Histopathological Changes in the Liver and Kidney of Male Rats Exposed to Gramoxone®

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Abstract: Gramoxone® is a Paraquat based herbicide formulation used for weed management. In the present study effects of Gramoxone® on histology of liver and kidney was assessed. Male rats were orally treated with Gramoxone® 5mg/kg body weight for 15 and 60 days and the result of treated rats was compared with control rats. Exposure depended histopathological changes were observed in liver and kidney of Gramoxone® treated rats. Result of this study indicates that Gramoxone® is toxic to liver and kidney and its usage is to be done with caution.

Keywords: herbicide formulation, paraquat, histology of liver and kidney

1. Introduction

Weeds compete with crop plants for space, nutrients and water. They reduces crop yield, land value and limits the choice of crops. Herbicides are used to destroy weeds. Consumption of herbicides occupy 44% of the total agrochemicals globally and 30% in India [1]. In India, usage of herbicides is rising due to increased nonavailability of skilled labours for manual weed removal, monoculture practices, and herbicides spraying are economical as compared to manual weeding.

Paraquat is used to control broad-leaved weeds and grasses in more than 100 different crops, including plantations [2]. In India, Central Insecticide Board and Registration Committee has approved the use of paraquat dichloride to control weeds in apple, cotton, grapes, maize, potato, tea, rice, rubber and wheat. However, in Assam and West Bengal paraquat application is high in tea plantation and Maharashtra, paraquat is used in sugarcane, cotton and fruit crops [3]. Besides agriculture paraquat is also used to kill weeds in industrial sites, roadsides, irrigation canals and home gardens.

Paraquat is a strong superoxide radicals producer. Production of superoxide radicals causes oxidative damage to membrane lipids and mitochondria of cells, resulting disturbances in the biochemical processes and cell death [4], [5] and [6].

Paraquat use is increasing because it is now being promoted as an alternative to herbicide glyphosate [7]. For immediate weeds killing paraquat is indiscriminately sprayed [8], [9]. Paraquat is detected in drinking water wells [10],[11], [12] and [13] also residues of paraquat have been found in cotton and sunflower seeds [14], onions [15], barley, wheat, rice, sorghum, cotton and potatoes [16].

Toxicity data on effects of paraquat on liver [17], [18], [19], [20], [21] and on kidney of rats, guinea-pigs, rabbits, and dogs [22], [23], [24], [25], [26], [27] are well documented. However, for weed management paraquat based formulations (PBF) are used. In formulations beside paraquat, solvent, surfactants and other components are present for effective spraying and weed killing. In market several PBF are available. Extensive data is need on effects of these PBF on human and animals.

Entry of exogenous chemicals in the body affects liver and kidney. Since these two are the organs of metabolism, detoxification and excretion of xenobiotics. The objective of the present study was to find out the effects of PBF Gramoxone® on histology of liver and kidney. This work will provide information on PBF toxicity to liver and kidney.

2. Materials and Methods

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 Institutional Animal Ethics Committee of S. P. Mandali’s Ramnarain Ruia College, Matunga, Mumbai 19.

Animals

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

Test Chemicals

Herbicide formulation used in this study was Paraquat dichloride based, Gramoxone® manufactured and marketed by Syngenta India Limited. It 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.

Treatment

Animals were divided into four groups with five rats in each group. Groups A and C rats are controls for 15 and 60 days, respectively. Groups B and D rats were treated with
Gramoxone® 5mg/kg body weight of the animal by gavage for 15 and 60 days, respectively. During the study animals had free access for food and water.

**Tissue collection**
At the end of experimental days animals were sacrificed. Immediately after scarifying animals were dissected carefully with minimum damage and liver and kidney were collected to study histopathology.

**Histopathological study**
Liver and kidney tissues were fixed in 10% neutral formalin for 24 hr to prevent autolyses and to maintain the natural state of the tissue cells. After fixation, tissues were washed in water to remove the excessive fixative. The washed tissues were then passed via graded series of ethyl alcohol and cleaned with xylene. The tissues were then embedded in paraffin (BDH M.P.56 C) to form the tissue blocks. The blocks were then cut using a microtome, with sections 5-6 µm and latter mounted on a clean glass, slides. The sections were stained routinely with Haematoxylin and Eosin. Tissue sections were studied for histopathological changes using LX 400 Labomed Microscope at 40X and 100X magnifications. The photographs of tissue for histological observations were obtained using microscope camera PRO Series 1080P HDMI.

**3. Results and Discussion**

Histopathological changes in the organs provide a useful data concerning changes in the cellular structure of organ earlier than external changes. Therefore, in the present study effect of PBH Gramoxone® on histopathology of liver and kidney was assessed.

Exposure to paraquat causes structural changes in the liver. Extensive vacuolization, increased sinusoidal space and blood congestion in the central vein [28], necrotic cells [17], intracellular edema and lipid accumulation [29] has been reported. In the present study, histopathological changes observed in the liver of Group B rats treated with PBH Gramoxone® 5me/kg for 15 days was decrease in diameter of the central vein, liver cord dissary, loss of cytoplasm and congestion (Fig.2) as compared to Group A (Fig.1).

However, Group D rat exposed to Gramoxone® for 60 days, disarray of hepatocyte, cellular infiltration, karyomegaly, granular degeneration, and haemorrhages were noted in liver (Fig.3) as compared to Group C (Fig. 4).

![Figure 1: Light micrograph of the liver form male Wistar rat (Control) after 15 days illustrates normal liver cell architecture with central vein surrounded by hepatocytes. (H & E X100)](image1)

![Figure 2: Light micrograph of the liver form male Wistar rat orally treated with Gramoxone® for 15 days illustrates decrease in the diameter of the central vein, liver cord dissary, loss of cytoplasm and congestion (H & E X100).](image2)

![Figure 3: Light micrograph of the liver form male Wistar rat (Control) after 60 days illustrates normal liver cell architecture with central vein surrounded by hepatocytes. (H & E X100)](image3)

![Figure 4: Light micrograph of the liver form male Wistar rat orally treated with Gramoxone® for 60 days illustrates disarray of hepatocytes, cellular infiltration, karyomegaly, granular degeneration and haemorrhages (H & E x400).](image4)
In the literature survey, the reported histological changes in the kidney on exposure to paraquat are renal tubular degeneration [10],[30], atrophy of glomeruli [31],[32], glomerular degeneration and hemorrhage [33], distal convoluted tubules lined by swollen cells with loss of cytoplasm, and pyknotic nuclei [17], leukocyte infiltration [34]. In this study increase in the size of the glomerulus, cellular infiltration and haemorrhages was observed in the kidney of Group B animals treated with Gramoxone® for 15 days (Fig.6) as compared to kidney of Group A animals (Fig.5) and loss of urinary space around glomerulus, tubular degeneration, cast formation and hemorrhages were observed in the kidney of Group D male rats treated with Gramoxone® for 60 days (Fig. 8) as compared to kidney of Group C rats (Fig.7).

**Figure 5:** Light micrograph of the kidney form male Wistar rat (Control) after 15 days illustrates normal kidney cell architecture tubules, glomerulus surrounded by Bowman’s capsule. (H & E x400)

**Figure 6:** Light micrograph of the kidney form male Wistar rat orally treated with Gramoxone® for 15 days illustrates increase in the size of the glomerulus, cellular infiltration and haemorrhages (H & E x100).

**Figure 7:** Light micrograph of the kidney form male Wistar rat (Control) after 60 days illustrates normal kidney cell architecture tubules, glomerulus surrounded by Bowman’s capsule. (H & E x100)

**Figure 8:** Light micrograph of the kidney form male Wistar rat orally treated with Gramoxone® for 60 days illustrates loss of urinary space around glomerulus, tubular degeneration, cast formation and hemorrhages (H & E x100).

Histopathological changes in the liver and kidney of the rats on oral exposure to PBF Gramoxone® indicate that, like paraquat, its formulation Gramoxone® is also toxic to the structure of these two organs. This effect is duration dependant. Longer exposure to Gramoxone® increases the intensity of histopathological changes.

4. Conclusion

This study result showed that PBF Gramoxone® is hepato and renal toxicant. However, further work is needed to understand the mechanism of Gramoxone® toxicity. The use of Gramoxone® must be supervised so is to prevent the discharge of components of the Gramoxone® in the environment and nontarget organism exposure to these components.

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References


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