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Biochemical And Hematological Changes In Male Wistar Rats Following Subacute Exposure To Pesticide

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Abstract
Pesticides are toxic chemicals or biological agents used to control or eliminate pest populations in the environment, such as insects, weeds, rodents, and fungi. This study aims to detect the biochemical and haematological changes in male Wistar rats following subacute pesticide exposure using a type of organophosphate pesticide called 'Terminator'. 25 male Wistar rats were divided into five different groups. Group A was used as the control group (pesticide was not applied to this group), Group B was exposed for a period of one hour (Low dose), Group C was exposed for a period of two hours (Medium dose), Group D was exposed through skin contact (applying pesticide directly to the skin), Group E was exposed to pesticide for a period of four hours (High dose). This experiment lasted for 14 days after which blood samples were collected by means of ocular puncture and divided into two parts, one was centrifuged and serum was collected while the other part was used for hematological indices and organs were harvested to access organ weight. Data was analyzed using Analysis of variance (ANOVA) followed by post Hoc LSD comparison and values were considered significant at p<0.ss05. The result showed significant increase in liver biomarkers, kidney biomarkers, Hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, relative liver weight and relative kidney weight. From this study, it can be deduced that exposure to Pesticide (Terminator) alters biochemical and some hematological states, necessitating protective equipment use and regular health evaluations for pesticide applicators and risk populations, and prompting professional help.
Keywords: *Pesticide, Organophosphate, Biochemical, hematological*

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I. Introduction

A pesticide is a toxic chemical substance or a mixture of substances or biological agents that are intentionally released into the environment in order to avert, deter, control and/or kill and destroy populations of insects, weeds, rodents, fungi or other harmful pests. Pesticides work by attracting, seducing and then destroying or mitigating the pests. Pests can be broadly defined as the plants or animals that jeopardize our food, health and / or comfort (Kaur et al 2019).

Pesticides are the most effective means of pest eradication all over the world, but their use has reached an alarming rate due to a number of adverse effects on non-target organisms (Bhushan 2013).

Source (Mondew, 2023)

Fig. 1 An image of the pesticide(organophosphate) used.

Pesticides are substance or mixture of substance which differ in their physical, chemical and identical properties from one to other. Hence, they are classified based on these properties. Some pesticides are also categorized into various classes depending on the needs. Presently, three most popular classifications of pesticides which are widely used is classification based on the mode of entry, pesticide function and the pest organism they kill, the chemical composition of the pesticide. Based on toxicity of pesticides, WHO classified them into four classes: extremely dangerous, highly dangerous, moderately dangerous and slightly dangerous (Kaur et al 2019).

Based on chemical composition, pesticides are classified into four main groups namely; organochlorines, organophosphorus, carbamates and pyrethrin and pyrethroids(Raghav 2013).

Organophosphates are chemical compounds formed through the esterification process involving phosphoric acid and alcohol. These chemicals serve as primary components in herbicides, pesticides, and insecticides and have extensive application in manufacturing plastics and solvents(Adeyinka et al 2023).

Organophosphate insecticides, such as diazinon, chlorpyrifos, disulfoton, azinphos-methyl, and fonofos, have been used widely in agriculture and in household applications as pesticides**(**Dyro et al.2020).

According to a research carried out in Thailand**,** Exposure to different pesticides may impact organ functions due to their cytotoxic effects, and multiple biomarkers may be used to monitor the early adverse effects of pesticides. Numerous studies have revealed that individuals who are exposed to pesticides experience significant hemotoxic effects. A study found significant hematological changes in Thai rice farmers. However, little is known about the hematological effects of pesticide exposure in Thailand (Bunsri 2023).

Repeated exposure to pesticides can cause a variety of human health problems, particularly among farmers. The existence of some disturbances of biochemical parameters in farmers should encourage to test the hypothesis of a link between pesticide exposure and the appearance of biochemical disorders in clinical trials(Bayili et al. 2020).

II. Materials And Method

Materials

Male wistar rats
Syringes
Saw dust
Cages
Cotton wool
Pelleted feed (growers feed)
Terminator(Organophosphate)
Plain bottles
Microscope
Dissecting kit
Chloroform AR JHD; (Guangdang Guanghua Chemical Factory,China)
Electronic Weighing balance M-methlar model M3111 China.
Hang gloves
Nose masks

EDTA bottles
Capillary tubes
Centrifuge
Micro-haematocrit centrifuge
Micro-pipette
Haematocrit reader
Feed plates
Water cans
Air tight room

Location

This study was carried out in the animal house, department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnewi campus, Nnamdi Azikiwe University.

Ethical Approval

Ethical approval was obtained from the faculty of basic medical science, college of health science Nnewi campus Nnamdi Azikiwe University.

Collection And Preparation Of Organophosphate Pesticide (Terminator)

The Terminator pesticide was purchased from Onitsha market

Animal Procurement, Care And Treatment

The experimental animals were obtained from Animal house, Department of Human Physiology, college of health sciences, Nnamdi Azikiwe university, Nnewi in Anambra state. The rats were housed in Standard plastic cages and metal cages in a temperature controlled room at 25±2ºC, under a 12/12h light/dark cycle, and were maintained on normal laboratory chow (Hybrid and Top feed, Nnewi) and water ad libitum. Animals were acclimatized for a time frame of three weeks before administration of the terminator pesticide. Rats handling and treatments conformed to the guidelines of the faculty of Basic Medical Science, College of Health Science and Technology, Nnamdi Azikiwe University, Nigeria for laboratory animal care and use.Animals weighed between 100-170g at first arrival and had no history of drug consumption.

Lethal Dose (Ld50) Determination

The median lethal dose (Ld50) of *terminator* was carried out in the department of Physiology, Faculty of Basic medical sciences, Nnamdi Azikiwe University Okofia Campus . This was determined using the Dietrich Locke method (1983).

Experimental Design

25 male wistar rats were divided into five different groups to check for the biochemical and hematological changes on male wistar rats following subacute pesticide exposure.
Group A was used as the control group (pesticide was not applied to this group)
Group B was exposed for a period of one hour (Low dose)
Group C was exposed for a period of two hours (Medium dose)
Group D was exposed through skin contact (applying pesticide directly to the skin)
Group E was exposed to pesticide for a period of four hours (High dose)

All animals were exposed to the same quantity of pesticide in an airtight room measuring height of 263cm, length of 178 cm and width of 78 cm except for Group D which was exposed through skin contact.

25 ml of *terminator pesticide* was injected with a syringe into 5 chunks of cotton wool(5ml per chunk of cotton wool) and placed in strategic corners of the room except group D which underwent tropical exposure. 25ml was f *terminator pesticide* was injected into two chunks of cotton wool (5ml per chunk) and applied all over the body of the experimental animal.

Experimental protocol

Before administration, the rats were weighed and their weights ranged from 100_120g .They were then divided into five experimental groups labelled A_E. Group A had 5 rats and served as control, they were administered only water and feed all through the experiment as shown in the table below.

Table 2.1 Grouping of animals

GROUP	MATERIAL ADMINISTERED	DURATION (TIME)	DURATION (DAYS)

A	25ml(25ml per 263cm by 178cm chamber)	_____	14
B	25ml(25ml per 263cm by 178cm chamber)	1 hour	14
C	25ml(25ml per 263cm by 178cm chamber)	2 hours	14
D	Skin(tropical) 25ml(25ml per 263cm by 178cm chamber)	pesticide was applied on the skin and left throughout the day	14
E	25ml(25ml per 263cm by 178cm chamber)	4 hours	14

Termination Of The Experiment And Blood Sample Collection

This experiment lasted for 14 days.Twenty four hours after last administration of *terminator pesticide*,the animals were weighed with the weighing balance to get the final weight before collection of blood samples and testes.Blood samples were collected by ocular puncture after anesthetizing the animal with chloroform according to Parasunama *et al 2010.* 10ml of blood were collected from two rats in each group including control group and divided into two bottles (5ml each). 5ml was put in a plain bottle for serum collection blood was centrifuged for 20 minutes at 1000 (rpm) after 5 minutes of clotting and serum was retrieved using a pippete. The remaining 5ml was put inside an EDTA bottle and used for hematological indices .

Organ Collection

After collection of blood samples,the animals were placed on a dissecting board and dissected. The liver and kidney was detached from surrounding attachments and weighed with an electronic analytical and precision balance and recorded.

III. Results

Table 4.1: Effect of Terminator pesticides exposure on serum creatinine, urea, uric acid, and relative kidney weight

	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Relative Kidney weight (g)
	MEAN±SEM	**MEAN±SEM**	**MEAN±SEM**	**MEAN±SEM**
Group A (control)	36.91±1.46	0.18±0.03	5.49±0.47	0.49±0.06
Group B (25mls of TIS for 1-hr)	48.09±3.92#	0.26±0.04#	10.09±0.23*	0.38±0.02#
Group C (25mls of TIS for 2-hrs)	54.90±2.18*	0.38±0.06*	9.89±1.77*	0.35±0.04*
Group D (25 mls of TIS topically)	64.15±7.12*	0.41±0.10*	15.21±0.82*	0.38±0.01#
Group E (25mls of TIS for 4-hrs)	51.87±1.35*	0.29±0.03#	10.22±0.91*	0.36±0.00*
F-ratio	6.608	2.566	11.998	2.324

Data was analysed using ANOVA followed by post Hoc Fisher's LSD comparison and data were considered significant at *p<0.05*. *: significant, #: not significant.

Table 4.1 result showed an increase in the mean serum creatinine levels in groups B, C, D, and E compared to group A, with significance in groups C, D, and E (p=0.008, p=0.001, 0.021), and group B (*p=0.068*) had no significance. The serum urea result showed a non-significant increase in groups B and E (*p=0.376,* p=0.550) and groups C and D (*p=0.043,* p=0.024) had a significant increase when compared to group A. The serum uric acid result showed a significant increase in groups B, C, D, and E (p=0.008, p=0.011, p=0.000, p=0.007) when compared to group A. The mean relative kidney weight showed a non-significant decrease in groups B and D (p=0.070, p=0.059) while groups C and E (p=0.023, p=0.032) had a significant decrease when compared to group A.

Table 4.2: Effect of Terminator Pesticide on Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP) and Relative Liver Weight

	Aspartate Transaminase (IU/L)	Alanine Transaminase (IU/L)	Alkaline Phosphatase (IU/L)	Relative Liver weight (g)

	MEAN±SEM	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group A (control)	15.00±1.00	13.33±0.88	77.31±1.09	3.22±0.14
Group B (25mls of TIS for 1-hr)	20.66±0.88#	17.00±1.52#	81.66±1.76#	3.97±0.16*
Group C (25mls of TIS for 2-hrs)	26.66±2.40*	13.66±0.88#	97.32±1.96*	3.45±0.28#
Group D (25 mls of TIS topically)	30.33±3.28*	20.33±2.40*	99.20±6.38*	3.03±0.02#
Group E (25mls of TIS for 4-hrs)	31.33±2.60*	19.00±2.08*	103.70±4.42*	3.30±0.18#
F-ratio	9.454	3.492	9.844	3.858

Data was analysed using ANOVA followed by post Hoc Fisher's LSD comparison and data were considered significant at *p<0.05*. *: significant, #: not significant.

Table 4.2 result showed an increase in the mean serum Aspartate Transaminase (AST) level in groups B, C, D and E when compared to group A, with significance in C, D and E (*p=0.004, p=0.001, p=0.000*) while group B(*p=0.104*) had no significance. The mean serum Alanine Transaminase (ALT) level showed an increase with significance in groups D and E (*p=0.014, p=0.038*) while groups B and C (*p=0.152, p=0.891*) showed no significance when compared to group A. The mean serum Alkaline Phosphatase (ALP) level showed an increase with significance in groups C, D and E (*p=0.003, p=0.002, p=0.001*) while group B (*p=0.425*) showed no significance when compared with group A. The mean relative liver weight showed a significant increase in group B (*p=0.015*) and an insignificant increase in groups C and E (*p=0.387, p=0.760*) while group D (*p=0.468*) showed insignificant decrease when compared to group A.

Table 4.3: Effect of Terminator Pesticide on White Blood Cell

	White blood cell (x10^9/l)
	MEAN±SEM
Group A (control)	4.31±1.65
Group B (25mls of TIS for 1-hr)	1.29±0.08#
Group C (25mls of TIS for 2-hrs)	4.29±2.13#
Group D (25 mls of TIS topically)	2.20±0.19#
Group E (25mls of TIS for 4-hrs)	6.75±1.25#
F-ratio	2.524

Data was analysed using ANOVA followed by post Hoc Fisher's LSD comparison and data were considered significant at *p<0.05*. *: significant, #: not significant.

Table 4.3 result showed a decrease in mean White blood cell in groups B, C and D while group E showed an increase with Groups B, C and D (*p=0.141, p=0.990, p=0.290*) showing non-significant decrease while group E (*p=0.226*) showed non-significant increase when compared with group A.

Table 4.4: Effect of Terminator Pesticide on Red Blood Cell, Pack Cell Volume and Haemoglobin

	Red blood cell (x10^12/l)	Pack cell volume (%)	Hemoglobin (g/dl)
	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group A (control)	5.17±1.04	36.16±1.85	12.96±0.80
Group B (25mls of TIS for 1-hr)	3.25±0.28#	32.33±2.18#	9.93±0.29*
Group C (25mls of TIS for 2-hrs)	6.59±1.51#	32.50±2.17#	9.98±0.73*
Group D (25 mls of TIS topically)	5.32±2.03#	33.33±1.66#	10.72±0.60#
Group E (25mls of TIS for 4-hrs)	5.63±0.83#	31.16±1.66#	11.21±1.31#
F-ratio	0.892	0.771	2.286

Data was analysed using ANOVA followed by post Hoc Fisher's LSD comparison and data were considered significant at *p<0.05*. *: significant, #: not significant.

Table 4.4 result showed an increase in groups C, D and E while group B showed a decrease in mean Red Blood Cell with no significance in increase in groups C, D and E (*p=0.454, p=0.933, p=0.805*) and also no significant decrease in group B(*p=0.317*) when compared to group A. The mean Packed cell Volume showed a decrease with no significance in groups B, C, D and E (*p=0.234, p=0.254, p=0.372 p=0.130*) when compared to group A. The mean Haemoglobin showed a decrease with significance in groups B and C (*p=0.026, p=0.028*) while groups D and E (*p=0.082, p=0.162*) showed no significance when compared with group A.

Table 4.5: Effect of Terminator Pesticide on Mean Corpuscular, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration

	Mean corpuscular volume (fl)	Mean corpuscular hemoglobin (pg)	Mean corpuscular hemoglobin concentration (g/dL)
	MEAN±SEM	**MEAN±SEM**	**MEAN±SEM**
Group A (control)	68.19±10.05	29.48±6.74	35.29±2.11
Group B (25mls of TIS for 1-hr)	35.31±3.14*	20.94±7.75#	27.16±0.80*
Group C (25mls of TIS for 2-hrs)	39.48±3.11*	12.75±0.52#	30.17±2.11#
Group D (25 mls of TIS topically)	28.26±8.23*	20.44±7.36#	31.07±1.30#
Group E (25mls of TIS for 4-hrs)	50.11±1.84#	19.17±1.33#	34.58±4.59#
F-ratio	6.296	1.102	1.637

Data was analysed using ANOVA followed by post Hoc Fisher's LSD comparison and data were considered significant at *p<0.05*. *: significant, #: not significant.

Table 4.5 result showed a decrease in the Mean Corpuscular Volume with significance in groups B, C and D (*p=0.004, p=0.008, p=0.001*) while group E (*p=0.066*) showed no significance when compared to group A. The Mean Corpuscular Haemoglobin showed a decrease with non-significance in groups B, C, D and E (*p=0.314, p=0.064, p=0.287, p=0.229*) when compared to group A. The Mean Corpuscular Haemoglobin Concentration showed a decrease with significance in group B (*p=0.048*) while groups C, D and E (p=*0.185, p=0.268, p=0.770*) showed no significance when compared to group A.

IV. Discussion And Conclusion

Discussion

The current study produced findings on the Biochemical and Hematological changes in male wistar rats following subacute exposure to pesticide.In this study it was noticed that there was significant increase in the urea (Groups C and D) and creatinine (Groups C, D and E) levels this finding is in disagreement with the findings of Adeoti et al. (2017) which says that there was no significance difference in the level of urea and creatinine while there was an increase in the uric acid in Groups B, C, D and E in this study which agrees with the study of of Nejad et al.(2012)

Pack cell volume and Hemoglobin showed an insignificant decrease in Groups B, C, D and E and a significant decrease in Groups C, D and E respectively this partially agrees with works of Khan et al. (2023) and Adeoti et al. (2017) they both recorded significant decrease while in this study, an insignificant decrease and a significant decrease was recorded. However the Red Blood Cells from this study showed no significant increase in Groups C, D and E while Group B showed an in significance decrease this partially agrees with the work of Adeoti et al. (2017) which recorded a decrease in Red Blood Cells.

White Blood Cells showed an insignificant decrease in Groups B, C and D while Group E showed an insignificant increase this findings disagrees with the findings of Mishra et al. (2014) which recorded an increase in White blood cells but if partially agrees with the works of Gaikwad et al. (2015) which recorded a significant decrease.

Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration showed a decrease which is in agreement with the findings of Nejad et al.(2012)

Conclusion

From this study, it can be deduced that exposure to Pesticide (Terminator) cause a shift in the normal biochemical and hematological state. Hence, Organophosphate chemicals despite being used as pesticides and insecticides pose a huge risk to human health. There is the need for general public sensitization on the dangers of exposure to organophosphate pesticides be it for agricultural or household use. The use of protective equipment by pesticide applicators as well as other risk populations should be encouraged. Individuals who are frequently exposed to low doses of organophosphates should periodically evaluate their health status to enable them to seek professional help and counseling.

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