DOI: https://dx.doi.org/10.4314/gjpas.v31i3.7

GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 31, 2025: 509-518
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EFFECTS OF PYRETHROID INSECTICIDE (BALLISTIC) ON SERUM LIPID PROFILE AND SOME SEX HORMONES LEVEL OF ADULT FEMALE ALBINO RATS

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(Received 14 February 2025; Revision Accepted 28 March 2025)

ABSTRACT

Ballistic is a pyrethroid insecticide containing deltamethrin and aromatic hydrocarbons as active ingredients. Twenty-four (24) female albino rats were divided into three groups of 8 animals. Rats of the control group I were fed normal rat feed and water, while rats of the treatment groups II and III were administered 0.5ml/kg body weight of ballistic insecticide from the stock solution of 0.1ml (low dose) and 0.15ml (high dose) respectively in addition to being fed normal rat feed and water *ad libitum* for 21 days. At the end of the experiment, animals were euthanized and blood samples collected for analyses. Results of this study showed a significant body weight reduction from 146.1±10.93g to 135.3±4.82g in group II and 172.9±6.42g to 160.3±1.45g in group III, whereas the control group recorded a significant body weight increase. This study recorded a significant ($p=0.001$; $p<0.05$) increase in the lipid profile of the two treatment groups. The respective values for group I, II and III were: 1.9±0.051mmol/L, 2.1±0.041mmol/L and 2.3±0.041mol/L for total cholesterol, 0.63±0.011mmol/L, 0.68±0.007mmol/L and 0.69±0.007mmol/L for triglyceride, 0.5±0.037mmol/L, 0.8±0.041mmol/L and 0.8±0.058mmol/L for low density lipoprotein, 0.28±0.008mmol/L, 0.31±0.004mmol/L and 0.34±0.004mmol/L for very low density lipoprotein. There was however a decrease in the high density lipoprotein of the treated rats (1.22±0.037mmol/L, 1.20±0.108mmol/L and 1.05±0.065mmol/L). Follicle stimulating hormones decreased significantly in the treatment groups while luteinizing hormones levels were significantly increased. This study has revealed that ballistic is cytotoxic and deleterious to the physical traits and some biochemical parameters of female albino rats.

KEYWORDS*:* Pyrethroid insecticide, ballistic, female albino rats, lipid profile, biochemical parameters, physical traits.

INTRODUCTION

Environmental pollution seems to be the most acute problem faced by humans in recent time. Majority of the problems posed by environmental pollutants are largely attributed to pesticides production and use. Pesticides are unique pollutants because they are deliberately released into the surroundings to cause toxicity in targeted pest species (Alaa-Edden *et al.,* 2019). Unfortunately, its inability to select its target organisms often leads to complications in humans and other non-target organisms. The use of insecticides and fertilizers has substantially alleviated food production to take care of the world's population (El-Nemr *et al.*, 2012; Bazrafshan *et al.*, 2013).

Pesticides are synthesized toxic chemical agents used for killing pest such as insects, rodents, fungi, and unwanted plants (WHO, 2017).

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Properly applied pesticides can be effective in managing harmful insects, fungi and weeds thereby increasing productivity and income. Nevertheless, hazardous insecticides in combination with climate change can pose significant risk to the survival of species and integrity of the ecosystem (Job and Nshabum, 2024). Their widespread use poses a risk to all animals and the surroundings because of their poisonous consequences and bioaccumulation of their residual features (Rasgele *et al.,* 2015). Pesticide poisoning had been recognized to be a main medical issue worldwide (Bertolote et al., 2006), People residing in proximity to farms and people disclosed to domestic usage of insecticides or consume meals containing pesticide residue, those in pesticide production companies, farmers and their households are fantastically prone to pesticide intoxication (Al-Gehani, 2013).

The vast use of pesticide to eliminate agrarian pests has turn out to be very challenging due to their ability to adversely impact on animals (Alspalan *et al.,* 2006). Work-related pesticide intoxication is a brand danger to individual's fitness and numerous reviews on work-related and environmental pesticide intoxication have been documented (Rasgele *et al.,* 2015: Cavusoglu *et al.,* (2011). Utilization of organic and inorganic substances in the form of insecticides and fertilizers in the area of agriculture has resulted to environmental pollution and created some health issues (El-Deeb *et al.,* 2007).

Pyrethroids make approximately 25% of worldwide insecticides sale (Zhang *et al.,* 2011). More than two hundred varieties of artificial insecticides exist (Latif *et al.,* 2013) and all of them possess numerous heavy metals. These metals when introduced into the bodies of organisms usually usually affect their growth and development, physiology, reproduction and survival (Hayat *et al.,* 2007). The toxicity of pyrethroids differs among organic species, because of the varying ways of metabolic degradation and excretion from the body (Aydin *et al.,* 2005; Andem *et al.,* 2016). Pyrethroids are among the most recent groups of pesticides in the market and account for a large percentage of pesticide employed domestically, in human and veterinary medicine (Diaz, 2015). Pyrethroids particularly 'Cypermethrin and deltamethrin are used to regulate pest which enhance food production (Usmani and Knowles, 2001), however their use has been reported to contaminate food as well as impacts the non-target species negatively (Das *et al.,* 2003).

According to Rehman *et al.,* (2014) pyrethroids are not severely toxic for people maintaining good work practices, but Schetgen *et al*., (2002); Heudort and Angerer (2001), revealed chemical residues of several pyrethroids insecticides in urine samples of some individuals of a settlement with no known exposure. Schettgen *et al*., (2002*)* stated that majority of the contamination observed was as a result of food contaminated with pyrethroids insecticides.

Haematological indices had been studied and there is usually a link between them and the general health indices of an individual. Haematological indices like the quantity and morphology of all the blood types are beneficial as prognostic and diagnosis tools (Celik *et al.,* 2015; Oyewole *et al.,* 2009; Owoeye *et al.,* 2011). The core reasons of mortality in the world had been related to cardiovascular-associated diseases (WHO, 2017), and in effect, a normal check at the serum cholesterol level is crucial to examine the hazard linked with the heart problems (Sharma and Kumari, 2018). Etim *et al.,* (2014) stated that the assessment of the haematological properties presents the possibility to medically look into the dietary, biological and diseases status of an organism.

The blood and other fluids are the foremost transport system of the human body and any modification resulting from organic or inorganic toxicants, pathogens, pressure or stress are shown in the blood image and in vital interior organs like liver and kidney (Ihedioha *et al.,* 2004). The goal of this work therefore, was to assess the effects of ballistics pesticide on the physical traits and some biological and chemical parameters of mature female albino rats.

MATERIAL AND METHODS

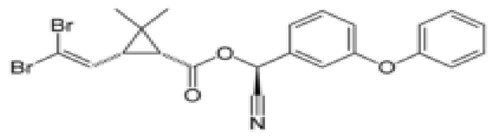
Procurement of the test compound

Pyrethroid insecticide (ballistic) was procured from Cross River State Agricultural Development Programme (CRADP), Ministry of Agriculture, Calabar.

Description of the test compound (ballistic)

Ballistic is an emergent and broad spectrum pyrethroids insecticide produced in New Zealand and supplied by ADAMA New Zealand Limited. It is used for the control of pest on a broad range of fruits, vegetable crops, trees, and ornamentals. It contains deltamethrin and aromatic hydrocarbons as active ingredients.

Deltamethrin is a distinctly powerful insect repellent used amongst different applications, for the manufacturing of insecticidal net, which together with interior residual spraying, are the primary vector management techniques encouraged for use by the World Health Organization (WHO) for the control of malaria. (WHO, 2016)



IUPAC NAME: Deltamethrin

Cyano(three-phenoxyphenyl) methyl (1R, R-3-(2,2-dibromoethen-1-yl)-2,2-dimethylcycloproppane-1-carboxylate

Aromatic compounds are biochemical organic compounds that include one or many pi electrons scattered all round them. Aromatic hydrocarbons, or arenes, are organic compounds containing solely carbon and hydrogen atoms (High Beam Encyclopedia)

Collection and transportation of experimental animals

Twenty-four (24) adult female albino rats (nine weeks old) were purchased from Zoology and Environmental Biology Animal House, University of Calabar and transported to Zoology and Environmental Biology Department's Laboratory, University of Calabar. Before the start of administration of ballistic to the rats, the animals were checked and weighed to ensure that they were healthy. On transfer to the experimental location the rats were allowed to acclimatize for one (1) week during which they were fed with standard rat feeds and water *ad libitum*.

Description of the experimental animals

Rattus norvegicus belongs to the order Rodentia and family Muridae. The rats have quick hair, a protracted bare tail, curved upright ears, bulging eyes, a pointed nose and pentadactyl foot. They are used for experimental purposes to study neuroanatomy, nutrition, endocrinology, genetics, behaviour etc.

Laboratory studies

Stock preparation and determination of treatment dose

0.1ml and 0.15ml of ballistic was diluted into 1000ml of distilled water each to form the stock solution of the low and high dose respectively, from the stock solution 0.5ml/kg body weight was determined as the treatment dose. Prior to the determination of the treatment dose, series of range finding tests following the guidelines of the Up-and- Down Procedure were performed to determine the dose that will not kill the organisms (Lipnick *et al.,* 1995).

Grouping and treatment of rats

Twenty-four (24) adult female rats were arbitrarily shared into three sets of 8 members and properly identified. The rats were housed in compartmented cages in a room under favourable conditions. The rats of control group had an initial mean weight of 173.8±6.35g and were nourished with regular rat feeds and water satisfactorily, whereas the toxicant was administered through oral administration to rats of the treatment groups II and group III. This experiment lasted for 21 days.

Euthanization and sample collection

After 21 days of treatment time, the rats were euthanized 12 hours after the previous intake of food; this was in agreement with the European Convention for the Protection of Vertebrate Animals and other Scientific Purpose Guideline (European Treaty Series No 123, 2005). All rats were anaesthetized with 90mg/kg ketamine (Van-Pelt, 1977), and dissected using designated dissecting kits. Blood samples were obtained through cardiac puncture and were stored in EDTA capped sample bottles to prevent clothing or coagulation.

Biochemical assay

Blood samples were spun at 2500rpm for 10 minutes using Wisperfuge Model 1384 centrifuge (Tamson, Holland). Serum samples were assayed for levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) using the microwell enzyme linked immunoassay (ELISA) technique utilizing the competitive binding principle; with analytical grade reagents from Syntron Bioresearch Inc. USA (Ekaluo *et al.,* 2010).

Total cholesterol (TC), Low density lipoprotein (LDL) cholesterol, High density lipoprotein (HDL) cholesterol and Triglycerides were assayed using the laboratory procedure manual. Method: Hitachi 704 Analyzer (Hopkins, 2004). The concentration of very low density lipoprotein cholesterol (VLDL) was analyzed using lipoprotein electrophoresis and ultracentrifugation method (Mark *et al.,* 1990).

Ethical clearance

Approval for this study was received from the Research Ethics Committee of the Faculty of Biological Sciences, University of Calabar and the experiment was carried out in line with the proposed guidelines set by World Health Organization (WHO, 2017). The rats were cared for according to the International Principles for Care and Use of Experimental Animals (NRC, 2011).

Data Analysis

Data collected during the study were expressed as mean ± SEM.

Analysis of variance (ANOVA) and a post-hoc test using Duncan multiple tests was applied for analysis of data. Probability level $p<0.05$ was regarded as significant.

RESULTS

Body weight variations of experimental rats

Results of daily administration of ballistic after 21 days on body weight changes showed that there was continues decline in body weight of the rats in the treatment groups (Group II and III). It was observed that weight changes in the treatment groups were concentration dependent with the high dose recording a higher decrease compared to the low dose. The control group however, displayed an increase in the mean body weight (Table 1). The average initial weight for the untreated group was 173.8 ±6.35g while the post treatment weight was 192.7 ±7.93g. The findings of this study revealed a marked drop in the mean final body weight gain of the treatment groups with the high dose (0.15ml) recording 172.9±6.42g mean initial weight and 160.3±1.45g mean final body weight gain. On the other hand, the low dose (0.1mL) recorded 146.1±10.93g mean initial body weight gain and 135.3±4.82g mean final body weight gain (Table 1). Statistical results on the initial body weight gain of the control and treatment groups showed that Group II (low dose) was statistically different ($p<0.05$) from Group I (control experiment) and Group III (high dose). Similarly, statistical results on the final body weight gain of rats in the control and experimental groups showed that there were significant differences between the three groups. In conclusion, the result of this study on body weight changes showed that ballistic insecticide caused a marked body weight reduction in the treatment groups when compared to the control (Table 1).

TABLE 1: Body weight changes of the rats in the control group and the treatment groups

Parameters	Control	Low dose (0.1mL)	High dose (0.15ml)	P-value
Mean Initial weight(g)	173.8±6.35 ^{a}	146.1±10.93 ^{b}	172.9±6.42 ^{a}	0.05
Mean final weight(g)	192.7±7.93 ^{a}	135.3±4.82 ^{b}	160.3±1.45 ^{c}	0.00

(Means with the same letter superscript on the same horizontal row are statistically the same and Results are described in mean plus/minus standard error of mean)

Effect of ballistic on follicle stimulating hormones (FSH) and luteinizing hormones (LH) of adult female albino rats

Follicle stimulating hormones (FSH) levels in this study showed significant decrease and the decrease was also concentration dependent. Rats in Group I (control group) recorded a mean level of 4.7mIU/ml follicle stimulating hormone, while rats of Group II treated with low dose of ballistic recorded mean follicle stimulating hormone level of 4.0mIU/ml whereas rats of Group III treated with high dose of ballistic recorded 3.5mIU/ml level of follicle stimulating hormone (Table 2). Statistical results showed substantial differences in the follicle stimulating hormone level of the control group and the two treatment groups. A post hoc test showed that the three groups were statistically different from each other.

There was an increasing effect of ballistic on the level of luteinizing hormone of the treated experiment. Rats in Group I (control group) recorded 7.3mIU/ml as the mean luteinizing hormone level, while those of the Group II (low dose) recorded the mean luteinizing hormone level of 22mIU/ml. Similarly, rats of group III (high dose) recorded 29mIU/ml as the mean luteinizing hormone level (Table 2). Statistical results showed substantial variation in the level of luteinizing hormone of the control group and the two treated groups ($p<0.05$).

TABLE 2: Effect of ballistic on follicle stimulating hormones and luteinizing hormones of female albino rats.

Parameters	Control	Low dose (0.1mL)	High dose (0.15ml)	P-value
FSH(mIU/ml)	4.7±0.051 ^{a}	4.0±0.091 ^{b}	3.5±0.149 ^{c}	0.00
LH(mIU/ml)	7.3±0.158 ^{a}	22±0.913 ^{b}	29±1.958 ^{c}	0.00

(Means with the same letter superscript on the same horizontal row are statistically the same and Results are described in mean plus/minus standard error of mean)

Effects of ballistic on the lipid profile of adult female albino rats

Total cholesterol (TC)

The result of this study on the total cholesterol (TC) concentration revealed a gradual increase in treated rats (Group II and III). It was observed that rats in Group I (control group) recorded a mean total cholesterol level of 1.9mmol/L while group II (Low dose) recorded 2.1mmol/L and group III (high dose) recorded 2.3mmol/L of the mean total cholesterol concentration (Table 3). Statistical results displayed substantial alterations in the total cholesterol concentration of the group 1 and the two treatment groups A post hoc test conducted to check statistical differences between groups showed that the three groups were significantly different from each other (Table 3).

Triglyceride (TG)

Results of triglyceride concentration of the control and two experimental sets showed a substantial rise. Group I recorded 0.63mmol/L mean triglyceride concentration while group II recorded 0.68mmol/L triglyceride concentration and group III recorded 0.69mmol/L (Table 3). Statistical results showed that group 1 was considerably different from the two treatment groups. A post hoc test showed no significant difference between group II and group III (Table 3).

Low density lipoprotein cholesterol concentrations (LDL)

The study showed that ballistic had an increasing effect on the low density lipoprotein levels (LDL) of the treatment groups. (Table 3). Rats in Group I (control group) had 0.5mmol/L mean concentrations of low density lipoprotein cholesterol whereas those of the treatment groups (Group II and III) recorded (0.8mmol/L). Statistical results revealed a substantial difference between the LDL cholesterol concentrations of group I and the treated experiment (group II and III) (Appendix 9).

Very low density lipoprotein concentrations (VLDL)

There was a gradual increase in the VLDL concentrations of the treatment groups. Animals in Group I (control group) recorded a mean total of 0.28mmol/L concentration whereas rats of Group II (exposed to low dose of ballistic) recorded a mean total of 0.31mmol/L while the rats in Group III (exposed to high dose of ballistic) recorded a mean total of 0.34mmol/L level of very low density lipoprotein concentrations (Table 3). Statistical results revealed substantial alterations in the concentration of VLDL of the Group I (control) and the two chemically-exposed groups. Further statistics showed that the three groups were significantly different from each other (Table 3).

High density lipoprotein cholesterol concentrations (HDL)

Unlike the results of other lipid profiles considered in this study, high density lipoprotein (HDL) concentration showed a gradual reduction in the chemically exposed groups and the decrease was concentration dependent. Rats in group I (control group) recorded a mean total concentration of 1.22mmol/L, those of Group II (exposed to low dose of ballistic) recorded a decrease in the mean high density lipoprotein cholesterol (1.20mmol/L), whereas rats of group III (exposed to high dose of ballistic) recorded a mean total of 1.05mmol/L level of high density lipoprotein cholesterol (Table 3). Statistical results showed that there were no substantial differences in the HDL concentrations of the group I and the other experimental groups (Group II and III).

TABLE 3: Effect of ballistic on the lipid profile of female adult albino rats

Parameters	**Control.**	**Low dose.** (0.1mL)	**High dos**(0.15ml)	**P- values**
TC (mmol/L)	1.9±0.051 [a]	2.1±0.041 [b]	2.3±0.041 [c]	0.00
TG (mmol/L)	0.63±0.011 [a]	0.68±0.007 [b]	0.69±0.007 [b]	0.00
LDL(mmol/L)	0.5±0.037 [a]	0.8±0.041 [b]	0.8±0.058 [b]	0.00
VLDL(mmol/L)	0.28±0.008 [a]	0.31±0.004 [b]	0.34±0.004 [c]	0.00
HDL(mmol/L)	1.22±0.037 [a]	1.20±0.108 [a]	1.05±0.065 [a]	0.24

(Means with the same letter superscript on the same horizontal row are statistically the same and Results are described in mean plus/minus standard error of mean)

DISCUSSIONS

In this study, oral administration of ballistic insecticide to female adult albino rats revealed changes in bodily activity such as, dullness, shivering, salivation, diarrhea and lack of urge for food with greater severity in the high dose group. Similar outcomes had been reported by Rekha *et al.,* (2013) who exposed albino rats to chlorpyrifos. Akhtar *et al.,* (2009), reported similar morphological changes when organophosphate pesticide (Chlorpyrifos) where administered to *Rattus norvegicus*. The findings of this work is also related to the observation of the following authors. Kammon *et al.,* (2010), Ambali *et al.,* (2011a), Ambali *et al.,* (2011b), Issa *et al.,* (2011), Bhadaniya *et al.,* (2012), Galakatu *et al.,* (2012), and Heikal *et al.,* (2012) who also worked on similar topics using same species as experimental animals.

This study also revealed a substantial decrease in body weight gain of the experimental rats. The weights of the rats were observed to decrease as the concentration was increased. This is also similar with results of Rekha *et al.,* (2013) who observed decrease in the body weight gain of the experimental animals after weeks of treatment with Chlorpyrifos. The findings of this study is also similar with that of Agiang *et al.,* (2017) who studied the "Assessment of the haematological indices of albino rats fed diets supplemented with jackfruit bulb, seed or a blend of bulb and seed". These results are also in agreement with the findings of the following authors Malik *et al.,* (2004) who worked with Chlorpyrifos toxicity in broiler chicken, Tripathi and Srivastava (2010), who studied long-term effect of oral administration of Chlorpyrifos on the kidney. Galakatu *et al.,* (2012), Mansour and Mossa (2011): Heikal *et al.,* (2012), Mossa and Abbassy (2012), Bhandaniya *et al.,* (2012) also recorded weight loss when experimental animals were exposed to different concentration of toxicants. The data on body weight reduction in this study are also in line with the findings of Hancock *et al*., (2007), Ambali *et al*., (2011a) who stated that the major rat strain observed was the decrease in the weight of their bodies. Umoren *et al.,* (2016) reported decrease food consumption and a corresponding decrease in body weight of rats exposed to palm wine. The findings recorded in this work however, is not in conformity with the reports of Rekha *et al.,* (2013) when rats were administered lower doses of Chlorpyrifos.

This study also investigated the lipid profile of the control and treatment groups. In other to avoid complications associated to the proper functioning of the heart, it is necessary to routinely check the lipid profile, as it is a known fact that increasing effects of the low density lipoprotein (LDL) cholesterol over time leads to damage of the arteries contribute to heart disease as well as increase the risk of developing stroke. This study has revealed a substantial increase in the total cholesterol (TC), triglyceride (TG), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) in adult female albino rats exposed to ballistic insecticide. On the other hand, high density lipoprotein (HDL) is known to be a healthy cholesterol as it helps to transport excess cholesterol out of the arteries to the liver for excretions. This study has recorded a reduction in the high density lipoprotein cholesterol of the chemically exposed animals and the decrease observed was concentration dependent. The statistical results however, did not show any significant change amongst the control and the chemically exposed sets of rats. These finding are similar to the observations of Anyanwu *et al.,* (2020) who reported a remarkable rise in the total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), and very low density lipoproteins (VLDL) and a decrease in the high density lipoproteins (HDL in rats exposed to low dose heavy metal mixture. The findings of this study are also similar to those of Larregle *et al.,* (2008) who worked on lipid absorption and digestion in liver of rats exposed to cadmium. Kaur and Sharma (2015) who studied the evaluation of biological and chemical changes caused by short-term and long-term concentration of cadmium in mice reported a similar result. The findings of this work on the decreased effects of high density lipoprotein (HDL) cholesterol corresponds with that of Umuren *et al.,* (2020) who studied The lipid lowering effect of aqueous extract of roselle on paracetamol-induced hepatotoxicity in wistar rats.

Results obtained for the hormonal assay revealed that ballistic caused a significant ($P<0.05$) reduction in follicle stimulating hormones (FSH). This report agrees with those of Anup *et al.,* (2007), Choi *et al.,* (2011), Karen *et al.,* (2012) and Mehran *et al.,* (2012) who reported an inverse association amongst caffeine consumption and the level of sex hormones. The report in this study however, revealed a substantial rise in the luteinizing hormone of the chemically exposed animals. This is also in line with reports of Amah-Taria *et al.,* (2016) who studied variations in the reproductive features of female rats. The findings also correspond with those of Uno *et al.,* (2015). The finding of this study does not agree with that of Onuka *et al.,* (2017) who studied "Evaluation of the effects of *tetrapleura tetraptera* extract and clomiphene citrate to determine influence of reproductive hormones and estrous cycle on leukocyte counts.

CONCLUSION

The findings of this study have revealed that ballistic caused a significant reduction in the final weight gain of the experimental rats.

More so, ballistic resulted in the significant alleviation of total cholesterol, triglyceride, low density lipoprotein, and very low density lipoprotein. The findings of this study however showed no significant alteration in the high density lipoprotein (HDL) of treatment animals ($p<0.05$). Furthermore, result of follicle stimulating hormones showed significant decrease in the treatment groups, while luteinizing hormones concentration of the treatment groups were significantly increased ($p<0.05$).

It is concluded in this study that ballistic insecticide possesses a potent cytotoxic effect on the physical traits and some biochemical parameters of adult female albino rats.

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