



Note

Effect of herbicide based formulations on structure and function of testis in rats

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Herbicides are known to add toxicity to the soil as their side effect. Farmers use either Paraquat based (PBF) or Glyphosate based (GBF) herbicide formulations for instant management of weeds. Here, we investigated the effect of PBF Gramoxone® and GBF Roundup® on the structure and function of the testis of the male Wistar rats at the pubertal or post pubertal stage. Male rats were gavaged with Gramoxone® (5 mg/kg body wt.) or Roundup® (250 mg/kg body wt.). The treatment period of 25 days starts on a postnatal day (PND) 28 to 53 (from pre-pubertal to the pubertal stage) or 60 days from PND 28 to 88 (from prepubertal to the post pubertal stage). Age-matched control animals received distilled water. The structure of the testes was evaluated by observing changes in its histology and the function of the testes was assessed by estimating serum follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone levels. No significant effect was observed in the structure and function of the testes of the pubertal stage rats on exposure to both herbicide formulations. However, shrunken and distorted seminiferous tubules with oligozoospermia in testes, a non-significant decrease in FSH and LH, and a significant reduction in testosterone levels were noted in the post pubertal stage rats. The results indicated that changes in the testes of post pubertal groups are due to a longer duration of exposure to the Gramoxone® or Roundup® than in pubertal groups, and also the effect of these two formulations was more on the testis structure and function than on the pituitary-testis axis.

Keywords: Follicle-stimulating hormone (FSH), Gramoxone®, Luteinizing hormone (LH), Post pubertal stage, Roundup®, Testosterone, Weed management

The presence of weeds reduces crop yields by 30-34%¹⁻³. For immediate weed management, herbicide formulations (HFs) are used as they are economically inexpensive compared to manual weeding^{4,5}. Paraquat based formulation (PBF) and Glyphosate based formulation (GBF) are the world's most profoundly used HFs⁶⁻¹⁰. Paraquat of PBFs produces free radicals that cause oxidative damage to the membrane lipids and mitochondria, resulting in

metabolic disturbances and the death of the weed¹¹. Glyphosate the main ingredient of GBFs competitively inhibits 5-enol-pyruvylshikimate-3-phosphate synthase, leading to disruption in the synthesis of aromatic amino acids¹². Due to protein deficiency weed growth ceases, followed by cellular disruption and death. Glyphosate also chelates micronutrients Ca²⁺, Mg²⁺, Cu²⁺, Fe²⁺, Co²⁺, Ni²⁺ and Zn²⁺ and interferes with biochemical pathways¹³ and the immune system of plants¹⁴.

Humans get exposed to PBFs and GBFs through air¹⁵, water¹⁶⁻¹⁸ and food^{7,19,20}. In recent years the concern about endocrine and reproductive toxicity due to exposure to PBFs and GBFs has increased^{7,21}. The testis is the main organ of the male reproductive system. It contains seminiferous tubules and in the interstitium of the testis, Leydig cells are present²². Each seminiferous tubule is lined with germ cells that develop into sperms under the influences of pituitary follicle stimulating hormone (FSH). Leydig cells produce testosterone under a feedback mechanism from the pituitary luteinizing hormone (LH).

Glyphosate and its formulation cause an increase in FSH²³, decrease in FSH²⁴, increase in LH and testosterone^{23,25}, and reduction in testosterone²⁶⁻³⁰. Also, studies are reporting, no significant effect on LH and FSH levels³¹ and testosterone³². These differences could be related to experimental design, duration of exposure, and type of GBF formulation tested. Paraquat and its formulation data on reproductive hormones are few^{33,34} as compared to GBFs. Considering these reports, here, we studied changes in the structure of testis along with its function (reproductive hormones) of pubertal or post pubertal rats on exposure to PBF Gramoxone® or GBF Roundup®. The structure was assessed by observing changes in the histology of testes and the function was evaluated by estimating FSH, LH and testosterone levels in the serum of the rats.

Materials and Methods

Animals and treatment

All the experimental procedure and sacrifice of rats in this study was carried out as per the guidelines and protocols (VP-140612-01 dated 31st January 2015) approved by the Institutional Animal Ethics

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Twenty days old Wistar (*Rattus norvegicus*) male rats were procured from the Bharat Serum Limited, Wagle Estate, Thane, Committee for the purpose of control and supervision of experimental on Animals (CPCSEA) -103/1999 and kept in the Animal house of Ramnarain Ruia College, Mumbai, CPCSEA No-315, under controlled temperature (20-24°C), humidity (40-50%), light (12 hour light/dark). Food and water are provided *ad libitum*.

Chemicals

Roundup® manufactured by Monsanto India Limited. It is a chemical composition of isopropylamine salt of a Glyphosate 41% (w/w) and other relevant (inert) ingredients 59% (w/w). Gramoxone® from Syngenta India Limited, contains paraquat dichloride 24% (w/w), nonylphenol ethylene oxide condensate 1% (w/w), cocoamine ethoxylate 4% (w/w), silicone defoamer 0.1% (w/w), acid blue 9 0.05% (w/w), triazolo (1,5.9)-pyrimidine 0.05% (w/w) and water.

Experimental protocol

After one week of acclimatization, rats were divided into six experimental groups with five animals in each group. Rats were orally exposed to Gramoxone® or Roundup® from postnatal day (PND) 28 to 53 (total 25 days) or from PND 28 to 88 (total 60 days). The treatment period PND 28 to 53 and PND 28 to 88 indicates animal exposure to the HF's from prepubertal to the pubertal stage or prepubertal to the post pubertal stage, respectively. Details of the experimental groups are as follows: Gr. I & IV, control animals for 25 and 60 days; Gr. II & V orally treated with Gramoxone® @5 mg/kg body wt. for 25; and Gr. III & VI orally treated with Roundup® @250 mg/kg body wt. for 25 and 60 days, respectively. Gramoxone® or Roundup® solutions were freshly prepared on a daily basis depending on the body weight of animals. At the end of treatment, period animals were sacrificed blood samples and testes were collected for estimation of hormone levels and histopathological changes, respectively.

Hormone estimation

FSH, LH, and testosterone levels in the serum were determined by a fully automated bidirectionally interfaced chemiluminescent immune assay method using Beckman Coulter Access at AYUSH Path Labs Private Limited (ISO 9001:2008 certified Laboratory), Koparkhairane, Navi Mumbai 400709.

Histopathological studies

Testes were fixed in 10% buffered formalin, embedded in paraffin sectioned at 5 µm. Sections were stained with hematoxylin and eosin to evaluate histopathological changes using LX 400 Labomed Microscope with camera PRO Series 1080P HDMI.

Statistical analysis

The data was analyzed using the Student's t-test, and values with $P < 0.05$ were considered significant.

Results

No mortality was observed during the experimental period. No significant change was observed in the levels of FSH, LH and testosterone (Fig. 1 A-C) of the animals of Gr. II and III compared to control Gr. I. Low-density sperms in the lumen and normal

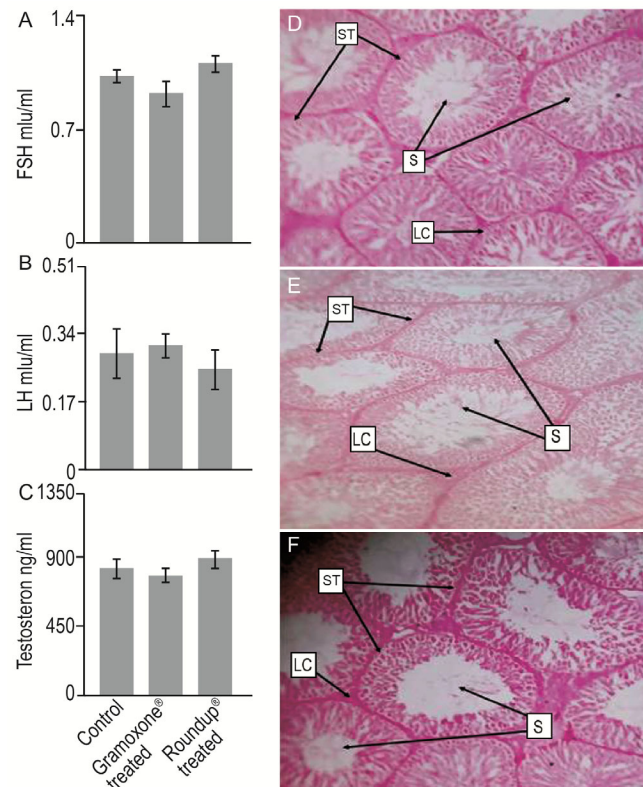


Fig. 1 — (A-C) Effect of Gramoxone® (5 mg/kg body wt.) and Roundup® (250 mg/kg body wt.) on hormone levels (FSH, LH and testosterone, respectively) of male Wistar rats for 25 days (PND 28 to PND53). Values are mean \pm SE for 5 rats, $P < 0.05$ compared to control. (D-F) Representative light micrographs of the testis stained by H & E (10X10). (D) Section of testis of control Gr. I showing normal Leydig cell (LC), normal seminiferous tubules (ST) with low density of spermatozoa (S); (E) Section of testis of Gr. II treated with Gramoxone® 5 mg/kg body wt. showing normal architecture of testis; and (F) section of testis of Gr. III treated with Roundup® 250 mg/kg body wt. showing close to normal architecture of testis.

histology were observed in the testes of the animals of Gr. I-III (Fig. 1 D-F). Non-significant decrease in FSH (Fig. 2A) and LH (Fig. 2B) levels of Gr. V and VI were observed as compared to control Gr. IV animals. However, a significant decrease in testosterone levels (Fig. 2C) in the animals of Gr. V and VI was observed as compared to Gr. IV. Rats in the control Gr. IV had normal histology of testis (Fig. 2D). Histopathological changes were observed in the testes of treated groups V (Fig. 2E) and VI (Fig. 2F) are shrunken and distorted seminiferous tubules, detachment of epithelium, dispersed Leydig cells, few spermatozoa as compared to Gr. IV (Fig. 2D).

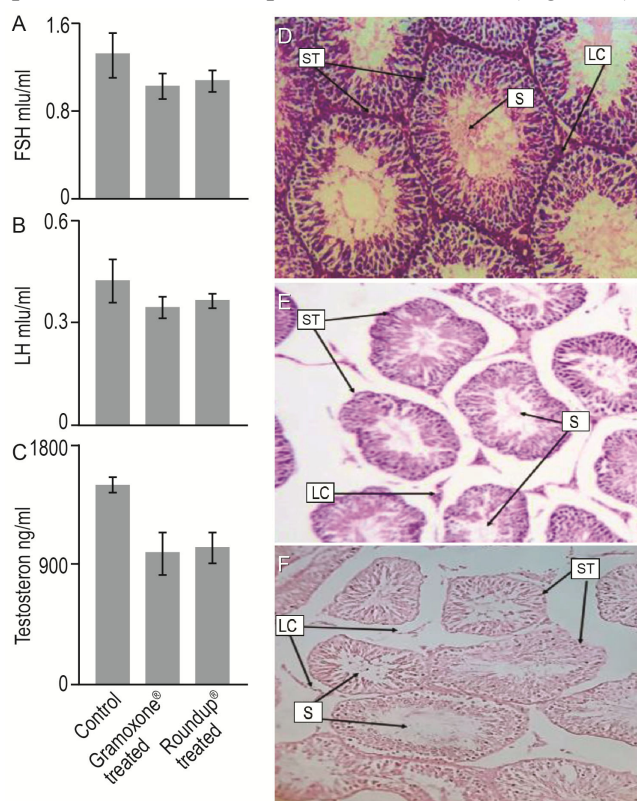


Fig. 2 — (A-C) Effect of Gramoxone® (5 mg/kg body wt.) and Roundup® (250 mg/kg body wt.) on hormone levels (FSH, LH and testosterone, respectively) of male Wistar rats for 60 days (PND 28 to PND 88). Values are mean \pm SE for 5 rats, $P < 0.05$ compared to control; and (D-F) Representative light micrographs of the testis stained by H & E (10X10). (D) Section of testis of control Gr. IV showing normal Leydig cell (LC), normal seminiferous tubules (ST), and many spermatozoa; (E) Section of testis of Gr. V treated with Gramoxone® 5 mg/kg body wt. showing dispersed Leydig cell (LC), shrunken and distorted seminiferous tubules (ST) with the detachment of epithelium and oligozoospermia; and (F) section of testis of Gr. VI treated with Roundup® 250 mg/kg body wt. showing dispersed Leydig cell (LC), shrunken and distorted seminiferous tubules (ST) with a detachment of epithelium and oligozoospermia or absence of spermatozoa.

Shrunken and distorted seminiferous tubules and dispersed Leydig cells were observed in the testes of many treated animals of Group V and VI (Table 1).

Discussion

The present study examined the changes in the testis of the rats orally exposed to two HFs (PBF Gramoxone® and GBF Roundup®). The treatment period was from prepubertal to the pubertal stage (25 days) or prepubertal to the post pubertal stage (60 days). Results of the present study indicate duration dependent changes in the testis on exposure to Gramoxone® as well as Roundup®.

Non-significant changes in the levels of reproductive hormones (FSH, LH and testosterone) and no changes in the histology of testis on exposure to Gramoxone® or Roundup® from prepubertal to the pubertal stage (25 days) as compared to controls indicate that both HFs do not affect the structure and function of the testis as well as pituitary-testis axis. However, exposure to Gramoxone® or Roundup® from prepubertal to the post pubertal stage (60 days), a non-significant decrease in gonadotropins (FSH and LH) and a significant decrease in the levels of testosterone level as well as histopathological changes in the testis indicate that Gramoxone® or Roundup® have an effect on structure and function of the testes and a lesser effect on the pituitary-testis axis.

The present study support reduction in the testosterone level on exposure to PBF^{33,34} or GBF²⁶⁻³⁰. This may be due to dispersed Leydig cells and also a nonsignificant decrease in LH. Since testosterone is secreted by Leydig cells of the testis under the influence of LH^{33,34}. Detachment of the germ layer of the testis may be due to a non-significant decrease in FSH level. The absence of sperms or few sperms in seminiferous tubules of the testis as compared to the testis of the control is due to a decrease in testosterone level because testosterone is also responsible for the formation of sperms.

Table 1 — Common histopathological changes observed in the testes of rats orally exposed to Gramoxone® and Roundup® for 60 days (PND 28 to PND 88). [Figure in the table indicates the number of rats in the group in which the changes were observed. Each group had 5 rats in total]

Histopathological changes	Gramoxone® Roundup®	
	(Gr. V)	(Gr. VI)
Shrunken and distorted seminiferous tubules	4	4
Detachment of epithelium	3	4
Dispersed Leydig cells	4	4
Few spermatozoa	4	5

Alteration in the structure and function of testis of the animals exposed to Gramoxone® or Roundup® from the prepubertal to the post pubertal stage may be due to increased bioavailability of Paraquat or Glyphosate of these HF in the testis as compared to animals exposed from prepubertal to the pubertal stage. Shrunken and distorted seminiferous tubules and dispersed Leydig cells may be due to the oxidative stress caused by paraquat^{7,35,36} of Gramoxone® or glyphosate^{29,30} of Roundup®.

Conclusion

This study revealed longer duration of exposure to commonly used herbicides Gramoxone® and Roundup® could be hazardous to the testis. For weed management, many brands of paraquat based formulations (PBFs) and glyphosate based formulations (GBFs) are used. In all such herbicide formulations, besides the main herbicide solvent paraquat or glyphosate, carrier, surfactant, antifoaming agent, sticker, and stabilizer are added to increase the efficiency. Damage to the testis as observed in the study could be due to the effect of paraquat and glyphosate alone or in combination with other components of formulation which needs further investigation.

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Conflict of Interest

Author declares no competing interests.

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