazifop-p-butyl for 14 Days

	Dose (mg/kg)	Duration (day)			
		1	7	14	7-d withdrawal
Neutrophils (%)	75.0	18.67 ± 2.08^{a3}	10.67 ± 1.53^{b2}	4.33 ± 2.08^{c1}	16.00 ± 2.65^{b23}
	37.5	18.67 ± 1.53^{a3}	10.67 ± 2.08^{b2}	7.33 ± 2.08^{c2}	16.67 ± 2.08^{b3}
	18.8	19.00 ± 1.00^{a1}	13.17 ± 2.08^{b1}	14.00 ± 2.65^{b1}	16.67 ± 1.15^{b1}
	0.0	18.67 ± 1.53^{a1}	26.33 ± 1.53^{a2}	23.67 ± 2.08^{a12}	25.33 ± 3.06^{a2}
Lymphocytes (%)	75.0	76.00 ± 1.73^{a1}	87.67 ± 1.53^{a2}	94.33 ± 2.89^{a2}	76.67 ± 2.52^{a1}
	37.5	74.00 ± 3.61^{a1}	83.67 ± 1.53^{a12}	91.00 ± 5.29^{a2}	77.33 ± 4.04^{a1}
	18.8	76.67 ± 2.31^{a1}	81.67 ± 3.21^{a1}	86.00 ± 2.65^{a1}	77.33 ± 5.69^{a1}
	0.0	74.67 ± 2.52^{a1}	67.33 ± 1.53^{b1}	72.33 ± 2.08^{b1}	68.33 ± 4.73^{a1}
Monocytes (%)	75.0	2.33 ± 2.08^{a1}	0.00 ± 0.00^{a1}	1.00 ± 1.73^{a1}	4.67 ± 1.15^{a1}
	37.5	2.33 ± 2.08^{a1}	1.33 ± 2.31^{a1}	1.67 ± 1.53^{a1}	2.33 ± 2.08^{a1}
	18.8	0.00 ± 0.00^{a1}	2.67 ± 0.58^{a1}	0.00 ± 0.00^{a1}	3.33 ± 3.06^{a1}
	0.0	1.33 ± 1.15^{a1}	2.00 ± 1.73^{a1}	1.67 ± 0.58^{a1}	4.67 ± 1.53^{a1}
Eosinophil (%)	75.0	3.33 ± 0.58^{ab1}	1.67 ± 1.53^{a1}	1.33 ± 1.15^{a1}	3.00 ± 1.00^{a1}
	37.5	6.67 ± 0.58^{a2}	2.33 ± 0.58^{a1}	1.67 ± 1.53^{a1}	3.33 ± 1.53^{a12}
	18.8	2.67 ± 0.58^{b1}	2.33 ± 2.08^{a1}	0.00 ± 0.00^{a1}	4.67 ± 2.52^{a1}
	0.0	5.67 ± 0.58^{ab1}	5.33 ± 1.15^{a1}	2.67 ± 1.15^{a1}	3.00 ± 0.00^{a1}

Significant differences were observed between mean ± SD values with different alphabet superscripts along a column for each parameter and values with different numeric superscripts across a row ($p < 0.05$).

Table 4: Biochemical parameters in rats after 14-day administration and 7-day withdrawal from Flauzifop-p-butyl

Parameter	Conc.	Duration (day)			
	(mg/kg)	1	7	14	7-d withdrawal
TP (mg/dL)	75.0	6.15 ± 0.26^{a2}	4.09 ± 0.16^{b1}	3.84 ± 0.72^{b1}	5.32 ± 0.26^{b2}
	37.5	5.81 ± 0.44^{a2}	4.09 ± 0.18^{b1}	3.09 ± 0.19^{b1}	5.31 ± 0.27^{b2}
	18.8	6.18 ± 0.05^{a2}	4.67 ± 0.58^{b1}	4.55 ± 0.11^{b1}	6.40 ± 0.16^{a2}
	0.0	6.18 ± 0.72^{a1}	7.00 ± 0.57^{a1}	6.23 ± 0.30^{a1}	6.55 ± 0.49^{a1}
Glucose (mg/dL)	75.0	63.67 ± 3.51^{a2}	53.00 ± 4.58^{b1}	54.67 ± 1.53^{b1}	68.33 ± 3.51^{a2}
	37.5	61.67 ± 2.08^{a2}	52.00 ± 2.00^{b1}	45.00 ± 6.24^{b1}	71.33 ± 1.53^{a2}
	18.8	74.67 ± 11.37^{a2}	51.67 ± 0.58^{b1}	44.00 ± 2.00^{b1}	70.00 ± 2.65^{a2}
	0.0	68.67 ± 17.01^{a12}	78.67 ± 3.51^{a12}	84.67 ± 1.53^{a2}	66.67 ± 3.06^{a1}
Urea (mg/dL)	75.0	40.00 ± 2.00^{a1}	73.33 ± 1.53^{a3}	91.00 ± 2.65^{a4}	53.00 ± 4.58^{a2}
	38.5	44.33 ± 1.53^{a1}	69.33 ± 4.16^{a3}	83.00 ± 3.61^{ab4}	50.67 ± 3.06^{a2}
	18.8	41.33 ± 1.53^{a1}	66.00 ± 2.00^{a2}	73.00 ± 2.65^{b2}	57.00 ± 2.65^{a2}
	0.0	42.00 ± 5.57^{a1}	44.67 ± 4.73^{b1}	42.67 ± 3.06^{c1}	41.67 ± 2.08^{b1}
Creatinine	75.0	4.74 ± 0.17^{a1}	6.34 ± 0.13^{a2}	7.15 ± 0.21^{a2}	5.33 ± 0.26^{a2}
(mg/dL)	38.5	4.60 ± 0.33^{a1}	6.04 ± 0.22^{a2}	6.16 ± 0.16^{a2}	5.66 ± 0.59^{a2}
	18.8	4.08 ± 0.16^{a1}	5.60 ± 0.51^{a2}	6.42 ± 0.38^{a2}	4.26 ± 0.06^{a1}
	0.0	4.11 ± 0.15^{a1}	3.11 ± 0.17^{b1}	4.14 ± 0.13^{b1}	4.38 ± 0.52^{a1}

Significant differences were observed between mean ± SD values with different alphabet superscripts along a column for each parameter and values with different numeric superscripts across a row ($p < 0.05$).

This is in accordance with the early evaluation of flauzifop-p-butyl, reduction in food intake could therefore be a result of interference with the normal physiology of digestion in the animals. Haematological indicators are parameters that are associated with blood and organs that make blood.[19] The evaluation of haematological parameters such as the amount and form of erythrocytes, leucocytes, thrombocytes, and haemoglobin level/pack cell volume is very important in toxicology and monitoring science.[18] Etim *et al.*[19] wrote that blood serves as a pathological indicator of an animal's exposure to toxins and other environmental factors. Blood components vary according to an animal's physiological state. As a result, the haematological indices reflect the majority of elements that influence the body's physiology. Any variation from the global normal haematological ranges in animals indicates a specific physiological abnormality or a risk to physiological health.

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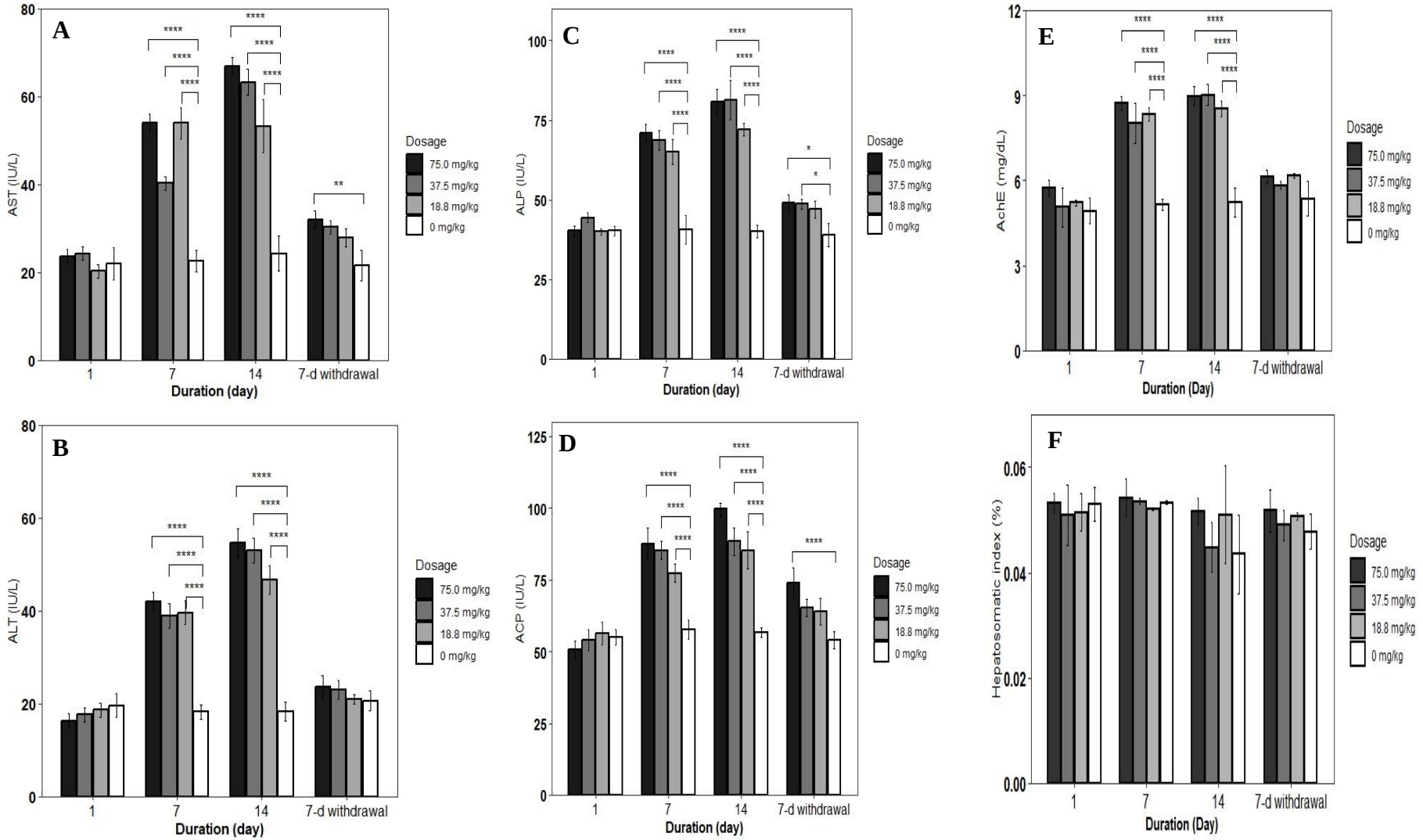

Figure 3:**A:** Aspartate transaminase (AST), **B:** Alanine transaminase (ALT), **C**: Alkaline phosphatase (ALP), D: Acid phosphatase (ACP),and **E**: AchE activities and the **F:** Hepatosomatic index (HSI) in rat after 14-day administered and 7-day withdrawal from Flauzifop-p-butyl.

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The significance of haematological parameters in toxicology research cannot be overstated. The pack cell volume (PCV) gives useful information about red blood cells (RBCs). A lower level of PCV indicates RBC loss, cell death, and/or bone marrow abnormalities. A higher PCV value often indicates dehydration and an increase in RBC production. Hb (haemoglobin concentration) tests are used to diagnose anaemia in both people and animals. Lower haemoglobin levels indicate fewer RBCs in the blood, which leads to inefficient oxygen circulation throughout the body. A low Hb level indicates several types of anaemia, thalassemia, iron deficiency, liver damage, cancer, and other conditions. Lung damage, heart disease, and other factors can all contribute to elevated Hb levels. White blood cells (WBCs) are widely recognised as crucial for safeguarding the body from infections and damage. The body releases white blood cells in response to an infection or inflammatory condition to aid in fighting against it. A higher range of WBCs indicates allergic responses, inflammatory conditions and infections such as bacteria, viruses, fungi, or parasites. Low WBC counts are a sign of autoimmune conditions, bone marrow damage, exposure to toxins, radiation, deficiency of vitamin B-12 and so on.[20,21] Blood platelets (otherwise known as the thrombocytes), the blood clothing cells, are very important in the diagnosis of health problems associated with the circulatory systems. The lower number of platelets could point to exposure to chemicals, certain types of cancer, kidney infection, or dysfunction.[22] It is therefore crucial to properly investigate the haematological indices of rats exposed to fluazifop-p-butyl. The administration of fluazifop-p-butyl (FPB) affected some of the haematological parameters. It resulted in a decrease in packed cell volume (PCV). The decline in PCV was only significant on day 14 at 37.5 mg/kg FBP compared to the control. Haemoglobin concentration (Hb) of the rat was not affected by 14-day exposure to FPB. The Hb values were not different between the control and each of the treatment groups for the exposure duration. Similarly, the RBC remained at a similar level on days 7, 14, and 7-day withdrawal as it was on day 1. The treatment had no effect on RBC for the duration of exposure and withdrawal. This result suggests a case of haemolysis of RBC due to sample collection or storage or a change in fluid-constituents balance in the blood. The decrease in PCV accompanied by no effects on Hb and RBC shows a false result of PCV in terms of the effect on the oxygen-carrying capacity, morphology, and the function of the red blood cells. Haemolysis of RBC due to sample collection or storage (*in vitro* hemolysis) could lead to a decreased PCV value.[23] In this case, measured haemoglobin is the most accurate indicator of the animal's oxygen carrying capacity, and the PCV value can be calculated by multiplying the haemoglobin by three. The platelet count decreased significantly only on day-7 at 18.8 mg/kg FPB compared to the control. This is not enough to infer a toxic effect on the platelet, failing to produce a consistent and/or dose-dependent effect. Thrombocytopenia occurs when the bone marrow fails to produce enough platelets, which can happen for a variety of reasons. Bone marrow can stop producing platelets due to many factors such as toxic chemicals, chemotherapy, and inadequate diet. Furthermore, acute thrombocytopenia can be linked to substances that cause aplastic anaemia or bone marrow disorders, in which blood cell synthesis is significantly reduced.[24-27] More research is needed to determine the effects of FPB on platelet production and distribution in the circulation.

A very obvious reduction in WBC occurred in rats exposed to FPB. WBC was similar between the groups on day 1, but on day-7, day-14 and even on 7-day withdrawal, WBC decreased significantly in those administered FPB ($p < 0.05$). The decline in WBC was duration and concentration-dependent, being most pronounced on day-14 at the highest FPB dose, and on 7-day withdrawal at 75.0 and 37.5 mg/kg FPB. A low WBC count means the body's inability to fight an infection or foreign agents. Fluazifop-p-butyl causing low WBC count might cause adverse effects on the entire immune system. Neutrophil count followed a similar pattern as WBC. Lymphocyte counts increased in those exposed to FPB. There was no obvious effect on monocyte and eosinophil counts. Basophil was not detected. Neutrophils are known to act as the immune system's first line of defense. Lymphocytes are much slower than neutrophils in action. Hence, a low value of the neutrophil-to-lymphocyte ratio (NLR) during the 14 days of exposure is a suggested case of defeat to the defence input by the immune systems. The RBC indices such as the MCV, MCH, and MCHC were not affected by exposure of the rat to FPB.

Biochemical parameters were affected due to the administration of FPB. On day 7 and day 14, total protein (TP) and glucose concentration reduced significantly while aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) activities as well as urea concentration increased significantly in rat administered FPB. Increase in the activities of some biochemical enzymes indicates the effect of the chemical on the liver of exposed rats. According to Mumoli *et al*.[28], a liver damage can be indicated by a rise in either ALT level more than three times of upper limit of normal (ULN), when ALP (alkaline phosphatase) level is more than twice ULN, or when the total bilirubin level is more than twice ULN when associated with an increased ALT or ALP. This is in agreement with Uchendu *et al*.[29,30]. Mayo who supported the assertion that increase in ALP, AST, or ALP activities may indicate liver damage. It may therefore be said that the administration of FPB may be hepatotoxic. Acetylcholinesterase activity was affected by exposure to rats to the herbicide. Acetylcholinesterase activity increased significantly at all doses of the herbicide used on day 7 and day 17 ($p < 0.0001$). The activity of acetylcholinesterase was seen to have returned to a similar level as the control on 7-day withdrawal. This agrees with the recent studies by many scholars on herbicides and pesticide chemicals. An increased AChE activity suggests an increase in the total capacity for hydrolyzing acetylcholine in the brains of the rats.

Conclusion

It may be concluded from the research findings that fluazifop-p-butyl, an aryloxyphenoxypropionate herbicide has some adverse effect on the behaviour of albino rats. The chemical also has the potential to increase acetylcholinesterase activities in the brain as well as some important serum enzymes, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) activities were all increased due to administration of FPB. On the haematological indices, it may be said that fluazifop-p-butyl has immune-depressing potential. This is because a decrease in total white blood cell count which was dose and duration dependent was obtained. A similar result was obtained for the neutrophil count. Red blood cell-related counts such as pack cell volume, haemoglobin concentration, RBC count, and RBC indices (MCV, MCH, and MCHC) were not affected by exposure to fluazifop-p-butyl.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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