FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 9 No. 1, January, 2025, pp 137 - 141
DOI: https://doi.org/10.33003/fjs-2025-0901-2956

EFFECTS OF GRADED DILUTED DOSES OF GRAMOXONE HERBICIDE ON PROGESTERONE, TESTOSTERONE, ALBUMIN AND TOTAL PROTEIN OF MALE AND FEMALE ALBINO RATS (*Rattus norvegicus*)

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ABSTRACT

The use of herbicides no matter how diluted it maybe, may have physiological effects on mammals. This work tends to examine the effects dilution factor of 10^4 of a 10-fold serial dilution of gramoxon herbicide graded into 0.25, 0.5 and 0.75 mg/kg body weight, orally administered to twenty male and twenty female rats has on progesterone, testosterone, albumin and total protein of the rats. The weights of the rats ranges between 79 - 119g and were divided randomly into group A, B, C and D. Each group has 10 animals, 5 male and 5 female. Group A is the control. Group A, B.C and D were fed with feed and water ad libitum. Group B, C and D were administered 0.25, 0.5 and 0.75mg/kg bw dose respectively. After 28 days the rats were sacrificed and blood collected into EDTA bottles for laboratory assessment. Computer based ANOVA excel showed that albumin and total protein revealed a significant difference ($p<0.05$) in group B (0.25mg/kg bw), C (0.5mg/kg bw) and D (0.75mg/kg bw) in both sex compared with control. Testosterone and progesterone level showed a significant decrease ($p<0.05$) compared with control in group B, C and D. The results clearly indicated that gramoxon herbicide has biochemical and reproductive effects on albino rats even at 10-fold dilution.

Keywords: Gramoxon, Albumin, Testosterone, Progesterone, Total protein

INTRODUCTION

The use of gramoxon herbicide (paraquat) by most local people is to control weeds and to reduce the strenuous physical practice of weeding by the use of cutlasses and hoes. Weeding is a process that involves bending or squatting on the farm(s) when carrying out the process. Weeding leads to back and leg pains, leg and finger injuries from cuts and it is time consuming. This physical process has drastically reduced in most communities in Delta State, Nigeria as a result of the use of herbicide to control weeds. Due to increase in population (Mehta, 2001) in the locality and urban areas, the need to increase food production is not a thing to think about twice because food is life. To increase food production, natural and organic fertilizers were replaced by chemical fertilizers and locally made pesticides were replaced by chemical pesticides and started many years back (Parayil, 1992). Gramoxone herbicide is one of the herbicides use in farming both peasant and commercial way of food production. Gramoxon herbicide has active ingredient called paraquat that has sub-acute toxicity to animals and man as reported (Dawson *et al.*, 2010). Paraquat which is the toxic ingredient in gramoxon herbicide is in the bipyridinium compounds and molecularly named as 1,1'-dimethyl 4,4'-bipyridinium (Raghu *et al.*, 2013). Because of the harmful nature of paraquat, the US of America authorized that only qualified farmers are allowed to utilize it (Cheryl and Poonam 2021). The Centre of Disease Control and Prevention (CDC) reported that, one can get exposed to paraquat through the ingestion of paraquat contaminated food, through inhalation, or skin exposure (CDC 2018). Paraquat (gramoxon herbicide) toxicity has chronic problem which can result to respiratory, hepatic, renal organs abnormalities and Parkinsonian lesions including fibrosis (He *et. al.* 2012). In male albino rats, gramoxon (paraquat) exposure leads to reduction in weight of genital materials such as testes, epididymis, seminal vesicles, prostate, sperm number and cell multiplication of spermatogonial stem cells (Hemayatkhah *et. al.*, 2008). The exposure to paraquat herbicide is toxic to progesterone in female albino rats by decrease in the hormone (Elham *et.al.*, 2015). This research examined the toxicological effects of gramoxon herbicide diluted in ten-fold with a dilution factor of 10^4 diluent, administered in graded 0.25, 0.5 and 0.75mg/kg bw sublethal doses on the albumin, total protein, testosterone and progesterone of male and female albino rats.

MATERIALS AND METHODS

Animals

All procedure adopted in the experiment in regards to animal handling complied with the International Guideline as stated by the Ethical Committee, Faculty of Science, University of Port Harcourt, Rivers State. The rats were purchased in the Animal House, Department of Animal and Environmental Biology, University of Port Harcourt and were transported to the laboratory of Animal and Environmental Biology, Dennis Osadebay University, Asaba Delta.

Acclimation

The animals were acclimated for 7 day so that they will get use to the laboratory condition of the laboratory of their new environment before the start of the experiment.

Number of rats used

A total of 20 male and 20 female albino rats weighing 79 - 119g were the number of animals used. Animals were procured in the Animal House, University of Port Harcourt, Rivers State. The purchase was done under standard laboratory conditions. Water and feeds were given at ad libitum.

The Purchase of gramoxon herbicide

Gramoxon herbicide was purchased from Choba Market, opposite University of Port Harcourt, Rivers State Nigeria.

Experimental design

Twenty 20 male and 20 female albino rats of variable weights 79-119g were randomly grouped into group A (control), B, C and D (treatment groups). A group contains 10 rats, (5 male and 5 female). Group B, C and D were treated with graded

0.25, 0.5, 0.75mg/kg b.w orally by gavage, using a 1ml syringe respectively. The graded amounts of doses were administered for 28 days to the animals and they were starved for one day prior to their decapitation.
At the end of 28 days, the animals under anaesthesia with chloroform were decapited and blood samples were collected and dispersed into Ethylenediaminetetraacetic acid (EDTA) tubes for total protein, albumin, testosterone and progesterone levels analysis.

Total protein analysis
The Biuret method as explained by Gornall *et al.* (1949) was used to determine protein level in the blood. Three (3) clean test tubes were labeled for blank, standard and sample accordingly. In each of the test tubes 0.02 ml of distilled H_2O, standard protein and serum were dropped accordingly. 1.0 ml of total protein Reagent 1 was added and stirred evenly and incubated at 25°C for half an hour. Absorbance of sample and standard were read against reagent blank at 500 nm and protein concentration calculated.

Albumin analysis
The albumin was analyzed employing the method of Doumas *et al.* (1971). Three test- tubes were labeled as blank, standard and sample. 10 ml of deionized water, standard albumin and serum was added into the test-tube respectively. 1.0 ml Reagent A (containing Bromocresol reagent) was then added into all tubes, stirred well and incubated at 25°C for 10 min. Absorbance of sample and standard were read against blank at 630 nm and albumin concentration calculated.

Testosterone and Progesterone analysis
Testosterone and progesterone levels were analyzed using the ELISA test kit (Tietz, 1986) and (Engvall, 2000) as used by (Airhomwanbor et. al, 2024)

Data Analysis
Data levels of albumin, total protein, progesterone and testosterone were analyzed using ANOVA computer based-excel software package and SPSS version 20. Data significant level was at P (0.05).

RESULTS AND DISCUSSION

Male total protein, albumin and testosterone
The total protein of the male rats exposed to 0.25mg/kg bw, 0.5mg/kg bw and 0.75mg/kg bw doses had a steady decrease according to the increase in doses compared to control group. Albumin experienced an increase in 0.5mg/kg bw dose, a decrease in 0.25mg/kg bw dose and no different in level in 0.75mg/kg bw with the control group. There was significant reduction in testosterone in male rats in an ascending order of administered doses of 0.25mg/kg, 0.5mg/kg and 0.75mg/kg compared with control (Table 1 and fig, 1, 2 and 3)

Table 1: Male total protein, albumin and testosterone results

Groups	Total protein g/l	Albumin g/l	Testosterone ng/mL
A (control)	95.00±1.78^a	37.20±0.76^a	1.92±0.21^a
B (0.25mg/kg)	86.00±2.28^b	35.20±0.96^c	1.31±0.29^b
C (0.5mg/kg)	86.00±4.25^b	38.60±0.92^d	0.27±0.02^c
D (0.75mg/kg)	88.40±5.00^c	37.20±0.37^b	0.25±0.03^c

Data are given as mean ± SEM of significance difference p>0.05

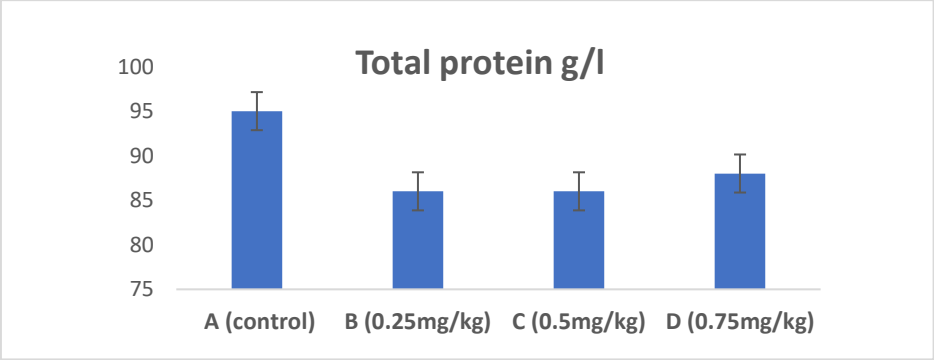

Figure 1: Bar chart of total protein showing the level of total protein and the error bar of each treatment

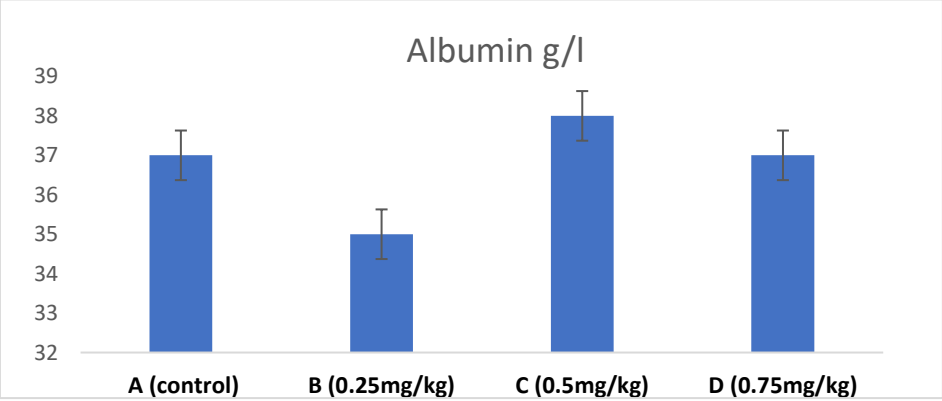

Figure 2: Bar chart of albumin showing the level of albumin and error bar of each treatment

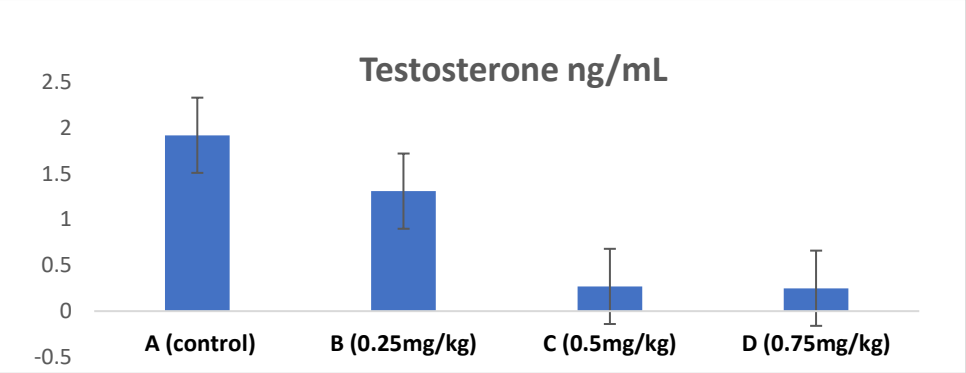

Figure 3: Bar chart of testosterone showing the level of testosterone and the error bar of each treatment

Female total protein, albumin and testosterone

There was a decrease in total protein level in all treated groups of various doses compared with control. Albumin had an increase in 0.5mg/kg bw dose and no significant variation in 0.25mg/kg bw and 0.75mg/kg bw doses compared with control. Progesterone experienced a significant decrease in group B (0.25mg/kg), C (0.5mg/kg) and D (0.7mg/kg) when compared with control. (Table 2 and fig.4, 5 and 6)

Table 2: Female total protein, albumin and progesterone results

Groups	Total Protein g/l	Albumin g/l	Progesterone ng/mL
A (control)	88.40±1.80[a]	36.40±0.40[a]	5.82±0.16[a]
B(0.25mg/kg)	85.40±1.72[b]	36.80±0.80[b]	5.09±0.11[c]
C(0.5mg/kg)	83.60±1.63[c]	40.00±2.16[c]	3.89±0.20[b]
D(0.75mg/kg)	85.64±1.63[b]	36.00±0.31[b]	3.47±0.47[b]

Data are given as mean ± SEM of significance difference p>0.05

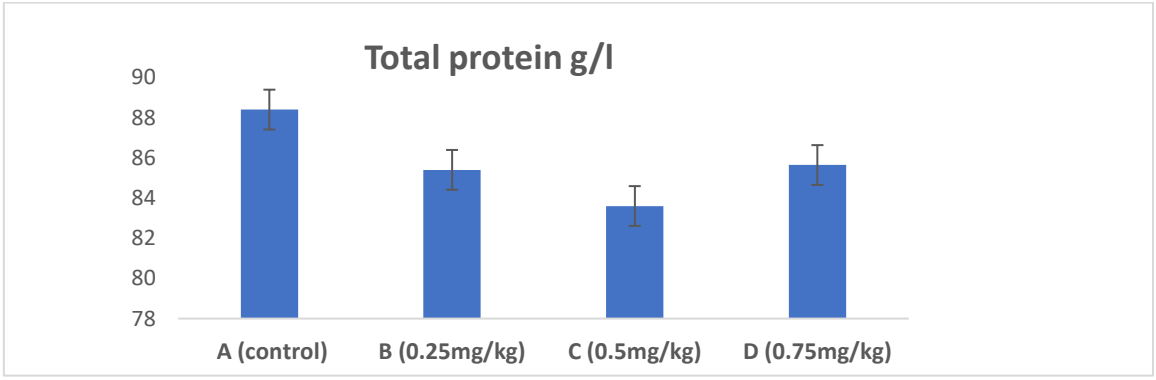

Figure 4: Bar chart of total protein showing the level of total and the error bar of each treatment

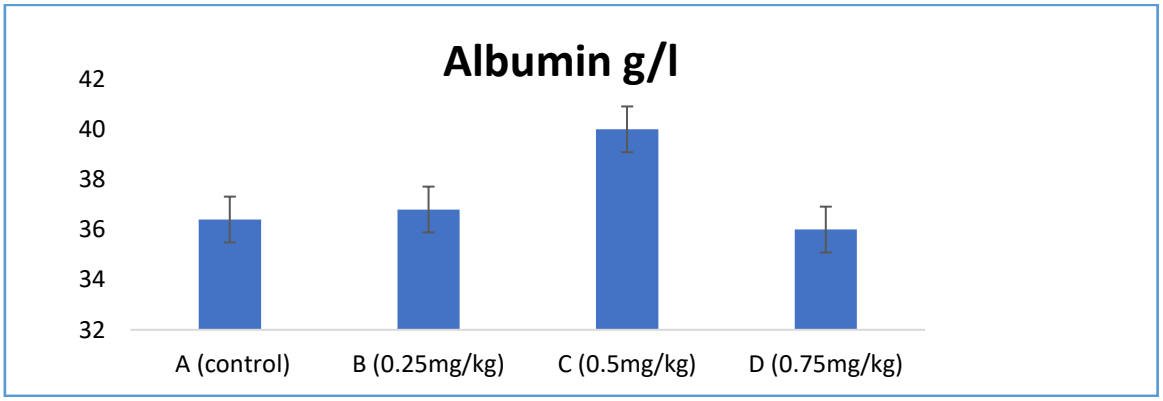

Figure 5: Bar chart of albumin showing the level of albumin and the error bar of each treatment

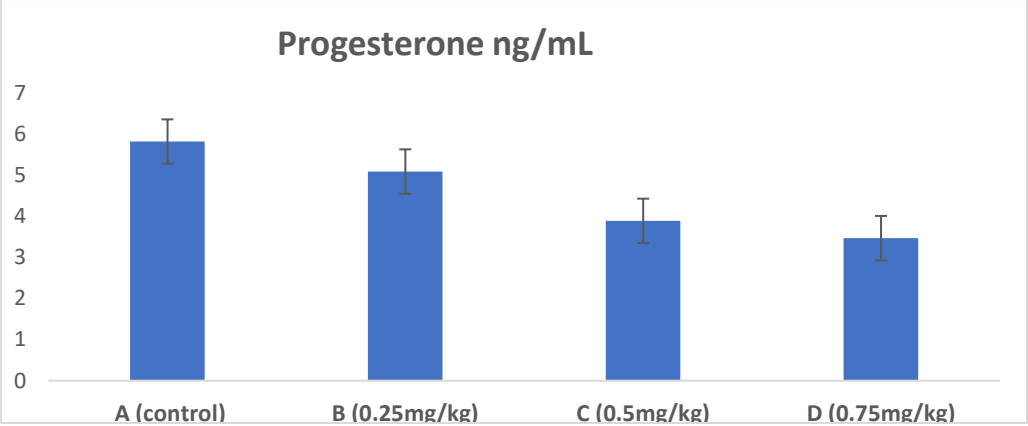

Figure 6: Bar chart of progesterone showing the level of progesterone and error bar of each treatment

Discussion

Gramoxon herbicide is a broad-based spectrum herbicide, that kills both useful and weed plants in the farm and also use to control weeds in the immediate surroundings in homes. In the farms especially in rural farms, animals may get affected when they feed on the plants in the farm after the use of the herbicide in the diluted form. Humans are also exposed to exposed to diluted gramoxon herbicide through inhalation of the herbicide fine molecules during spraying of the herbicide to control weeds on the farms without the use of personal protective equipment (PPE). This work is a further evaluation of gramoxone herbicide detrimental effect even though at a diluted and minute doses of the gramoxon herbicide have on total protein, albumin, progesterone and testosterone.

Total protein in both male and female showed a significant decrease compared with the control. Total protein generally serves as a measure of the amount of albumin and globulin. A decrease in total protein is a sign of kidney and liver problem. This finding is in line with the report of (Lalruatfela et. al 2014) that stated the exposure to paraquat result to reduction in total protein which would equally affect the albumin.

There was a significant reduction of testosterone level compared with the control after the 28 days of chronic exposure even at a ten-fold serial dilution and in minute graded doses. This supports the findings of (Fu *et. al.*, 2019) which reported that Long time exposure to pesticides in micro doses, result to major damage to animals and including human beings. The decrease of testosterone was supported by the reports of (*Aktas, et al.* 2012), that explained that the reduction of steroidogenic acute regulatory (StAR) enzymes by paraquat (active ingredient in gramoxone herbicide) an enzyme use for protein and the control of the movement of cholesterol inside the mitochondria for the production of testosterone was the cause of the decrease in testosterone level of the male rats. This work further indicated that gramoxon herbicide (paraquat as active ingredient) irrespective of its dosage will pose reproductive complication through the abnormal reduction of reproductive hormones and this will lead to reproductive challenges in male and female animals. Because of the close resemblance in physiology of albino rats and humans, hormonal abnormality resulted from exposure of albino rats in minute doses such as 0.25, 0.5 and 0.75 mg/kg bw at a dilution factor of 10^4 in a ten-fold serial dilution, one can extrapolate such effects to humans.

In this research there was a reduction in progesterone that support the findings of (Ryszard *et.al.*, 2016) where paraquat brought about the inhibition of progesterone. This research also supports the research done by (Elham *et, al*., 2015) on female albino rats exposed to paraquat herbicide which showed a significant decrease in progesterone.

CONCLUSION

It will be concluded that gramoxon herbicide diluted in a ten-fold serial dilution and a dilution factor of 10^4 of the diluent administered in graded minute doses of 0.25, 0.5 and 0.75mg/kg body weight has reproductive hormonal and biochemical effects.

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