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Research Article

EVALUATION OF TOXIC EFFECT OF 2,4-D (2,4-DICHLOROPHENOXYACETIC ACID) ON FERTILITY AND BIOCHEMICAL PARAMETERS OF MALE REPRODUCTIVE SYSTEM OF ALBINO RATS

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ABSTRACT

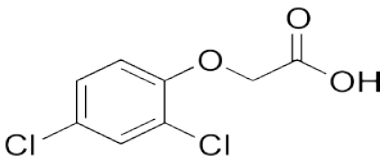
In present study herbicides 2,4-D dissolved in olive oil was administered orally to male rats at dose level 50, 100 and 150 mg/kg b.wt./day for 30 and 45 days. Its reproductive toxicity was evaluated on the basis of fertility, hormonal analysis and biochemical parameters. There was a significant decrease in the weight of testes and sex accessories, and reduction in sperm counts both in epididymis and testes in treated animals. Histology of testes showed degenerative changes in seminiferous tubules. The fertility test showed 30%, 60%, 80% and 50% 80% and 100% negative results for the respective dose levels and days. Testicular glycogen and sialic acid were reduced whereas testicular protein and cholesterol were increased. In addition, a significant decrease in serum testosterone, FSH and LH level was also observed. Present study indicated that 2,4-D showed toxic effects on male reproductive functions.

Keywords: 2,4-D, Testis, Testosterone, Sperm counts.

INTRODUCTION

Herbicide use has increased dramatically around the world over the past 6 decades [1]. By 2001 approximately 1.14 billion kilograms of herbicides were applied globally for the control of undesirable vegetation in agricultural, silvicultural, lawn care, aquacultural, and irrigation/recreational water management activities [2]. 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. Prolonged inhalation sometimes causes dizziness. Ingestion 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 [2]. Pesticides have been applied to fight against pests of plants, animals and humans [3].

A number of herbicides have been widely used now-a-days for the control of weeds. 2,4-D (2,4-Dichlorophenoxyacetic acid), one among these chemicals, is a chlorinated phenoxy compound used as an herbicide at high concentration to control many types of broad leaf weeds in lawns, gardens, agricultural fields and forestry [4].



2,4-Dichlorophenoxyacetic acid

The teratogenic, neurotoxic, immunosuppressive, cytotoxic and hepatoxic effects of 2,4-D have been well documented [5-7]. Herbicide 2,4-D increases lipid per oxidation in animal and human cells in vitro [8, 9]. 2,4-D has been shown to cause cellular mutations which can lead to cancer. This mutagen contains dioxins, a grousp of chemicals known to be hazardous to human health and to the environment [10]. Exposure to 2,4-D induce nephrotoxicity in rats during late pregnancy and early postnatal periods [11]. Mikov et al. reported that 2,4-D has a hypoglycemic effect in mice [12].

In rodents, this chemical also increases levels of the hormones progesterone and prolactin, and causes abnormalities in the estrus cycle [13]. In Minnesota, higher rates of birth defects have been observed in areas of the state with the highest use of 2,4-D and other herbicides of the same class. This increase in birth defects was most pronounced among infants who were conceived in the spring, the time of greatest herbicide use [14]. However, only few attempts have been made to observe the effects of 2,4-D on male reproductive system. Toxicants which affect the male reproductive system can act either directly or indirectly [15]. Thus, the present investigation was designed to determine the reproductive toxicity associated with 2,4-D in rats (*Rattus norvegicus*).

MATERIALS AND METHODS

Healthy adult male albino rats (*Rattus norvegicus*) weighing 150-200 gm were used for the experimentation. The animals were kept in clean polypropylene cages (measuring 12"x10"x8") with chrome plates grills and were maintain at room temperature (20°C±5°C) and uniform light dark cycle 14:10 hour light and dark cycle. Animals were mostly maintained on standard pellet diet procured from Ashirwad Industries, Chandigarh and occasionally on germinated /sprouted gram and wheat seeds as an alternate feed and fresh water *ad libitum*.

Test material and dose

Technical grade 2,4-D (2,4-dichlorophenoxyacetic acid) obtained from "Herbicides Industrial Pvt. Ltd.", Jaipur was used for experimentation. The herbicides was dissolve in olive oil and was administered male rats through oral incubations at the dose level of 50, 100 and 150 mg/kg b.wt./day for 30 and 45 days.

Experimental Design

Animals were divided into four groups having six animals each.

Group I animals received olive oil only kept as control.

Group II (A, B) animals received **50 mg/kg b.wt/day** of 2,4-D orally for 30 and 45 days respectively.

Group III (A, B) animals received **100 mg/kg b.wt/day** of 2,4-D orally for 30 and 45 days respectively.

Group IV (A, B) animals received **150 mg/kg b.wt./day** of 2,4-D orally for 30 and 45 days respectively.

At the end of the experimentation rats were weighed, sacrificed under light ether anesthesia. The male reproductive organs were removed, washed with distilled water, dried, and processed for biochemical and histopathological studies.

Fertility Test

The mating exposure test of all the animals was performed. They were cohabited with proestrous females in the ratio of 1:3. The vaginal plug and the presence of sperm in the vaginal smear were checked for positive mating. Females were separated, and resultant pregnancies were noted, when dam gave birth. The number and size of litters delivered were recorded.

Sperm Dynamics

The sperm motility in cauda epididymis and density of testicular and cauda epididymis was determined [16].

Biochemical Parameters

The Total protein[17], sialic acid[18], glycogen[19] and cholesterol[20] were assessed by standard method.

Hormonal Immune Assay

Testosterone, leutinizing hormone (LH), and follicle stimulating hormone (FSH) were estimated through chemiluminescence in fully automatic Advia Cemtaus Immuno Assay System.

Testicular histology

Testes of rats exposed to 2,4-D and control were fixed in Bouin's fixative for at least 48 h, 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

The data were analyzed statistically using Student's ''t'' test [21] and the significance of differences was set at $P<0.05$ (Significant), $P \leq 0.01$ (moderate significant) and $P \leq 0.001$ (highly significant) level.

RESULTS

Study showed no significant difference in body weight of normal and 2,4-D treated animals at the end of experimental period. However, the weight of testis, epididymis, seminal vesicle and ventral prostate were decreased significantly in treated rats (Table 1). The study revealed a marked reduction in sialic acid and glycogen contents of testis, whereas testicular cholesterol and protein contents were increased significantly (Table 2). The hormonal analysis showed a marked decrease in the testosterone, leutinizing hormone (LH) and follicle stimulating hormone (FSH) (Table 3). The sperm density in testis and cauda epididymis decreased significantly after 2,4-D treatment (Figure 1a & 1b). The sperm motility in cauda epididymis was also severely impaired (Figure 1c) and the fertility test showed 30%, 60%, 80% negative fertility and 50%, 80%, 100% negative fertility for respective doses and days (Figure 1d).

Table 1: Changes in organs weight after 2,4-D treatment

Treatment	Testis Mg/100g b. wt.	Epididymis	Seminal Vesicle	Ventral Prostate
Group I Control(Vehicle treated)	1285.08± 60.20	512.29± 33.31	427.33± 20.73	185.99± 16.98
Group II A (50mg/kg b.wt./30days)	1016.62± 39.23**	422.80± 17.30*	381.62± 13.89*	141.38± 4.90*
Group II B (50mg/kg b.wt./45days)	950.91± 48.22**	397.11± 15.22**	368.39± 10.42*	117.98± 9.34**
Group III A (100mg/kg b.wt./30days)	1005.18± 22.36**	409.19± 4.96*	374.08± 13.44*	139.13± 6.67*
Group III B (100mg/kg b.wt./45days)	894.56± 13.67***	388.31± 10.21**	361.48± 11.34*	110.19± 8.30**
Group IV A (150mg/kg b.wt./30days)	992.66± 22.10***	399.74± 15.09**	362.95± 12.00*	123.17± 9.74**
Group IV B (150mg/kg b.wt./45days)	891.35± 16.04***	368.23± 10.86**	354.62± 14.50**	93.16± 9.40***

(Mean ± SEM of 6 animals) (Group II A, B; III A,B; IV A, B compared with control)

* = P ≤ 0.05; ** = P ≤ 0.01; *** = P ≤ 0.001

Table 2: Biochemical changes in testes after 2,4-D treatment

Treatment	Protein Mg/g	Sialic Acid	Cholesterol	Glycogen
Group I Control (Vehicle treated)	248.79± 6.54	5.07± 0.10	5.30± 0.48	2.76± 0.16
Group II A (50mg/kg b.wt./30days)	316.89± 16.91**	4.38± 0.16**	10.99± 1.88*	2.34± 0.06*
Group II B (50mg/kg b.wt./45days)	332.86± 15.01***	3.99± 0.23**	11.63± 1.40***	2.01± 0.11**
Group III A (100mg/kg b.wt./30days)	332.64± 15.99***	4.21± 0.25*	11.75± 1.43***	2.14± 0.15*
Group III B (100mg/kg b.wt./45days)	334.93± 6.68***	3.96± 0.31**	11.82± 0.86***	1.55± 0.17***
Group IV A (150mg/kg b.wt./30days)	331.15± 9.26***	4.03± 0.24***	11.78± 1.16***	2.00± 0.09**
Group IV B (150mg/kg b.wt./45days)	341.01± 9.88***	3.90± 0.19***	12.65± 0.34***	1.02± 0.16***

(Mean ± SEM of 6 animals) (Group II A, B; III A,B; IV A, B compared with control)

* = P ≤ 0.05; ** = P ≤ 0.01; *** = P ≤ 0.001

Table 3: Radio immunoassay of testosterone, FSH, LH after 2,4-D treatment

Treatment	Testosterone	FSH	LH
	MIU/ml		
Group I Control (Vehicle treated)	2.8± 0.17	0.96± 0.10	2.10± 0.20
Group II A (50mg/kg b.wt./30days)	1.9± 0.24*	0.68± 0.09*	1.40± 0.16*
Group II B (50mg/kg b.wt./45days)	0.9± 0.11**	0.65± 0.11*	1.10± 0.20**
Group III A (100mg/kg b.wt./30days)	1.20± 0.54**	0.63± 0.09**	1.20± 0.19**
Group III B (100mg/kg b.wt./45days)	0.8± 0.25***	0.50± 0.06**	0.90± 0.17**
Group IV A (150mg/kg b.wt./30days)	0.90± 0.22**	0.48± 0.10**	1.00± 0.20**
Group IV B (150mg/kg b.wt./45days)	0.60± 0.13***	0.32± 0.09***	0.70± 0.08***

(Mean ± SEM of 6 animals) (Group II A, B; III A, B; IV A, B compared with control)

* = P ≤ 0.05; ** = P ≤ 0.01; *** = P ≤ 0.001

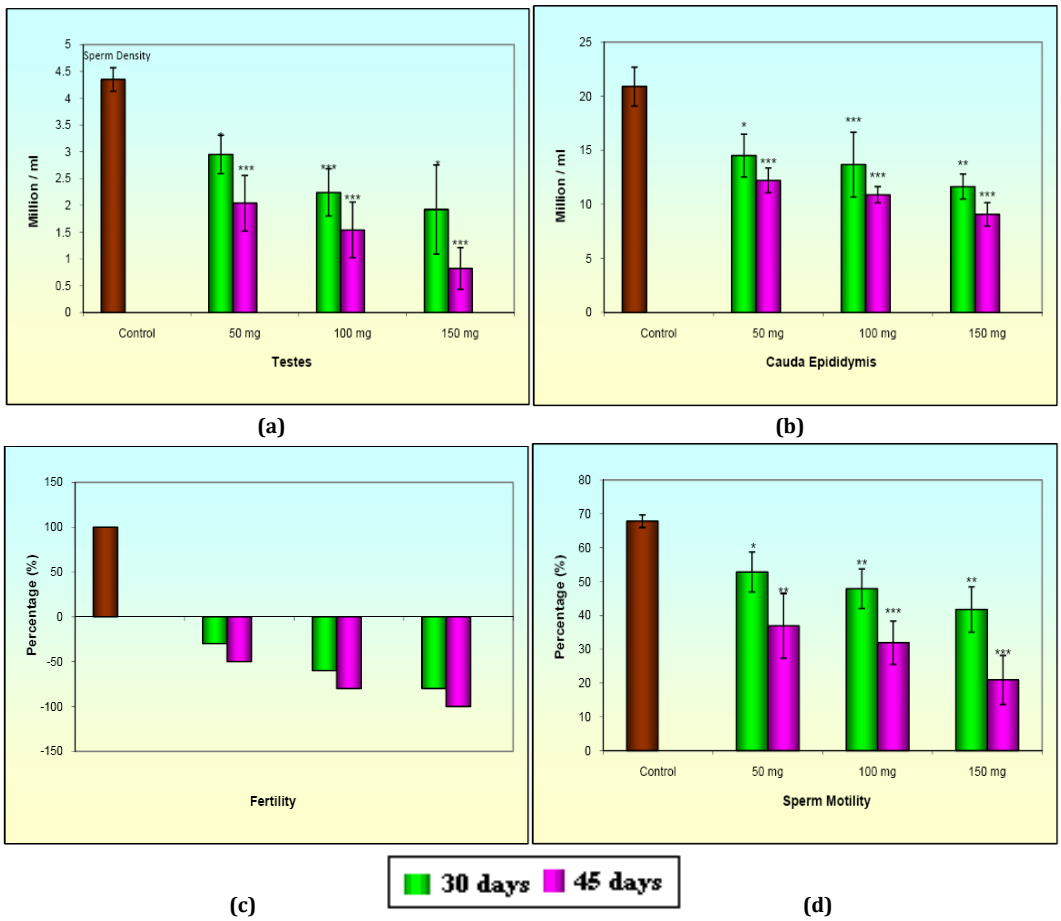
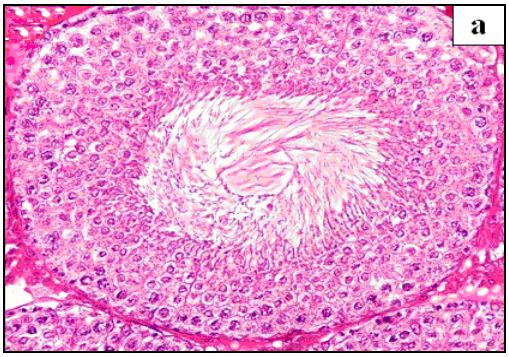
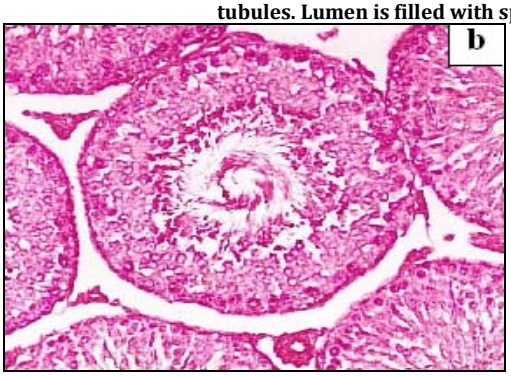


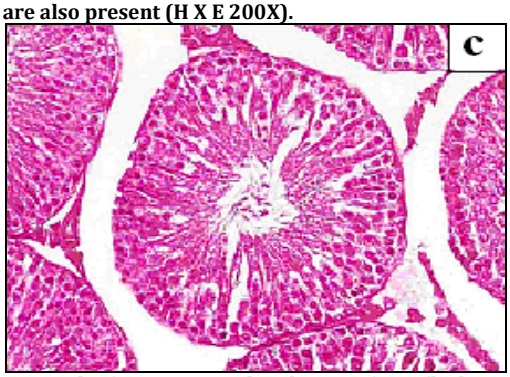
Fig. 1: (a) Altered sperm density in Testes; (b) Altered sperm density in cauda epididymis; (c) Altered sperm motility in cauda epididymis; (d) Altered fertility test after 2, 4-D treatment



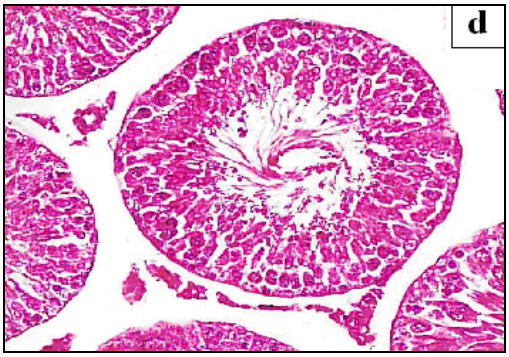
(a) Micro-photograph of control rat testes showing all the successive stages of spermatogenesis i.e. normal morphology of seminiferous tubules. Lumen is filled with sperm. Leydig cells are also present (H X E 200X).



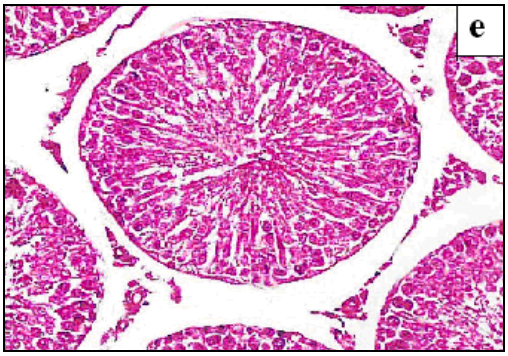
(b) Photograph of testes treated with 2,4-D 50mg/kg b.wt. for 30 days showing decreased number of spermatogenic cell. Lumen is filled with sperm debris. Leydig cells are few in number.



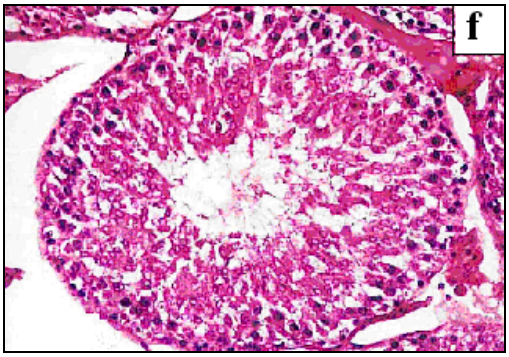
(c) Photograph of testes treated with 2,4-D 50mg/kg b.wt. for 45 days showing damaged seminiferous tubules with degenerated spermatozoa. Seminiferous tubular epithelium is irregular and degenerated.



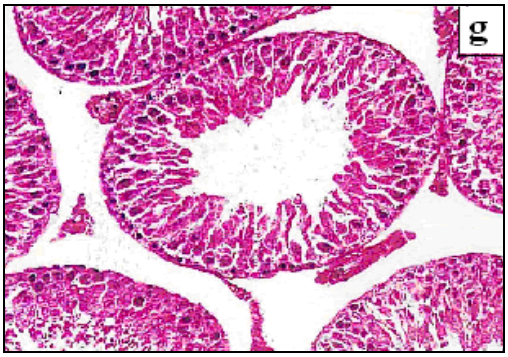
(d) Photograph of testes treated with 2,4-D 100mg/kg b.wt. for 30 days showing large space between the tubules. Sperms are very few in numbers. Spermatogenic arrest could be seen.



(e) Photograph of testes treated with 2,4-D 100mg/kg b.wt. for 45 days showing damaged seminiferous tubule containing degenerated spermatogenic cell. Lumen is full of cellular and spermatogenic debris.



(f) Photograph of testes treated with 2,4-D 150mg/kg b.wt. for 30 days showing severe degenerated changes. Germinal epithelium is irregular and loosened. Intertubular space having less connective tissue.



(g) Photograph of testes treated with 2,4-D 150mg/kg b.wt. for 45 days showing shrunken seminiferous tubules and increased intertubular space. Lumen is devoid of spermatozoa. Spermatogenic process distrupt.

Fig. 2:

Testicular histopathology

Histoarchitecture of control rat testes exhibits normal morphology of seminiferous tubule with all successive stages of spermatogenesis, lumen filled with spermatozoa and sertoli cells are present. While in treated rats, testicular cell population showed a decrease in number of spermatocytes and spermatids and majority of the tubules were deformed or of irregular shape. Interstitial space was comparatively loose. Lumen contains cellular debris. Seminiferous tubule exhibiting loosened tunica propria along with degenerated interstitial cells, degenerated spermatogonia and damaged sertoli cells. Lumen with less sperms and disrupted Leydig cells are also visible (Figure 2a-g).

DISCUSSION

The present study revealed that administration of 2,4-D (50,100 and 150 mg/kg b.wt./day for 30 and 45 days) to male albino rats resulted in acute testicular toxicity. The reduction in the weight of the testes may be due to decreased number of germ cells and elongated spermatids. 2,4-D reduce spermatogenic potential by reducing their number of sertoli cells, as weight of testes is largely dependent on the mass of differentiated spermatogenic [22, 23] or may be due to reduced tubule size, spermatogenic arrest and inhibition of steroid biosynthesis of leydig cells, a site of steroid biosynthesis [24]. Similarly due to low bio-availability of androgen the weight of accessory sex organs also decreases [25, 26] as all are androgen dependent, depleted androgen concentration might cause reduction in weight [27]. Biologically active gonadotropins are essential for normal sperm production, growth, development and maturation of testes and cauda epididymis [28]. The suppression of gonadotropins may decrease sperm density in testes and cauda epididymis [29]. The negative fertility test may be attributed to lack of forward progression and reduction in density of spermatozoa and altered biochemical milieu of cauda epididymis [30, 31]. Several secretory product of epididymis may affect which may reduce sperm motility and fertility [32].

Administration of 2,4-D also changes the biochemical parameters of the reproductive tract. Increased testicular cholesterol concentration may be correlated with its non-utilization by the system leading to a fall in circulating androgen due to anti androgenic activity [33, 34]. Increased concentration of cholesterol in testes may be the result of its non-utilization leading to the reduction of the production of testosterone, the main hormone involved in the control of fertility of animals including rats [35].

Reduction in glycogen level after the administration of 2, 4-D inhibited the glycogen synthesis which eventually decrease spermatogenesis [36, 37]. Decrease in the testicular sialic acid concentration is due to the antispermatogenic activity or reduced androgen production [38]. Elevation in the total Protein content in testes may be due to hepatic detoxification activity which results in the inhibitory effect on the activity of enzyme involved in the androgen biotransformation [39, 40].

Reduction in the serum testosterone clearly demonstrated the inhibitory effect of 2, 4-D on secretion of pituitary gonadotropins (FSH and LH) and in turn on testosterone biosynthesis. The low level of testosterone arrests spermatogenesis [41, 42]. Reduction in the FSH and LH affects the development and function of testes and inhibit the development of spermatogenesis and seminiferous tubule [43]. Thus it is concluded that herbicide 2,4-D is highly toxic to reproductive function and alter the fertility of animals.

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