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HEMATOLOGICAL, BIOCHEMICAL ALTERATIONS, AND CHANGES IN HISTOLOGICAL ARCHITECTURE OF SOME TISSUE OF MALE WISTAR RATS EXPOSED TO 2,4-D HERBICIDE

Ujjwala S Deshmukh* and P M Ramteke

*Government Vidarbha Institute of Science and Humanities. Amravati (M.S.), India.

Corresponding Author

Ujjwala S Deshmukh
Email:- ujjwaladeshmukh@rediffmail.com

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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. Albino Wistar male rats were given daily oral dose of 25% of LD50 of the 2,4-D (12mg/kg/body weight) dissolved in tap water for 120 days. Haematological analysis shows significant increase in TLC particularly in Eosinophill with slight decrease in Monocyte, while no alterations were found in RBC count and haemoglobin %. Biochemical analysis show significant increase in urea, uric acid and creatinine level. The histological study shows alterations in architecture of liver, kidney and testis of rats. In treated rats, hepatic cell of liver appear degranulated, hepatic cord becomes disarrayed and hydropic degeneration of some hepatocytes was observed. Sections of kidney showed inflammatory changes in the glomeruli with increased cellularity, capillary hyperemia, exudation, hypertrophy, tubular degeneration and the glomerular membrane was slightly thickened. Interstitial nephritis and edema was also visible along with extensive vacuolization in renal tubules. The testis exhibited shrinkage of seminiferous tubules.

Keywords: Herbicides, 2, 4-D, Histology, Haematology, biochemical analysis, liver, kidney, Testis.

INTRODUCTION

Herbicides 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, silvicultural, 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 human beings. Hepatotoxic, genotoxic and neurotoxic effects of different pesticides have been evaluated in many *in vivo* and *in vitro* studies. The 2,4-Dichlorophenoxyacetc acid (2,4-D) is an herbicide widely used to control the growth of broadleaf plants. It is chemically derived from phenoxyacetic acid (Tayeb *et. al.,* 2010). 2, 4-D mainly used on cereal crops such as wheat, corn, oats, rye, barley and the cane crops (U.S. Environmental Protection Agency 2002).

According to Joshi (2012) the teratogenic, neurotoxic, immunosuppressive, cytotoxic and hepatoxic effects of 2,4-D have been well documented. Herbicide

2,4-D 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 mutagen 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.

So considering the effect of herbicide, work was carried out to study the effect of sub-chronic exposure of 2,4-D on albino male Wistar rats with reference to variation in histology of liver, kidney and testis.

MATERIAL AND METHOD

Healthy male Wistar albino rats were procured from Sudhakarrao 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 *adlibitum*. 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 as liver, testis & kidney of 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 thickness, and stained with Hematoxylin and Eosin (H X E) for light microscopic examination.

Statistical analysis

In each assay, the experimental data represent the mean of six independent assay ± Standards Deviation. Mean were compared using the Student t-test. Differences were considered significant at the level $p < 0.05$ and very significant at the level $p < 0.01$.

RESULTS

Rats were exposed to 2,4-D by giving a dose 25% of LD50 i.e. 12 mg/ kg/ body weight/day, for 120 days. Shrinked testis (comparative reduction in weight), stomach filled with fluid having pungent smell, ulceration on small intestine, edema and swelled kidneys were observed in all most all experimental rats. As compared to control rats, variations in haematological parameters were observed in rats exposed to 2,4-D, (table I). Significant increase in TLC and significant decrease in Eosinophill of all 2,4 D- exposed rats was found, whereas no variations were observed in Hb%, R.B.C, Neutrophils and Lymphocytes.

Biochemical analysis of blood of control as well as 2, 4-D exposed rats was done to compare the effect of 2,4-D; results given in Table I. Significant increase in Urea, Creatinine and Uric acid level has been observed in experimental rats. While no variations in SGOT and SGPT level was observed.

Histological changes:

Effect of 2,4-D herbicide on Liver architecture

Hepatocytes of control rats were polygonal in shape, mononucleate or binucleate (Fig1a). 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).

Effect of 2,4-D herbicide on Kidney architecture

Kidney of 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 and tubular degeneration. The glomerular membrane was slightly thickened. Interstitial nephritis & edema was visible in the section. Extensive vacuolization in renal tubules was also seen (Fig. 2b).

Effect of 2,4-D herbicide on Testis architecture

The testis of control rats shows normal cellular architecture with normal germinal epithelial cells, primary and secondary spermocytes in control rats (Fig. 3a). The testis of 2,4-D treated rats exhibited shrinkage of seminiferous tubules and sperms appear to be agglutinated (Fig. 3b).

Table 1. Haematological results of control and 2,4-D treated rats

Parameters	Control	2,4-D treated rats
Hb%	12.25±2.74	13.25±1.057
TLC thousand/ cu mm	4516±2183	7683.33**±511.53
RBC millions / cu mm	11.08±0.91	11.48±0.287
Neutrophils%	35.66±23.02	29±3.405
Monocytes%	4±1.67	1.5±0.547
Eosinophils%	2.33±0.82	1**±0
Lymphocytes%	58±22.62	66.83±3.710

Values are given as mean of six separate animals' ± standards deviations. *p<0.05; **p<0.01 (Student's t-test)

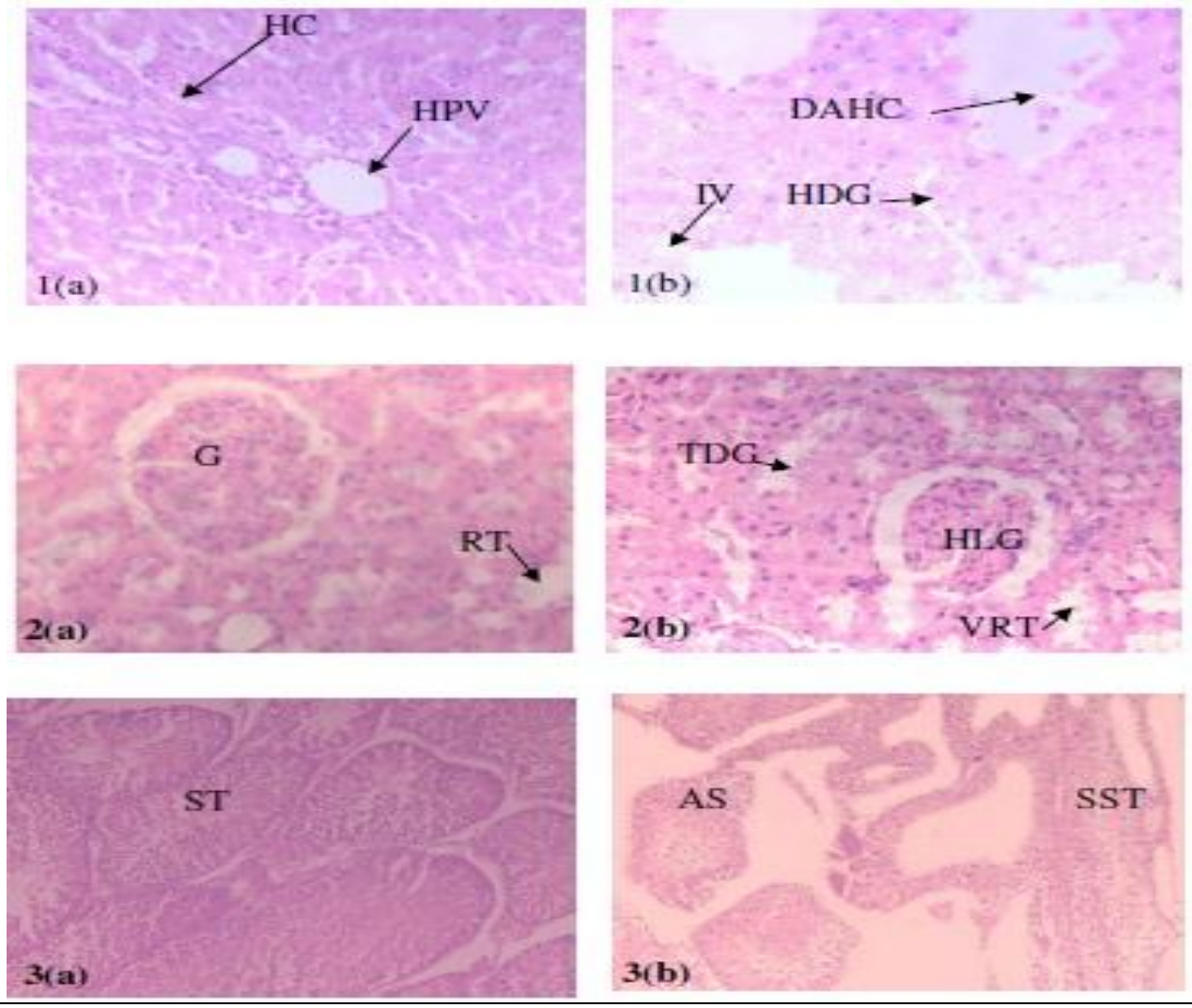
Table 2. Biochemical analysis results of control and 2, 4-D treated rats

Parameters	Control rats	2,4-D treated rats
Urea	16.16±2.639444	45.66±6.15359**
Creatinine	0.45±0.187083	1.08±0.332205**
SGOT	139.33±94.92453	181.5±298.1743
SGPT	54.5±16.89675	109.5±157.9845
Uric acid	0.916±0.547723	6.426±4.322618**

Values are given as mean of six separate animals ± standards deviations. *p<0.05; **p<0.01 (Student's t-t).

Fig 1(a). T.S. Liver of control Wistar albino rat (male) showing regular hepatic cords, Hematoxylineeosin (HE) X 400, HPV- Hepatic portal vein, HC- Hepatic cord. Fig 1(b): T.S. Liver of experimental rat (male) showing DAHC- disarray of hepatic cords, IV- Intense vacuolization, HDG- Hydropic degeneration. Fig 2(a): T.S. Kidney of control albino rat (male), (HE) X 400. G- Glomerulus, RT- Renal tubule. Fig 2(b): T.S Kidney of experimental rat (male), (HE) X 400. TDG- tubular degeneration, HLGHypertrophied and lobulated glomerulus, VRT- Vacuolisation in renal tubular cells. Fig 3(a) : T.S Testis of control albino male wistar rat, Hematoxyline- eosin X 400, ST- Seminiferous tubule. Fig 3 (b): T.S. Testis of experimental rat showing shrinked seminiferous tubules (HE) X 100, SST – Shrinkedseminiferous tubules, AS- Agglutinated sperms.

Histological effects of 2, 4- D on Wistar albino male rats (liver, kidney and testis).



DISCUSSION

Administration of oral dose of 2,4-D to rats for 120 days showed the observable effect on the behavior of rats. Hyperactivity was seen just after the administration of dose for 5-10 minutes. After 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 mortality. According to Pesticide information profile (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.

As compared to control rats, variations in haematological parameters were observed in 2,4-D exposed rats results are given in Table I. There is significant increase in TLC and significant decrease in Eosinophill of all 2, 4-D exposed rats has been found. No variations were observed in Hb%, R.B.C, Neutrophill and Lymphocytes percents in experimental rats. Same result were observed by Kobal and Budihna, (1999) a sub-acute toxicity test using 2,4-D, (10 mg.kg-1and100mg.kg- 1) and 4-chloro-2-methylphenoxyacetic acid (MCPA, 15 mg.kg-1 and 150 mg.kg-1) was carried out on Wister rats (n = 60) and New Zealand White rabbits (n = 30) of both sexes. The administration of herbicides 2, 4-D or MCPA did not significantly affect the red or white blood cell counts neither in rats nor in rabbits, as compared to controls. However, there were great differences in leukocyte counts within all groups.

Biochemical analysis of blood of control as well as 2,4-D exposed rats was done to compare the effects of 2,4-D; results are given in Table II. Significant increase in Urea, Creatinine and Uric acid level has been observed in experimental rats. While no variations in SGOT and SGPT level was observed. Similar results were observed by Morgulis *et al.* (1998) effect of 2,4 -D in chicks dosed with 100, 300, 500, or 600 mg 2, 4-D/kg b.wt., by the oral route. Clinical, laboratory and histopathological methods were used. After acute exposure, this herbicide decreased motor activity and induced muscular weakness and motor incoordination; decreased weight gain; increased serum creatine kinase, serum uric acid and creatinine levels.

After dissection, experimental rats showed shrinked testis (comparative reduction in weight), swelled kidneys with tumor/edema, lesions on liver, stomach filled with fluid having pungent smell and ulceration on small intestine in all most all experimental rats. According to United state Environmental Protection Agency (2005), the primary target organs of the chemical 2,4-D are eyes, thyroid gland, kidney, adrenal, ovaries and testis. According to Kobal and Budihna (1999), the macroscopic examination of organs at the pathoanatomical dissection of the rats treated with 2,4-D at doses of 100 mg. kg-1, and with MCPA at doses of 150 mg.kg-1 revealed slightly inflamed and swollen mucous membranes of the stomach and small intestines.

Hepatocyte of control rats were polygonal in shape, mononucleate or binucleate. In 2,4-D herbicide treated rats, the cell appear degranulated. Hepatic cord becomes disarrayed. Hydropic degeneration of some hepatocytes is also evident. 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 Cunninghan- Burns (1985) observed focal necrosis of the hepatocytes in rats following treatment with 2,4-D. Kidney of a control rats revealed normal renal tubule and glomerulus. 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.

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. 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. According to Tayeb *et. al.* (2015) sub-acute exposure to different doses of 2,4 dichlorophenoxyacetic acid (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.

The testis of 2,4-D treated rats exhibited shrinkage of seminiferous tubules and sperms appear to be agglutinated. However, normal cellular architecture is seen showing normal germinal epithelial cells, Primary and secondary spermatocytes in control rats. In this context, Server *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 LD50 induced considerable alteration in Haematological as well as in biochemical parameters and architecture of the liver, kidney & testis of rats. Therefore widespread use of this herbicide in public place and agriculture field is to be prohibited or restricted.

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