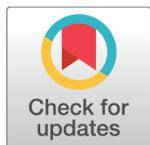


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Changes in Male Rat Thyroid Gland Exposed to Roundup[®] and Gramoxone[®]

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Abstract

Objectives: To study the effects of glyphosate based herbicide formulation Roundup[®] and paraquat based herbicide formulation Gramoxone[®] on the structure and function of the thyroid gland. **Methods:** Two groups of male rats were orally treated with a dosage of Roundup[®] (250 mg/kg) and Gramoxone[®] (5 mg/kg) respectively, from the 29th day of their age to 89th day. Control animals were administered with distilled water. At the end of the experimental period, blood samples and thyroid lobes were collected for estimation of thyroid hormones and histopathological changes, respectively. The function of the thyroid gland was evaluated by estimating thyroid hormones (thyroid stimulating hormone, thyroxine, and triiodothyronine) level, and the structure of the thyroid gland was assessed by observing histopathological changes. The treated group's results were compared with the control group. **Findings:** Non-significant increase in thyroid stimulating hormone and a significant decrease in thyroxine and triiodothyronine levels, as well as moderate desquamation of cells into the follicular lumen and cellular infiltration in thyroid structure were observed. Results of this study provide evidence on histopathological changes in the thyroid gland along with the decrease in thyroxine and triiodothyronine levels on exposure to Roundup[®] and Gramoxone[®]. Roundup[®] is less thyrotoxic compared to Gramoxone[®]. **Applications:** Ignoring the guidelines of the Insecticide Boards and manufacturer's instructions, Roundup[®] and Gramoxone[®] formulations are indiscriminately used for immediate weed killing. The human population is exposed to constituents of herbicide formulations through the air, water, and food. In this context, the result of the present study suggests that exposure to Roundup[®] and Gramoxone[®] formulations can be a risk to thyroid damage.

Keywords: Herbicide; Roundup[®]; Gramoxone[®]; Thyroid Hormones; Histopathology; thyrotoxic

1 Introduction

Geographical region and iodine supplements are the main reasons for increasing thyroid diseases in the population^(1,2). Besides this, exposure to environmental chemicals is directly or indirectly involved in the interferences in thyroid function⁽³⁻⁸⁾.

Commercial herbicide formulations contain main herbicide chemical and many surfactants and adjuvants. Easy availability of herbicide formulations and their economic inexpensiveness compared to manual weeding^(9–12) makes the use of formulations highly popular. However, this releases many chemicals of the formulation in the environment. In recent years the concern about the endocrine and reproductive toxicity of the chemicals of herbicide formulations has increased^(13,14). However, the toxicity of herbicide formulations on the thyroid is controversial because of little data.

Glyphosate-based herbicide formulation (GBHF) and Paraquat-based herbicide formulations (PBHF) are broad-spectrum commercial herbicide formulations and are heavily used worldwide for quick weed killing. Few study data is available on the influences of glyphosate and GBHF on thyroid activity^(15–19). Compared to pure glyphosate and GBHF, the exposure impact of pure paraquat chemical and PBHF on thyroid toxicity is less well known. Hence, studies are needed to demonstrate GBHF and PBHF are thyrotoxic.

The present study attempt was to find out the effect of GBHF Roundup[®] and PBHF Gramoxone[®] on thyroid-stimulating hormone [TSH], thyroxine [T4], and triiodothyronine [T3] levels along with the changes in the histology of the thyroid gland because structural changes provide strong evidence of the extent of damage to the thyroid gland.

2 Materials and Methods

Twenty days old healthy Wistar strain (*Ratus norvegicus*) male rats were procured from the Bharat Serum Limited, (103/1999/Committee for the purpose of control and supervision of experimental on Animals (CPCSEA), Wagle Estate, Thane. Animals were kept in the Animal testing center of Ramnarain Ruia College, Mumbai (CPCSEA No-315) under conventional conditions such as relative humidity 50±10%, temperature 27± 2°C, and with 12:12 hours light and dark cycle, artificial illumination is provided during the day. Animals were fed *ad libitum* and acclimatized for seven days before the commencement of the study.

All the experimental procedures and sacrifice of rats were carried out as per the guidelines and protocols approved by the Institutional Animal Ethics Committee, S. P. Mandali's Ramnarain Ruia College (Protocol number-140612-01 dated 31st January 2015).

2.1 Experimental protocol Chemicals

The herbicide formulation used in the present study was Roundup[®] manufactured by Monsanto India Limited and its chemical composition is isopropylamine salt of a Glyphosate 41% (w/w) and other relevant (inert) ingredients 59% (w/w). The second herbicide formulation was Gramoxone[®] manufactured and marketed by Syngenta India Limited. Gramoxone[®] contains paraquat dichloride 24% (w/w), phenol 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.

a) Animal grouping

After one week of acclimatization, male rats were assigned into three groups of five rats each group, which were treated by gavage with sterile distilled water (control group), Roundup[®] (250mg/kg) and Gramoxone[®] (5mg/kg) respectively, for 60 successive days (the pilot study was carried out for the selection of dosages). During the experimental period, animals had free access to food and water. At the end of the experimental period, animals were sacrificed, and immediately blood samples and thyroid lobes were collected for analysis.

b) Hormone analysis

TSH, T3, and T4 in the serum were determined by using Luminescence-based ELISA kits at AYUSH Path Labs Private Limited (ISO 9001:2008 certified Laboratory), Koparkhairane, Navi Mumbai 400709.

c) Thyroid lobes for histopathological studies

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

2.2 Statistical analysis

Data are mean ± SEM. The results were analyzed using Student's T-test, P<0.05 was considered as significant.

3 Results and Discussion

The thyroid gland is an endocrine gland whose follicular cells biosynthesis and secretes thyroid hormones [T4 and T3] in several stages⁽²⁰⁾. Secretion of T4 and T3 hormones are regulated by a negative feedback mechanism in coordination with pituitary TSH⁽²¹⁾. The thyroid gland controls the growth, development, and metabolism of the body.

Increasing usage and exposure to herbicide chemicals may disturb thyroid hormone levels^(22,23). Changes in the thyroid gland produce physiological modulation in the body. Therefore, in the present study, male rats were orally treated with GBHF Roundup[®] and PBHF Gramoxone[®] to assess the effects of these two formulations on histology of the thyroid gland along with levels of T4, T3, and TSH. There was no mortality during the experimental period.

3.1 Changes in the thyroid hormones levels

After sixty days of exposure to both herbicide formulations, a significant decrease in T4 and T3 hormone levels [Figure 1. A and B] and non-significant increases in TSH levels (Figure 1.C) were observed compared to the control group. A non-significant increase in TSH levels on exposure to both formulations (Figure 1.C) was due to the decrease in T4 and T3 hormone levels.

Glyphosate and GBHF Roundup Bioflow, and Kalach 360 SL decrease T4 and T3 hormone levels with an increase in TSH level^(16–18), and the result in the present study with the GBHF Roundup[®] agrees with this. However, GBHF Roundup Transorb decreases the TSH level with no change in the T4 and T3⁽¹⁹⁾. The discrepancy⁽¹⁹⁾ may be due to the type of glyphosate, and composition of GBHF. Since herbicide formulations contain many surfactants and adjuvants that make these formulations more toxic than technical grade herbicide chemicals^(24,25).

A significant increase in TSH in conventional farmers who sprayed paraquat⁽²²⁾ and hypothyroidism has been reported among women on exposure to paraquat⁽²³⁾ and the result in the present study with the PBHF Gramoxone[®] support this.

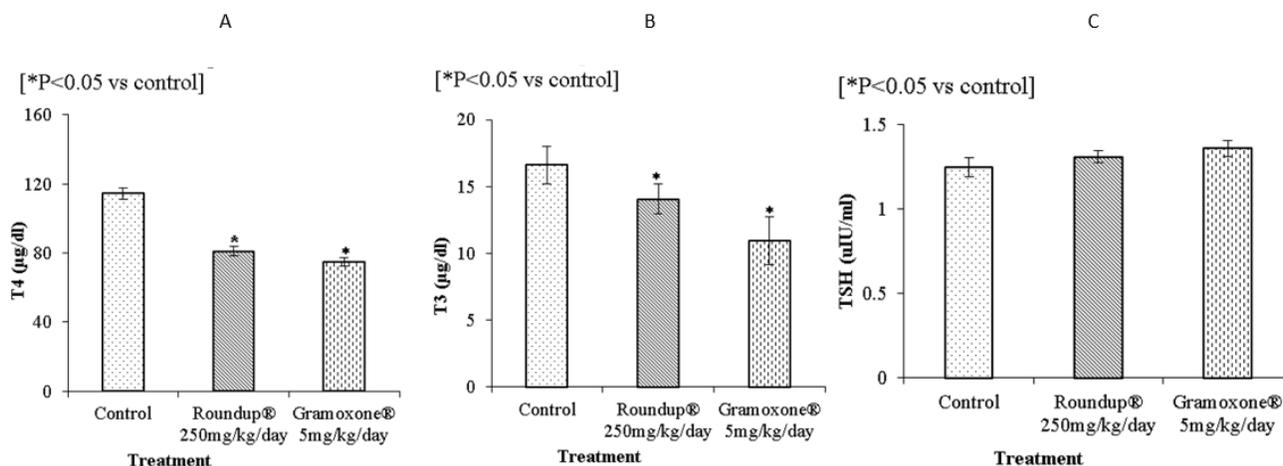


Fig 1. A. T4 of control, Roundup[®] and Gramoxone[®] exposed male rats, B. T3 of controls, Roundup[®] and Gramoxone[®] exposed male rats, C. TSH levels of controls, Roundup[®] and Gramoxone[®] exposed male rats

3.2 Histopathological changes in the thyroid gland

The control animal's thyroid gland showed normal thyroid follicles with clear colloid [Figure 2. A]. Compared to control animals, histopathological changes observed in the thyroid glands of herbicide formulations exposed animals [Figure 2. B and C] were cellular infiltration and desquamation of the follicular cells into follicular lumen.

Most of the reported effects of GBHF are on thyroid hormone levels and enzymes involved in the pathway of T4 and T3 biosynthesis^(16–19). Less importance was given to histopathological changes in the thyroid on exposure to GBHF. Few data is available on GBHF Kalach 360 SL⁽¹⁷⁾ and the present study confirms and provides data on histopathological changes in the thyroid on exposure to GBHF Roundup[®].

There is a lack of data on the effect of the commercial PBHF on thyroid structure. The present study result provides histopathological evidence in the thyroid tissue on exposure to PBHF Gramoxone[®] [Figure 2. C].

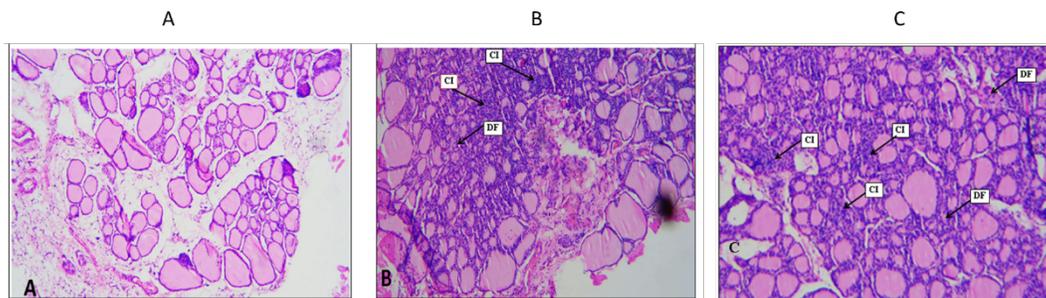


Fig 2. A. Control rat thyroid tissues after 60 days showing normal thyroid follicles with clear colloid [Haematoxylin and Eosin stained x100], B. Histopathological section of rat thyroid tissues after 60 days exposure to Roundup® 250mg/kg showing desquamation into follicular lumen (DF) and cellular infiltration (CI) [Haematoxylin and Eosin stained x100], C. Histopathological section of rat thyroid tissues after 60 days exposure to Gramoxone® 5 mg/kg showing desquamation into follicular lumen (DF) and mild cellular infiltration (CI) [Haematoxylin and Eosin stained x100].

In the present study, continuous exposure of male rats to Roundup® and Gramoxone® may have increased the chances of the bioavailability and bioaccumulation of chemical components of the formulations in the thyroid follicular cells. Glyphosate and paraquat induce oxidative stress^(26,27). Dual oxidase maturation factors 1 and 2 are present in the membrane of follicular cells. These factors are important in the biosynthesis of the T4 and T3⁽²⁸⁾. Oxidative stress may be the reason for the degradation of these factors as well as cellular infiltration and desquamation of follicular cells in the lumen of thyroid follicles.

Results of the present study also showed that similar types of histopathological changes in the thyroid of male rats treated with both the herbicide formulation. However, the decrease in T4 and T3 level of Gramoxone® treated animals was more as compared to Roundup® treated group, suggesting that the efficiency of Gramoxone® producing oxidative stress is more than Roundup®.

4 Conclusion

Data from the present study showed that exposure to Roundup® and Gramoxone® formulations severely affects the levels of T4 and T3 along with moderate changes in the structure of the thyroid gland. Oxidative stress may be the reason for the changes in the thyroid gland. Gramoxone® is more thyrotoxic compared to Roundup®. Changes in the thyroid were due to the main herbicide ingredient of the Roundup® and Gramoxone® or due to the cumulative effect of surfactants and adjuvants of the formulation needs to be further investigated.

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