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Possible Risk Effect of Nopest® Pesticide Exposure on Non-target Organism

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Abstract:- Healthy male albino wistar rats (*Rattusnorvegicus)* were exposedto oral administration of 0.2ml of Nopest® (DDVP) pesticide once daily for 28 consecutive days at increasing concentration of 0.75, 1.5 and 3.0mg/ml to ascertain possible oxidative stress and toxic effects on the liver of the animals exposed with the different concentrations of the pesticide. There was significant increase ($p<0.05$) in the body weight for all the weeks of exposure and for all the groups as compared to the control group except at week four (101.4±5.51),(122.6±14.3) and (161.0±19.75) for group A,B, and C respectively. The liver function analysis was conducted to determine the level of change in Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP). Analysis was also conducted for antioxidant enzymes: superoxide dismutase (SOD), glutathione (GSH), ascorbic acid (AA), and Malondialdehyde (MDA) in order to determine oxidative stress. There was significant increase in the levels of the antioxidants ($p<0.05$) which suggested that oxidative stress occurred in the exposed rats.

Keywords:- *oxidative stress, non-target organism, pesticide*.

I. INTRODUCTION

Today, man faces numerous issues related to the catastrophic impacts of pests on his valuable plants, stored food, and goods all over the world. These pests could harm agricultural animals directly or transmit disease germs to them. These pests live with us in our homes, storage facilities, offices, industries, and fields, causing discomfort, loss of agricultural output and stored food, material pollution, and individual health(Ujowundu*et al*., 2020). Bedbugs, ants, cockroaches, housefly, mosquito, beans beetles, grain weevils, aphids, leaf-worm, fleas, and leaf hopper are just a few examples of these pests.Ecto-parasites and endo-parasites are both present in these pests. Several pest management and control approaches have been implemented in order to eliminate or limit their menace, as well as to ensure that man, his domestic animals and his agricultural products, are safe in the environment(Yassin*et al*., 2015). Biological, chemical, cultural, and physical control strategies are some of the pest control approaches used.

Biological control entails eradicating or reducing the population of pests by using natural enemies such as predators, parasites, or pathogens. The use of *Bacillus thuringiensisisraelensis* (Bti) for mosquito control is a good example (Bruhl *et al*.,2020).Physical control entails changing the environment using methods such as sound, asphyxiation, dehydration, temperature adjustment, and the use of electromagnetic media, as well as handpicking and killing the pest.

Chemical control entails the use of chemicals, commonly referred to as pesticides, to eliminate pest agents. Chemical methods have been widely adopted by the general public in both developed and developing countries, with little regard for the pesticides' hazardous effects on humans once absorbed into the body system by inhalation or accidental consumption(Aditya*et al*., 2012).

Organophosphate, carbamates, and organochlorine insecticides are three prominent pesticide families. Organophosphate insecticides impact the brain system by affecting the enzyme that controls the neurotransmitter acetylcholine. They were invented in the early 1800s, but it wasn't until 1932 that their effects on insects, which are identical to those on humans, were identified. However, indiscriminate usage and application of these pesticides to the targeted pest have resulted in ailments such as breast cancer, sterility, liver issues, and oxidative stress (Hasio, 2015; Benedetti *et al*., 2004). Nervous excitation, tremors, convulsions, and death are all symptoms of pesticide poisoning (Eddelston, 2008).Organophoshate pesticides are widely employed in agricultural and domestic pest control, accounting for over half of all insecticidal use worldwide(Thompson, 2011, Naidoo and Rother, 2016). Their use is almost often associated by widespread toxicity in non-target organisms, such as humans. Dichlorvos (2, 2-dichlorivinyl dimethyl phosphate), commonly known as DDVP, is the most widely used organophosphate insecticide in the world (Henshaw and Iwara, 2018).

Nopest, snipper, boom, fly-bate, derikol, and other brands of 2,2-dichlorivinyl dimethyl phosphate are sold in Nigeria under various brand names (Owoeye*et al*., 2012). It functions as an acetylchlorinestrase inhibitor when taken into the body through any route such as the skin, mouth, or nose, causing symptoms such as weakness, headache, blurred vision, salivation, perspiration, nausea, vomiting, diarrhea, stomach cramps, and frequent urination (Gilden*et al*., 2010). Pesticide absorption produces oxidative stress, which is defined as a mismatch between the systemic expression of reactive oxygen species and a biological system's ability to quickly detoxify reactive intermediates or repair the harm they cause.

The oxidative stress phenomenon is caused by an imbalance in the free radical/antioxidant equilibrium in

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favor of free radicals, which can lead to a variety of pathological events in the liver. Cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, and myocardial infarction are all known to be linked to oxidative stress in humans (Pizzino*et al.*, 2017). Reduced exposure to oxidizing environmental pollutants, increased levels of endogenous and exogenous antioxidants, or minimizing the creation of oxidative stress through stabilizing mitochondrial energy production and efficiency can all help to avoid the dangers of oxidative stress. (Poljsak, 2011).Nopest® is a brand of 2,2-dichlorovinyl dimethyl phosphate (DDVP) that is used to manage pests of public health, field crops, flowers, stored grains, vegetable fruit crops, and palms all over the world. These insecticides if not handled appropriately, may be accidently ingested during application. When used against domestic pests such as mosquitoes, cockroaches, and other home pests, it may fall on food and clothing. Furthermore, individuals apply this pesticide inadvertently against pests that attack stored food products such as beans (weevil), rice, and other grains, without being aware of the potential hazardous effects.

As a result, the goal of this research is to see how Nopest® insecticide affects oxidative stress in albino wistar rats (Rattusnorvegicus).

II. MATERIALS AND METHODS

A. *ETHICAL STATEMENT*

Ethical permit was obtained from the research and ethics committee of the School of Biological Sciences (SOBS), Federal University of Technology Owerri. The animals were examined and allowed to acclimatize for two weeks before the formal experiment commenced.

B. *Sourcing, Housing and Feeding of Animals*

Albino wistar rats *(Rattusnorvegicus*) weighing between 110 and 130g were used as experimental animals. The animals were obtained from the animal house of the Zoology Department, University of Nigeria, Nsukka, Enugu state Nigeria. They were allowed to acclimatize with the new environment (Animal house of the Department of biological sciences, Federal University of Technology, Owerri). The animals were kept in cages and placed in a well-ventilated room conditions (temperature 26±1^{o}C; photoperiod: 12 hour light and dark cycle each throughout the experimental period. The rats were allowed free access to food (rat pellet) and water.

C. *Preparation of Animals*

At the end of acclimatization period, the animals were weighed and distributed randomly into four groups of six animals each. The first 3 groups were exposed to the test substance at different concentrations while the fourth group was not exposed to the test substance and was marked as control. The cages of the exposed groups were labeled A, B and C, while the control cage was labeled D. the exposure lasted for 28 consecutive days.

D. *Test Substance*

The test substance used for this experiment was NOPEST ®pesticide, a brand of Dichlorvos, commonly known as DDVP (2, 2 – dichlorovinyl dimethyl phosphate) pesticide. It was obtained from chemical line in Ekeonunwa market Owerri in Imo state and was diluted serially in water and administered to the test animals at different concentrations of 3.0, 1.5 and 0.75mg/ml. the test substance was administered orally using feeding tube.

E. *Preparation of Serial Dilution*

0.6ml of the stock solution of Nopest® pesticide was measured using a 2ml syringe into a conical flack (250ml). 200ml of distilled water was measured using measuring cylinder and was added into the conical flask in order to obtain a concentration of 3.0mg/ml solution of DDVP.100ml was taken from 3.0mg/ml solution obtained and was stored in a bottle labeled A. 100ml of water was added to the 100mg/l of the solution to obtain a concentration of 1.5mg/ml. 100ml of this new concentration was also taken and stored in a sterile bottle labeled B. another 100ml of water was added to 100mgs/l obtained from B to produce a concentration of 0.7mg/ml. 100ml of this solution was also collected and stored in a bottle labeled C.

F. *EXPERIMENTATION*

Groups A, B, and C were exposed to the pesticide solution once daily for 28 consecutive days. Group D(which served as control group) was not exposed to any form of treatment but were properly fed and taken care of throughout the study. Group A, B and C animals were given 0.2ml of the pesticide solution of concentrations 3.0mg/ml, 1.5mg/ml and 0.7mg/ml respectively, using a feeding tube. The cages housing the animals were cleaned daily, food and water supplied freely. The animals in the different cages were weighed once in a week and average weight properly recorded. At the end of exposure, three (3) rats each were collected from the four cages and sacrificed. Blood samples were collected by cardiac puncture, using 5ml syringe with 0.50mm needle into EDTA bottle.

G. *Studies on hepatotoxicity*

Serum activity of liver function marker enzymes such as ALT, ALP, and AST was measured according to manufacturer's instructions using liver enzyme activity test kits (Bio Merieux France).

H. *Oxidative Stress Parameters*

The method of Fridovich (1989)was used to determine Superoxide dismutase (SOD) activity. The blood plasma sample was properly diluted (20times). A 0.2ml portion of diluted sample was added to 2.5ml of 0.05m phosphate buffer, pH 7.8. The mixture was equilibrated in the spectrophotometer before adding adrenalin solution. The reaction started with the addition of 0.3ml of freshly prepared epinephrine solution (0.059%) to the mixture followed by quick mixing by inversion in the curette. The reference cuvette therefore contain 3.5ml buffer, 0.3ml of adrenalin and 0.2ml of sample. The increase in absorbance was taken at 480nm for 150sconds at 30 seconds interval. Absorbance was calculated using: $A= ECl$. Where E=molar absorptivity ($4020M^{-1}cm^{-1}$), C= Concentration, l=light path (1).

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Estimation of MDA concentration was estimated using the method described by Devasagayam*et al* (2003). The blood plasma was diluted 20 times, Acetic acid 1.5ml (20%; PH 3.5), 1.5ml of thiobarbituric acid (0.8%) and 0.2ml of sodium dodecylsulphate (8.1%) was added to 0.1ml supernatant and heated at 100^0c for 60min. Mixture was cooled and 5ml of n-butanol – pyridine (15:1), 1ml of distilled water was added and vortexed vigorously. After centrifugation at 1200g for 10min, absorbance was measure at 532nm using spectrophotometer. Absorbance was calculated using the relationship: A=ECl, where E= Molar absorptivity ($1.5\times10^5M^{-1}cm^{-1}$) C= Concentration, l=light path (1)

Vitamin c in serum was assayed by the method of Wilson and Gullan (1969). Exactly 0.5ml of serum was added to 2ml of freshly prepared TCA (6g/100ml) in test tubes and mixed well on a vortex mixer. This mixture was centrifuged for 10minutes at 2500rpm. 1.2ml of the clear supernatant was pipetted into the test tubes. The standards are prepared in duplicate. 1.2ml of TCA (6g/100ml) was added to two test tubes to use as blank. 0.4ml of dimitrophenyl hydrazine –thiourea-copper sulphate (DTCS reagent was added to all the tubes, which were capped, mixed and incubated in a water bath at 37^0c for 3hours). The tubes were removed from the water bath and chilled for 10minutes in ice bath, while mixing slowly. 2ml of cold $12MH_2SO_4$ was mixed and the mixture checked to make sure it did not exceed room temperature. The spectrophotometers were adjusted with the blank to zero absorbance at 520nm and the absorbance of standard and samples read. The concentration of vitamin C was calculated using the formula. 1ml of dye = 0.0143µg/AA. Concentration of Ascorbic acid = 0.0143 x Absorbance.

Concentration of glutathione was determined following the method of Raja *et al* (2007). Blood sample was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.01ml of this supernatant, 2ml of phosphate buffer (pH 8.4), 0.5ml of 5, 5-dithio, bis (2-nitrobenzoic acid) and 0.4ml double distilled water was added. Mixture was vortexed and the absorbance read at 412nm within 15mins.Absorbance of glutathione was calculated from the standard calibration curve (y=Mx) prepared by plotting absorbance of standard glutathione concentrations against their standard (known) concentrations when subjected to the same experimental conditions.

I. STATISTICAL ANALYSIS

The results of the antioxidants and liver enzymes were expressed as mean ± standard deviation, and the test of statistical significance was carried out using analysis of variance (ANOVA) at 95% confidence interval ($P<0.05$). All statistical calculations were performed with SPSS version 18 for windows.

J. Results of Body weight

There was a significant increase in body weight of exposed rats from week one to week three, and then a marked decrease in body weight for the fourth week compared to that of the control. This is shown I the table below.

Groups	Weeks				
	0(g)	1(g)	2(g)	3(g)	4(g)
A	137.40±1.18	139.38±11.45	143.0±11.70	153.6±13.92	101.4 ± 5.1
B	131.2±16.10	132.8 ± 17.22	137.0±16.44	175.8±24.59	122.6 ± 14.3
C	149.2±20.71	154.4 ± 20.91	158.0±20.86	165.8±20.14	161.0 ± 19.74
D	114.8±21.26	115.8 ± 20.70	119.2±22.04	123.2±21.75	126.6 ± 21.4

Table 1: Body weights of rats exposed to Nopest® pesticide (DDVP) once daily for 28 days

Values are expressed as Mean ± SEM.

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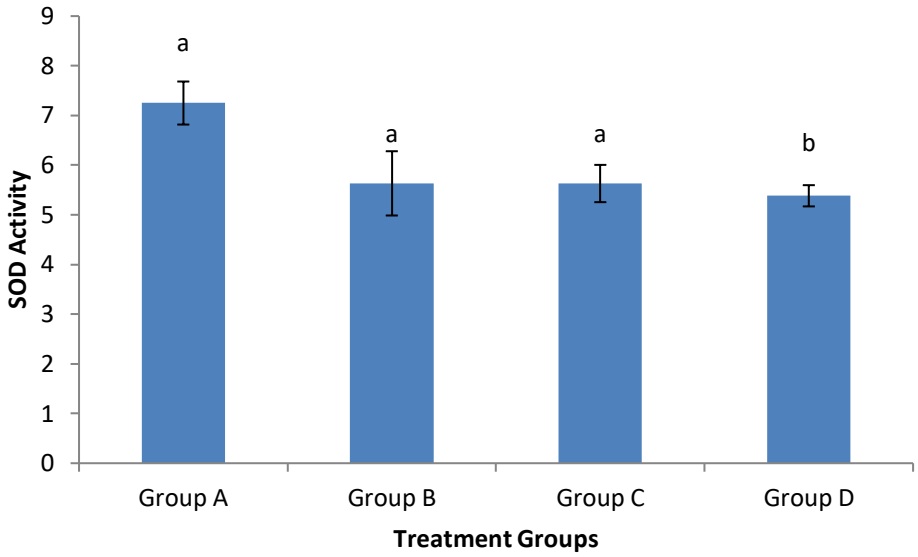

Fig. 1: Effect of oral administration of Nopest® pesticide on SOD activity in rats

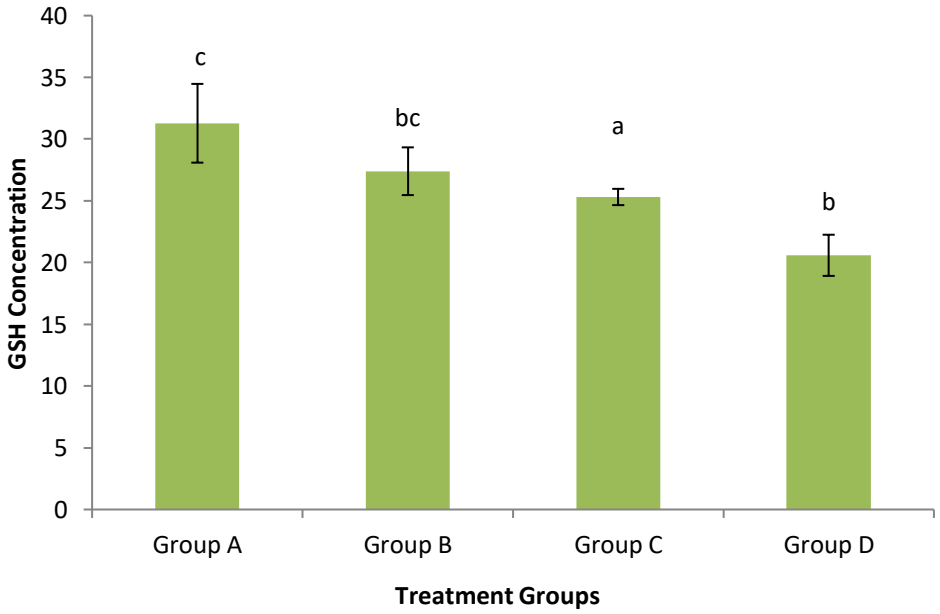

Fig. 2: Effect of oral administration of Nopest® pesticide on GSH concentration in rats

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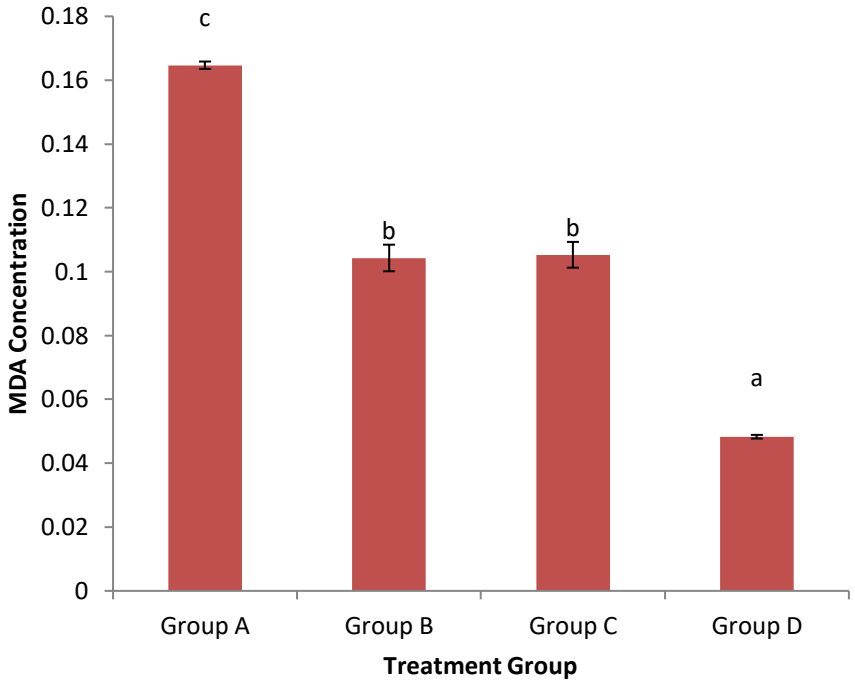

Fig. 3: Effect of oral administration of Nopest® pesticide on MDA concentration in rats

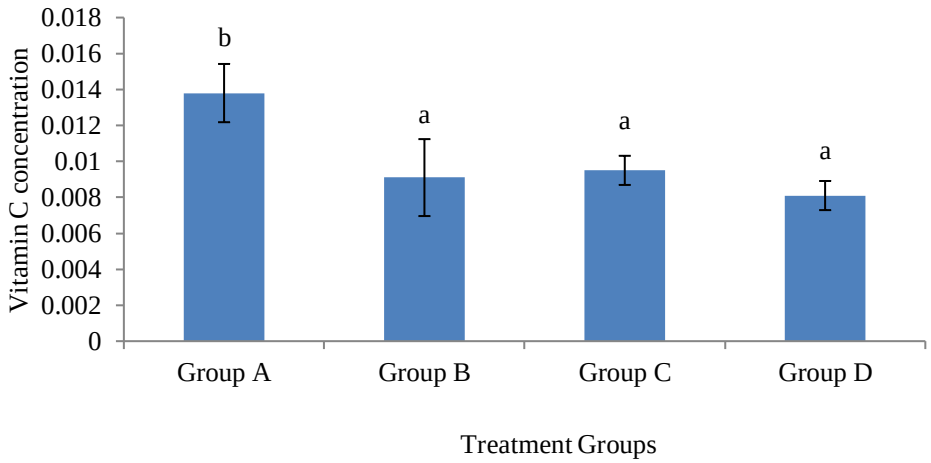

Fig. 4: Effect of oral administation of Nopest® pesticide on Vitamin C concentration in rats

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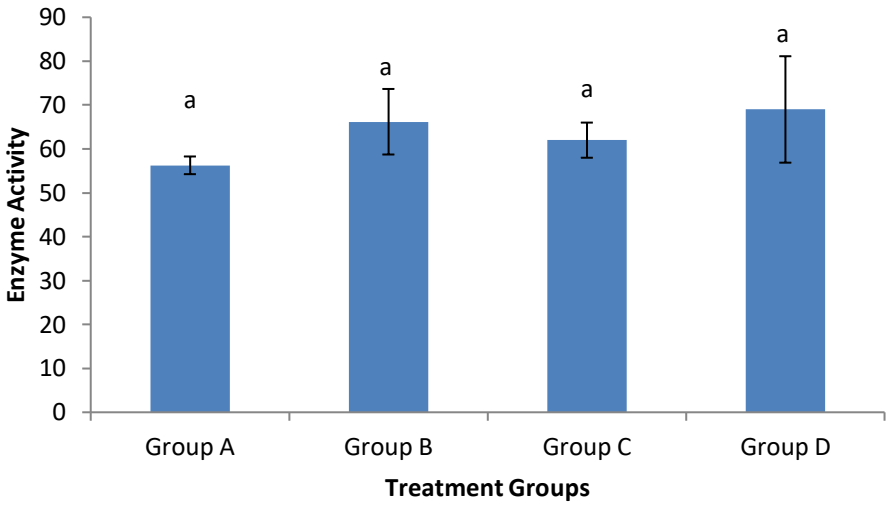

Fig. 5: Effect of oral administration of Nopest® pesticide on the activity of AST in rats

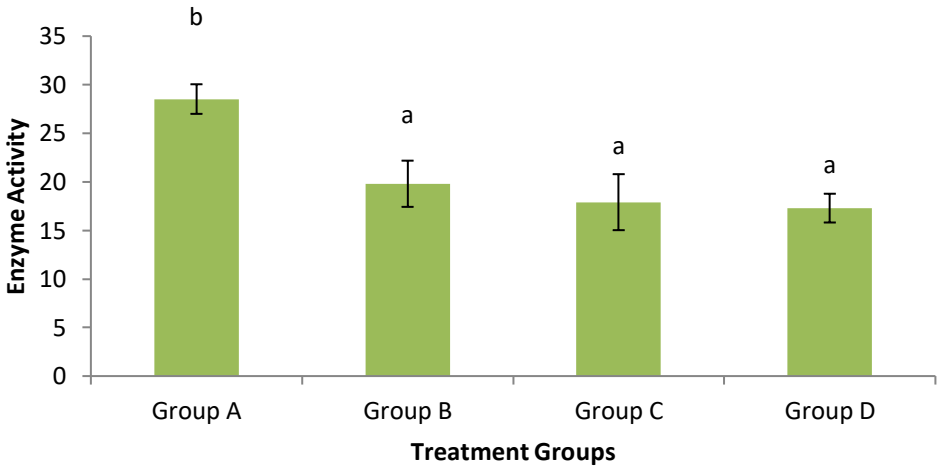

Fig. 6: Effect of oral administration of Nopest® pesticide on the activity of ALT in rats

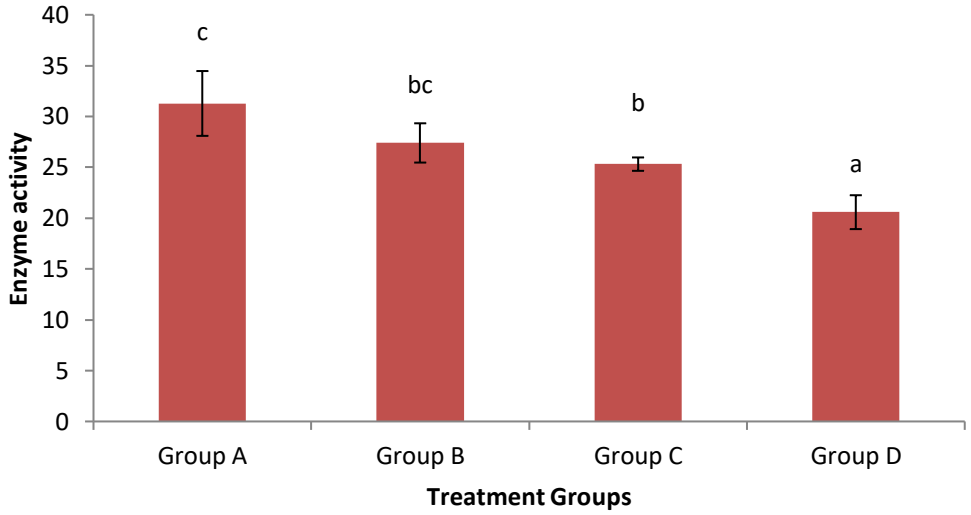

Fig. 7: Effect of oral administration of Nopest® pesticide on the activity of ALP in rats

III. DISCUSSION

The body weight of exposed rats increased from week one to week three, and then decreased significantly for the fourth week as compared to the control group. The rise in body weight in the exposed rats could be a result of the toxicant Norpest's detrimental effects. Ibrahim *et al* (2012), on the other hand, found a substantial drop in body weight in rats exposed to lead acetate, which was dose dependent. Changes in weight can be caused by a variety of variables, one of which is metabolic imbalance.

Serum enzyme levels are used as indicators of an individual's overall health, particularly in cases of hepatocyte injury and oxidative stress (Khan *et al*., 2009; Vasanth*et al*., 2012). As a result of normal cell turnover, small levels of intracellular enzymes are present in the blood. Increased amounts of enzymes are released and their concentrations in the blood rise when cells are damaged.

The increase in liver enzymes indicated that the Norpest pesticide was hazardous to the exposed rats' livers. Nwankwo*et al* (2019); Atef (2010) and Ajibose (2012), who subjected wistar rats to organophosphate insecticides, showed similar effects. A damaged liver is usually indicated by high ALT values, whereas malnutrition is usually indicated by low ALT levels (Nwankwo*et al*., 2019).

Furthermore, elevated MDA levels in Norpest-treated groups indicate lipid peroxidation caused by free radicals created by the insecticide (Ujowundu*et al*., 2020).

IV. CONCLUSION

According to the findings of the current investigation, Norpest has the potential to cause liver damage. These negative changes in liver function could be linked to reactive oxygen species-induced oxidative stress (ROS).

V. RECOMMENDATION

Based on the findings of this study, I recommend that pesticide applicators using Nopest (DDVP) for domestic, commercial, industrial, and agricultural pest management follow the manufacturers' guidelines/application instructions to the letter. To avoid absorbing the chemical, people should use protective gear such as hand gloves, boots, helmets, nasal masks, eye glasses, coats, and other items while applying it. They should also get regular medical checkups and laboratory tests to determine the antioxidant and liver enzyme levels in their blood.

- CONFLICT OF INTEREST: There are no conflicts of interest stated by the authors.
- AUTHORS CONTRIBUTION: All of the authors contributed equally to the final paper and gave their approval.

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