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Research Article
Injurious Effects of Glyphosate on Sperm Profile and Testicular Tissues of Wistar Rat

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Abstract

Background and Objective: Despite the agricultural importance of glyphosate, there are concerns bordering on their effects on the environment and man. This study was, therefore, designed to analyze the adverse effects of glyphosate on sperm profile and testicular architecture of Wistar rat. **Materials and Methods:** Twenty four mature male Wistar rats were divided into 4 groups (A-D) of 6 rats each in a completely randomized design. Group A served as the control, groups B, C and D received 100, 200 and 300 mg kg^{-1} b.wt., of glyphosate, respectively. Treatments were administered via oral gavage for 2 months after which the animals were sacrificed under chloroform anesthesia 24 h after the last dose. Sperm and testes samples were collected for examination and analyses. Data obtained were analyzed by using one way Analysis of Variance (ANOVA). **Results:** The results revealed a significant ($p<0.05$) and dose dependent decrease in testes weight, mean sperm motility, viability and count and an increase in sperm head abnormality of rats administered glyphosate treatment compared to the control. Testes photomicrograph of glyphosate treated rats showed mild to severe degeneration of sertoli cells, eroded interstitial cells and atrophy of some spermatogonia cells which was indications of testicular toxicity. **Conclusion:** Therefore, glyphosate adversely affected sperm quality, sperm quantity and destroyed the cellular architecture of the testes of Wistar rats. These findings indicate that glyphosate could induce infertility in males.

Key words: Spermatoxic effect, testicular toxicity, herbicides, glyphosate, fertility, wistar rat

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Herbicides are substances used to control plants that are not wanted (weeds). Selective herbicides control specific weeds leaving the desired crop relatively unharmed, while the non-selective herbicides kill every plant within its reach. Herbicides have acute toxicity resulting from rapid ingestion of a significant quantity and chronic toxicity resulting from environmental and occupational exposure over a long period of time. Some herbicides cause series of health effects ranging from skin rashes to death. The routes of exposure include direct consumption, improper application resulting in the herbicide coming in contact with people or wildlife and inhalation of aerial sprays. Herbicides can be transported via leaching or surface runoff to contaminate groundwater or distant surface water sources and the properties that increase the likelihood of transport include persistence and high water solubility[1].

Glyphosate is a broad spectrum systemic herbicide and crop desiccant with the IUPAC name N-phosphonomethyl glycine. It is a water soluble aminophosphonic analogue of glycine which inhibits the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase. It is used to kill weeds including annual broad leaf and woody plants and grasses which compete with crops. It was first sold for agricultural use under the trade name Roundup. Glyphosate is reportedly the most used herbicide in agriculture and the second most used in homes and gardens[2]. Between 1970 and 2016, there have been a hundred fold increments in the frequency and volume of application of glyphosate based herbicides globally with further increases expected in the future[3]. Glyphosate adsorbs strongly to soil and the residues are generally immobile in soil. It also has the potential to contaminate surface waters through erosion[4].

Despite the approval of glyphosate by regulatory bodies, concerns bordering on their effects on the environment and man persist[5]. This study was, therefore, designed to analyze the adverse effects of glyphosate on sperm profile and testicular architecture of Wistar rat.

MATERIALS AND METHODS

Experimental materials: The study was carried out in the animal house of the Department of Genetics and Biotechnology, University of Calabar, Nigeria from September-December, 2018. Glyphosate was purchased from the Department of Agrochemicals, Ministry of Agriculture, Calabar, Nigeria.

Experimental animals/procedure: Twenty-four sexually mature male Wistar rats with body weight ranging from 180-200 g were purchased from the Department of Physiology, University of Calabar, Nigeria. The animals were housed in aluminum cages covered with wire mesh. They were fed growers mash and allowed unrestricted access to clean water. They were handled in accordance with the guidelines for care and use of laboratory animals as stipulated by the Animal Genetics research committee of the Department of Genetics and Biotechnology.

Following 7 days of acclimatization, the animals were divided into 4 groups (A-D) of 6 rats each, in a completely randomized design. Animals in group A served as the control and received feed and water only. Animals in groups B, C and D received 100, 200 and 300 mg kg^{-1} b.wt., of glyphosate, respectively. Treatments were administered via oral gavage for 2 months after which the animals were sacrificed under chloroform anesthesia 24 h after the last dose was given.

Estimation of sperm profile: Testes and epididymis were surgically removed and weighed by using an electronic weighing balance (Scout-pro: 3000 g). The epididymis were placed in physiological saline in the ratio of 1:10 (w/v) and macerated to release sperm cells. After pipetting, the suspension was filtered with an 80 µm stainless wire mesh and then used for sperm parameters analysis[6].

Estimation of sperm motility: Sperm motility was estimated by placing 2 drops of sperm suspension on a sterile slide and then placing a cover slip over it. Five slides were prepared for each sample in quick succession and examined. The number of motile cells divided by the total number of spermatozoa counted under ×40 objective lens was determined and expressed in percentage[7].

Determination of sperm viability: Sperm viability was determined using the eosin-nigrosin staining technique. Two drops of sperm suspension was mixed with an equal volume of stain. The mixture was smeared on sterile slides and allowed to air-dry. Five air-dried smears were prepared for each sample and examined by using x40 objective lens of light microscope[6]. Live sperm cells appeared whitish, while dead ones took up the stain and appeared pinkish. The number of live cells divided by the total number of cells examined was expressed in percentage[7].

Estimation of sperm head abnormality: To estimate sperm head abnormality, 2 drops of sperm suspension was mixed with an equal volume of 1% eosin Y solution. The mixture was smeared on sterile slides and allowed to air-dry. Five air-dried smears were prepared for each sample and examined with ×100 objective lens of light microscope. The number of abnormal sperm heads in every 200 spermatozoa was determined and expressed in percentage[6,7].

Estimation of sperm count: A cover slip was placed on the improved Neubaeuer[3] (2.5×10^4 mm, Hawksley, England) hemocytometer and a fine pore capillary tube was used to charge it with sperm suspension[6,7]. Sperm cells were examined and counted under ×40 objective lens of light microscope. Sperm count was expressed in million-cells mL^{-1}.

Histology of testes: Testes tissues were fixed in formal saline, dehydrated with graded concentration of alcohol, immersed in chloroform for 30 min and later in xylene for 15 min to remove alcohol. Tissues were immersed in molten paraffin wax twice an hour, de-waxed, transferred into embedding moulds, cut into 2-4 µm diameter sections with a microtome (Shandon AS325) and floated in water bath to flatten the sections. These sections were then placed on albumenized slides, heat fixed, de-waxed in xylene and dehydrated in alcohol. The slides were stained with haematoxylin for 20-30 min, rinsed in clean water, counter stained with 1% eosin for 5 min, fixed with alcohol and cleared with xylene. Prepared slides were examined with ×40 and ×100 objective lenses of light microscope and then photomicrographs were taken.

Statistical analysis: Data obtained from this study were subjected to one way Analysis of Variance (ANOVA) by using Predictive Analytic Software (PASW) version 18.0. Least Significant Difference (LSD) was used to separate means that were significant at $p<0.05$.

RESULTS

Weight of testes and epididymis: The result revealed a significant ($p<0.05$) and dose dependent decrease in testes weight of rats administered glyphosate compare to those in the control group. The mean testes weight of rats in the control group was 2.02 g while, the least testes weight (0.82 g) was recorded in rats administered 300 mg kg^{-1} b.wt., of glyphosate (Table 1). Similarly, there was a significant ($p<0.05$) and dose dependent decrease in mean epididymal weight of rats administered glyphosate compared to those in the control group. Least epididymal weight (0.58 g) was recorded in rats administered 300 mg kg^{-1} b.wt., of glyphosate, whereas, the epididymal weight of rats in the control was 1.04 g (Table 1).

Sperm profile: Mean sperm motility, viability and count of rats administered glyphosate treatment reduced significantly ($p<0.05$) and dose dependently compared to those in the control group. The mean sperm motility, viability and count for the control group were 81.2 and 65.0% and 18.4 million cells mL^{-1}, respectively. However, the least mean sperm motility (48.4%), viability (48.0%) and count (10.4 million cells mL^{-1}) were recorded in rats administered 300 mg kg^{-1} b.wt., of glyphosate (Table 1). Conversely, mean sperm head abnormality increased significantly ($p<0.05$) and dose dependently in rats administered glyphosate compared to those in the control. Rats administered 300 mg kg^{-1} b.wt., of glyphosate had the highest sperm head abnormality (8.30%) compared to those in the control which had the least abnormality (1.84%).

Histology of the testes: Photomicrograph of the testes of rats in the control is shown on Fig. 1. The result revealed numerous seminiferous tubules of various sizes and shapes containing spermatogonia at various levels of maturation. The interstitium within which are leydig cells appeared to be normal. Basement membranes are intact, as sertoli cells are seen. Thus, the testes integrity of rats in the

Table 1: Effects of glyphosate on sperm profile, testicular and epididymal weight of male Wistar rats

Sperm parameters	A	B	C	D
Sperm motility (%)	81.2 ± 0.80^d	70.2 ± 2.22^c	61.6 ± 2.01^b	48.4 ± 2.87^a
Sperm viability (%)	65.0 ± 1.84^d	58.8 ± 0.86^c	54.0 ± 1.34^b	48.0 ± 1.96^a
Sperm abnormality (%)	1.84 ± 0.24^d	4.27 ± 0.31^c	6.30 ± 0.62^b	8.30 ± 0.46^a
Sperm count (10^6 mL^{-1})	18.4 ± 0.50^d	15.8 ± 0.37^c	13.8 ± 0.66^b	10.4 ± 0.51^a
Testes weight (g)	2.02 ± 0.04^d	1.68 ± 0.08^c	1.26 ± 0.05^b	0.82 ± 0.23^a
Epididymis weight (g)	1.04 ± 0.04^c	0.88 ± 0.04^b	0.78 ± 0.04^b	0.58 ± 0.04^a

Values are presented as mean±standard error mean, Means followed by the same case letter (a, b and c) along the horizontal array indicate no significant difference ($p<0.05$), Letters a, b, c and d represent least significant difference used to separate the means, Group A: Served as the control, Group B: Received 100 mg kg^{-1} b.wt., of glyphosate, Group C: Received 200 mg kg^{-1} b.wt., of glyphosate, Group D: Received 300 mg kg^{-1} b.wt., of glyphosate

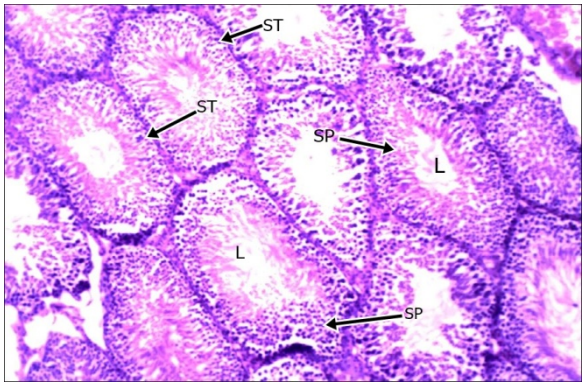

Fig. 1: Testis photomicrograph of rats in the control group, (H/E ×100)
ST: Seminiferous tubules, L: Lumen SP: Spermatogonia

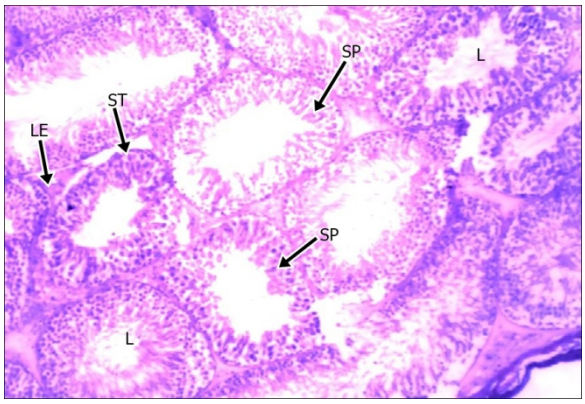

Fig. 2: Testis photomicrograph of rat administered 300 mg kg^{-1} b.wt., of glyphosate (H/E ×100)
ST: Seminiferous tubules, L: Lumen SP: Spermatogonia

control was not compromised. On the contrary, glyphosate caused varying degrees of lesions on the cellular architecture of the testes. Testes photomicrograph of glyphosate exposed rats showed mild to severe degeneration of sertoli cells, eroded interstitial cells and atrophy of some spermatogonia cells which are indications of testicular toxicity (Fig. 2).

DISCUSSION

The results revealed a significant ($p<0.05$) and dose dependent decrease in testicular and epididymal weights of rats administered glyphosate compared to the control. The decrease in testes weight could be due to reduction in tubule size, number of germ cells or spermatid[8]. Decrease in the level of serum testosterone, follicle stimulating and luteinizing hormones could affect gonad weight which is in tandem with other findings[9].

Sperm profile analysis revealed that mean sperm motility, viability and count of rats administered glyphosate reduced significantly ($p<0.05$) and dose dependently compared to the control. Reduction in sperm count of exposed rats could be due to decrease in testosterone level. Low levels of follicle stimulating and luteinizing hormones could also inhibit spermatogenesis[10]. Decrease in sperm count is in tandem with the findings of other researchers[11-14]. Furthermore, reduction in sperm motility could be caused by decrease in mitochondrial activity, altered fructose synthesis or corrosion of microtubule structure of spermatozoa[15,16]. The inhibition of sperm motility in rats administered glyphosate may be as a result of low ATP level[17,18]. In this study, reduction in sperm viability corroborated reduction in sperm motility and the reduction could be due to the impact of glyphosate on the epididymis of exposed rats[19].

Conversely, mean sperm head abnormality increased significantly ($p<0.05$) and dose dependently in rats administered glyphosate compared to those in the control. Sperm abnormalities induction suggested possible point mutations in reproductive cells[20]. These abnormalities usually occur during transit, maturation and storage[21]. Reactive oxygen species which is reportedly induced by pesticides is known to adversely affect sperm motility, viability and increase sperm head abnormalities[22,23]. The decrease in sperm motility, viability and increase in sperm head abnormalities observed in this study were in agreement with the reports of Zidan[12] and Sai *et al.*[24].

Histopathological examination of the testis, which is an accessory organ responsible for producing spermatozoa and sex hormones revealed degenerative changes in germ cells and germinal epithelium induced by glyphosate exposure. This is in tandem with the findings of previous researchers who reported variable degree of degeneration and accumulation of cellular debris in the seminiferous tubules up to total cellular destruction after exposure to different pesticides[25,26]. Testicular damage arising from glyphosate exposure in this study corroborated the observed lowered fertility of exposed rats. The implication of this study is that glyphosate exposure can inhibit fertility in males. Similar investigation should be carried out in female Wistar rat to evaluate the effects of the herbicide since this study was limited to male reproductive indices.

CONCLUSION

This present study demonstrates that glyphosate adversely affected sperm quality and quantity and

degenerated the cellular architecture of the testes of Wistar rats. These findings, therefore indicate that glyphosate could induce infertility in males. However, further investigation should be conducted on the effects of glyphosate on a broader range of physiological indices.

SIGNIFICANCE STATEMENT

This study unveiled the spermatotoxic effect and testicular toxicity of glyphosate in exposed Wistar rat. Thus, this study will aid researchers to uncover the critical areas of reproductive toxicity of glyphosate that other studies were not able to explore.

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