Histological changes caused due to intoxication of 2, 4-D herbicide in Wistar albino rats

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ABSTRACT

The phenoxy herbicide 2, 4-D (dimethyl-amine salt DMA) is widely used herbicide to control the growth of weeds and broadleaf plants because of its selective nature. To cope up with weeds problem farmers are using tremendous amount of herbicides frequently, these herbicides without affecting crop plants may get accumulated in it as well as may leached to water through soil. Unknowingly human beings may get exposed to such chemicals. The attempt was made to analyze the sub chronic effect of herbicide, 2, 4-D on Albino Wistar male rats. Albino rats were given oral dose of 25% of LD50 of the 2, 4-D (12mg/kg/body weight) dissolved in tap water for 120 days. Shrunken testis (comparative reduction in weight), ulceration/edema on small intestine and swelled kidneys were observed in all experimental rats. The histological study shows alterations in liver, kidney and testis of rats. In 2, 4-D herbicide treated rats, hepatic cell in liver appear degranulated, hepatic cord becomes disarrayed and hydropic degeneration of some hepatocytes was observed. Examination of kidney sections showed inflammatory changes in the glomeruli with increased cellularity, capillary hyperemia, exudation, hypertrophy, tubular degeneration. The glomerular membrane was slightly thickened. Interstitial nephritis & edema was also visible along with extensive vacuolization in renal tubules. The testis exhibited shrinkage of seminiferous tubules.

KEY WORDS: HERBICIDES, 2, 4-D, HISTOLOGY, LIVER, KIDNEY, TESTIS.

INTRODUCTION

Weeds are the most serious threats among the farmers. Weeds compete with the crops for soil nutrients, sunlight and space, harbor insects, pests and microorganisms, some weeds release poisonous substances into the soil that are harmful to plants, human beings and animals. That means, they reduce farm and forest productivity, displace and degrade species. Herbicides, commonly known as weed killers, have become an important part of landscape maintenance because chemical weed control often is the more economical way than hand or mechanical weeding. Some of these act by interfering with the growth of the weed and are often
synthetic “imitations” of plant hormones (Kellogg et al., 2000). Herbicides are also a primary method of vegetation management in industrial areas, along highways and other non-cropland areas. There are many different types of herbicides available in the market. Some are of selective type, like Pendimethalin, 2, 4-D, Pursuit etc. selectively toxic to weeds. While Paraquat, Glyphosate are non-selective herbicides (Pfeiffer, 2011). Herbicide use has increased significantly around the world over the past six decades. By 2001 approximately 1.14 billion kilograms of herbicides were applied globally for the control of unwanted vegetation in agricultural, lawn care, aquaculture and irrigation/recreational water management activities. In general, herbicides are low to moderate in toxicity towards humans and animals, because most herbicides target chemical pathways that animals do not possess (e.g., photosynthesis), however, there are exceptions; many can be dermal irritants since they are often strong acids, amines, esters, and phenols. Inhalation of spray mist may cause coughing and a burning sensation in the nasal passages and chest. Extended inhalation sometimes causes dizziness. Intake will usually cause vomiting, a burning sensation in the stomach, diarrhea and muscle twitching. Herbicides represent 36% of global pesticide use, followed by insecticides (25%), fungicides (10%) and other chemical classes (Joshi et al., 2012).

Pesticides have been useful to fight against pests of plants, animals and humans. Hepatotoxic, genotoxic and neurotoxic effects of different pesticides have been evaluated in many in vivo and in vitro studies. The 2, 4-Dichlorophenoxyacetic acid (2, 4-D) is an herbicide widely used to control the growth of broadleaf plants. It is chemically derived from phenoxyacetic acid. The major uses of 2, 4-D is on cereal crops such as wheat, corn, oats, rye, barley and the cane crops (U.S. Environmental Protection Agency 2002) Tuschl and Schwab, 2003, Tayyab et al., 2010 and 2015.

According to Suresh Joshi (2012) the teratogenic, neurotoxic, immunosuppressive, cytotoxic and hepatoxic effects of 2, 4-D have been well documented. 2, 4-D herbicide increases lipid peroxidation in animal and human cells in vitro. 2, 4-D has been shown to cause cellular mutations which can lead to cancer. This mutation contains dioxins, a group of chemicals known to be hazardous to human health and to the environment. Exposure to 2, 4-D induces nephrotoxicity in rats during late pregnancy and early postnatal periods. Mikov et al., (2010) reported that 2, 4-D has a hypoglycemic effect in mice.

Farmers are facing the problem of labors, weeds are reducing the produce in the fields, therefore farmers have been shifted towards the synthetic herbicides, they are using tremendous amount of herbicides; with the recurrence of weeds variety of herbicides are repeatedly used. These herbicides may not affect the crop plant but may get accumulated in it, may percolate in nearby water body through soil. Possibility of exposure and chronic accumulation of such chemicals cannot be rejected. However considering the toxic effect of herbicide, work was carried out to study the effect of sub-chronic exposure of 2, 4-D on albino male Wister rats with reference to variation in histological architecture of liver, kidney and testis, as very limited work is carried out rats or other mammals.

MATERIAL AND METHODS

Healthy male Wister albino rats were procured from Sud hakarao Naik Pharmacy College, Pusad, with an average weight of 170±10 gm. Rats were acclimatized to laboratory condition. During acclimatized rats were provided with food and water ad libitum. The animals were kept in clean polypropylene cages (measuring 12”x10”x8”) with chrome plates grills. The rats were grouped in to two groups, six rats in each group; one group was kept as control, while other as experimental. Experimental rats were given 25% of LD50 (12mg/kg /body weight) oral dose of the 2, 4-D dissolved in tap water for 120 days. The control rats were sacrificed on 120th day. Whereas experimental rats were sacrificed on 121st day after giving 120 days oral dose according to norms of ethical committee (1060/ac/07/CPCSEA). The tissues like liver, testis & kidney of male rats were removed, fixed in Bouin’s fixative for 24 hrs, processed by paraffin wax impregnation method, cut using a rotary microtome at 5 μm thicknesses and stained with Hematoxylin and Eosin (H X E) for light microscopic examination.

RESULTS AND DISCUSSION

Administration of oral dose of 2, 4-D to rats for 120 days has shown the observable effect on the behavior of rats. Hyperactivity was seen just after the administration of dose for 5-10 minutes. After substantial exposure to oral dose, rats showed symptoms of lethargy, red nasal and ocular discharge, severe dehydration, reduced feed and water intake, and pasty diarrhea with pungent smell. Hair fall, loss of weight also been observed but no any mortality was observed.

According to EXOTOX NET (1996), 2, 4-D is a respiratory system irritant that can cause prolonged difficulty in breathing, coughing, burning, dizziness and temporary loss of muscle coordination. When experimental animals were sacrificed for tissue, it was observed that the stomach was filled with fluid having pungent smell, also it has been observed that testis were shrunk (com-
HISTOLOGICAL CHANGES

**Effect of 2, 4-D herbicide on Liver architecture**

Liver hepatocytes of control rats were polygonal in shape, mononucleate or binucleate (Fig 1a). In 2, 4-D herbicide treated rats, the cell appear degranulated. Hepatic cord becomes disarrayed. Hydropic degeneration of some hepatocytes was also observed (Fig 1b). Gorzinski *et al.*, (1987) reported necrobiotic changes in the form of hepatocellular cytoplasmic swelling and homogeneity in rats.
received 15 mg/kg b.w. of 2, 4-D per day. Hallenbeck and Cunningham- Burns (1985) observed focal necrosis of the hepatocytes in rats following treatment with 2, 4-D. Makinde et al., (2015) reported fish were exposed to 0.00, 1.40, 1.44, 1.48 and 1.52mg/l of 2, 4-D amine cause advancing phase of hepatic necrosis in the liver.

Effect of 2, 4-D herbicide on Kidney architecture

Kidney of a control rats revealed normal renal tubule and glomerulus (Fig. 2a). Examination of kidney sections of 2, 4-D treated rats showed inflammatory changes in the glomeruli with increased cellularity, capillary hyperemia, exudation, hypertrophy, tubular degeneration. The glomerular membrane was slightly thickened. Interstitial nephritis and edema was visible in the section. Extensive vacuolization in renal tubules was also seen (Fig. 2b). Hard et al., (1999) reported that hyperplasia of renal tubular epithelium implies an increase in the number of lining cells inside the tubules i.e. without proliferation beyond the basement membrane Tayeb et al., (2015) reported sub-acute exposure to different doses of 2, 4 dichlorophenoxoacetic (2, 4-D) on rat kidney. Forty animals were divided into four equal groups and treated with different doses of 2, 4-D: 0, 15, 75 and 15mg/kg body weight per day via oral gavage for 28 consecutive days. The histopathological study revealed tubular damages, glomerular alterations, vascular congestion and increased number of pyknotic nuclei in kidneys of all 2, 4-D treated groups.

Effect of 2, 4-D herbicide on Testis architecture

The testis of 2, 4-D treated rats exhibited shrinkage of seminiferous tubules and sperms appear to be agglutinated. (Fig.3b). However, normal cellular architecture is seen showing normal germinal epithelial cells, Primary and secondary spermatocytes in control rats (Fig. 3a). In this context, Sever et al., (1997) found that 2, 4-D reduced sperm counts and increased abnormalities in sperm humans.

CONCLUSION

The result obtained in the present investigation reveal that the sub chronic dose of 2, 4-D herbicide i.e., 25% of LD 50 induced considerable alteration in architecture of the liver, kidney & testis of rats. Biochemical and haematological alterations were also observed by various workers. Effects on endocrine regulation as well as influence on reproduction need to be studied thoroughly. It can be concluded that widespread use of such herbicides in public places and agriculture fields are to be prohibited or restricted. There is a need to aware the people about the hazardous effects of herbicides.

REFERENCES


Tuschi H. and Schwab C. (2003). Cytotoxic effect of the herbicide 2, 4-Dichlorophenoxyacetic acid in hepg2 cell. J. Food and Chemical Toxicology 41: 385-393.

