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EVALUATION OF SOME PHARMACOLOGICAL MEASURES FOLLOWED BY THE TREATMENT OF HERBICIDE OXYFLUORFEN IN FEMALE MICE (*MUS MUSCULUS)*

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ABSTRACT:
Herbicides are the largest class of pesticides used in the world today and so their harmful effects can be witnessed in both agriculture and on humans. In the present study the pharmacological measures have been evaluated on the mice by treating it with oxyfluorfen herbicide. The adverse effects were showed up in mice after it being injected with this herbicide. In the treated group the estrous cycle and histological assessment along with some biochemical assays were studied. There was irregularity in the reproductive cycle of the mice. The histological observation was done in the ovary, liver and adrenal. All of these organs had changes compared to control group. The lipid profile also shown alterations along with thyroid and steroid harmone profile.
Keywords: Oxyfluorfen, ovary, adrenal, liver, oestrous cycle, hormonal assays and bioassays.

INTRODUCTION:

Herbicides, commonly called as weed killers are used to kill unwanted plants. Selective herbicides kill specific targets, while leaving the desired crop relatively unharmed. Some of these acts by interfering with the growth of the weed and are often synthetic mimics of natural plant harmones (Behera, et al. 2010). Some studies on phenoxy herbicides were too few to accurately assess the risk of many types of cancer from the herbicides, even though evidence was stronger that exposed to herbicides is associated with increased risk of soft tissue sarcoma and non-hodgkin lymphoma (Keservani, et al. 2018, Jarouliya, U. and Keservani, R.K., 2017a,).

A few herbicides are highly toxic and some are genotoxic and also carcinogenic in mammalian bioassays. Oxyfluorfen is a pre-emergent and post-emergent broadleaf and grassy weed herbicide. It is a member of the diphenyl ether group of herbicide. It is a herbicide that is not genotoxic and practically non-toxic. Oxyfluorfen is of low acute toxicity.

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Hence the present study is undertaken to see effect of oxyfluorfen compound on some physiology of mice (Jarouliya, U. and Keservani, R.K., 2017b).

MATERIALS AND METHODS:

Healthy adult female swiss albino mice weighing about 28 to 32 gm were obtained from the mice colony, Department of Zoology, Karnatak University Dharwad. They were kept in polypropelene cages (29x220x140mm) with stainless steel grill top and bedded with paddy husk and were maintained at a temperature of 28 ± 2^0 at 50-60% humidity and 12h light-dark cycle for atleast a week before the experiment. The experimental protocol was approved by CPCSEA guidelines for the care and use of small laboratory animals.

The animals were randomly divided into three experimental groups, with each group consisting of five mice and given the following treatments.

The drug was injected intraperitoneally in the lower right or left quadrant of abdomen. Oxyfluorfen drug (2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl) of 1ml is dissolved in 15ml and 20ml of distilled water (stock solution)

Group I: Served as control

Group II: 5 mice received 0.3ml (20ml stock solution) i.e; 500mg/kg body weight Oxyfluorfen for 14 days daily. A total of 14 intraperitoneal injection were given to mice.

Group III: 5 mice received 0.3ml (15ml stock solution) i.e; 70mg/kg body weight Oxyfluorfen for 14days. Total 14 injections were given to mice intraperitoneally

The body weight and estrous cycle were recorded throughout the experiment from day 1. At the end of second week ,the mice were sacrificed 24 hrs after the last treatment followed by overnight fasting. The mice were administered with light ether anesthesia under sterile conditions.

Adrenal, liver and ovary samples were dissected, trimmed off connective tissues, washed using normal saline to eliminate blood contaminations. The organs were thus subjected for histopathological assessment and biochemical assays. The blood sample was withdrawn through the jugular vein and collected along with the serum from both control and treated animals after 2 weeks of treatment for biochemical studies.

- Bioassays of E_2,T and T_3 and T_4
- Biochemical assays of lipid profile and liver enzymes.

STATISTICAL ANALYSIS:

Statistical tools were employed to analyze the data systematically. Data were expressed as MEAN±SE. The level of statistical significance was set at $p<0.05$ and $p<0.01$. Comparison of normally distributed variables among groups were made using SPSS and the values were recorded.

RESULTS:

Ovary:

As for as estrous cycle concerned it was found to be irregular in treated mice compared to the control group (Table 1,2,3 and graph1, figure 1 a and b). The histology of ovary, liver and adrenal showed up drastic changes in treated mice. The number of primary follicles, growing follicles, corpus luteum, graafian follicle were reduced. There were presence of cystic follicle

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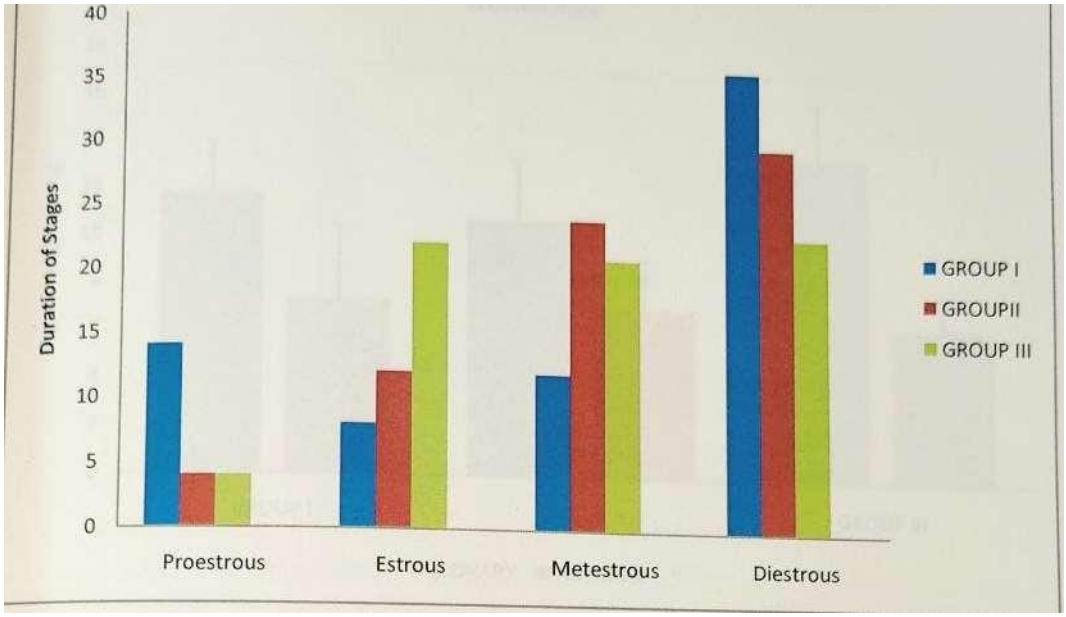
(Fig 3, C), antral follicles without oocyte (Fig 2, C) and disorganized histoarchitecture of ovary was observed. Antral follicle undergoing initial stage of atresia. Graafian follicle was with disrupted cumulus oophorous and without oocytic nuclei (Fig 2, D). The ovary of treated mice revealed an increase in the stroma (Fig 3, D). Ovarian lesions were observed in treated groups.



Graph 1: Estrous cycle in control and Oxyfluorfen treated mice

Table 1. Estrous cycle in Group II mice.

Date	Mice-1	Mice-2	Mice-3	Mice-4	Mice-5
13/11/13	D.E	D.E	P.E	M.E	E
14/11/13	M.E	D.E	E	D.E	M.E
15/11/13	M.E	D.E	E	D.E	M.E
16/11/13	M.E	E	E	M.E	E
17/11/13	D.E	M.E	M.E	P.E	D.E
18/11/13	D.E	E	M.E	E	D.E
19/11/13	D.E	E	D.E	M.E	D.E
20/11/13	D.E	D.E	M.E	M.E	M.E
21/11/13	P.E	D.E	D.E	M.E	M.E
22/11/13	M.E	D.E	M.E	M.E	E
23/11/13	M.E	D.E	M.E	M.E	E
24/11/13	D.E	D.E	M.E	E	E
25/11/13	M.E	M.E	E	D.E	D.E
26/11/13	D.E	M.E	M.E	M.E	D.E
27/11/13	E	D.E	P.E	D.E	D.E

P.E: Proestrous, E: Estrous, ME: Metestrous, DE: Diestrous.

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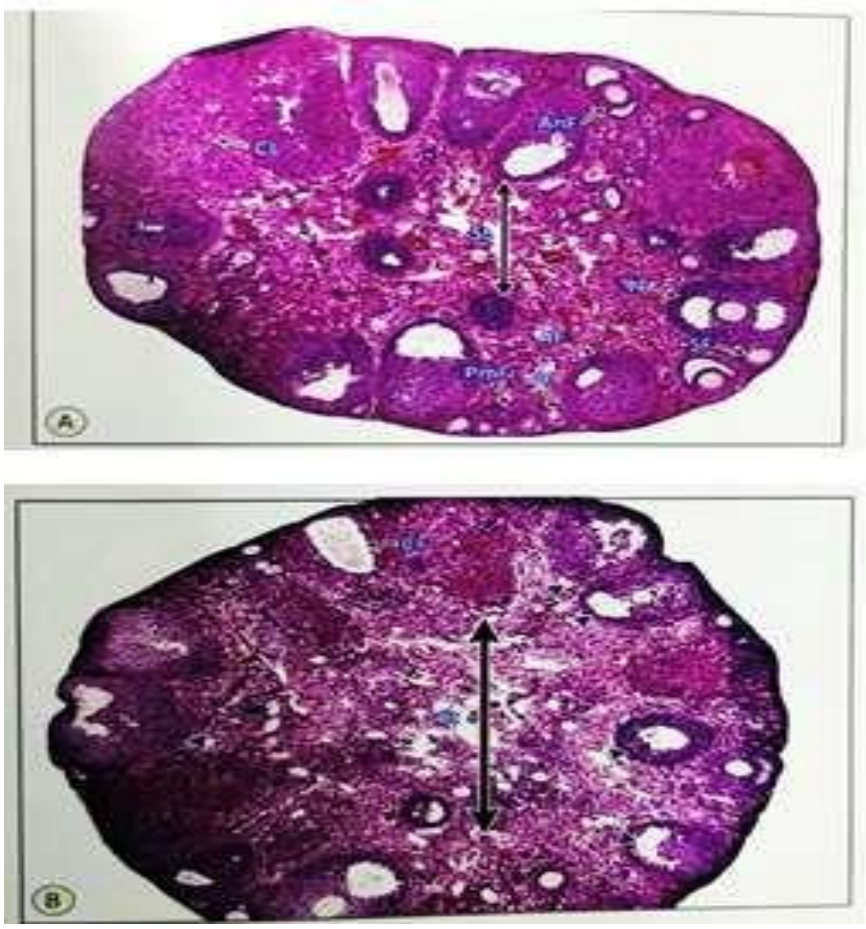

Figure 1: A: Control ovary; B: Treated ovary

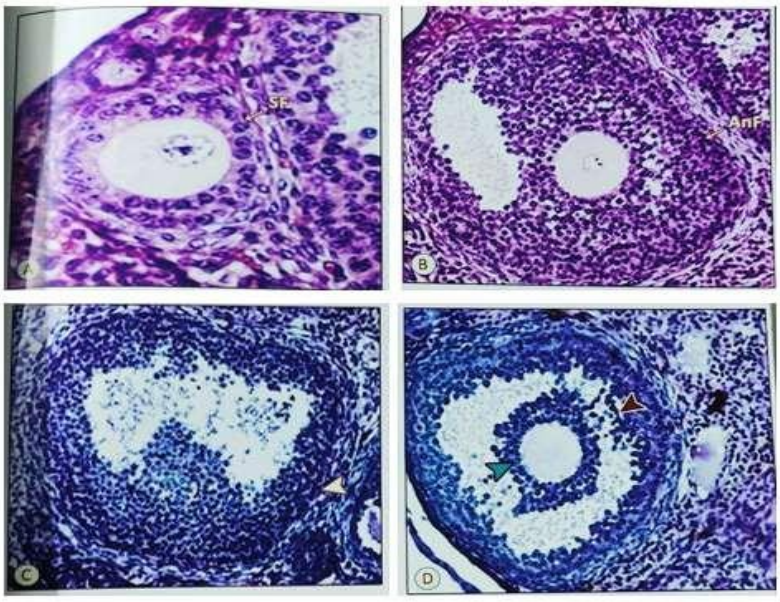

Figure 2: A, B: Control ovary; C, D: Treated ovary

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Table 2. Estrous cycle in Group III mice.

Date	Mice-1	Mice-2	Mice-3	Mice-4	Mice-5
13/11/13	M.E	P.E	D.E	D.E	D.E
14/11/13	M.E	E	D.E	D.E	M.E
15/11/13	E	M.E	M.E	D.E	E
16/11/13	M.E	D.E	P.E	D.E	E
17/11/13	D.E	D.E	E	D.E	E
18/11/13	M.E	D.E	E	D.E	D.E
19/11/13	P.E	D.E	E	M.E	M.E
20/11/13	E	D.E	E	E	M.E
21/11/13	E	D.E	M.E	E	E
22/11/13	E	D.E	D.E	M.E	E
23/11/13	D.E	D.E	D.E	M.E	P.E
24/11/13	M.E	M.E	D.E	M.E	M.E
25/11/13	D.E	M.E	M.E	D.E	E
26/11/13	D.E	E	M.E	D.E	E
27/11/13	D.E	E	E	E	E

P.E: Proestrous, E: Estrous, ME: Metestrous, DE: Diestrous.

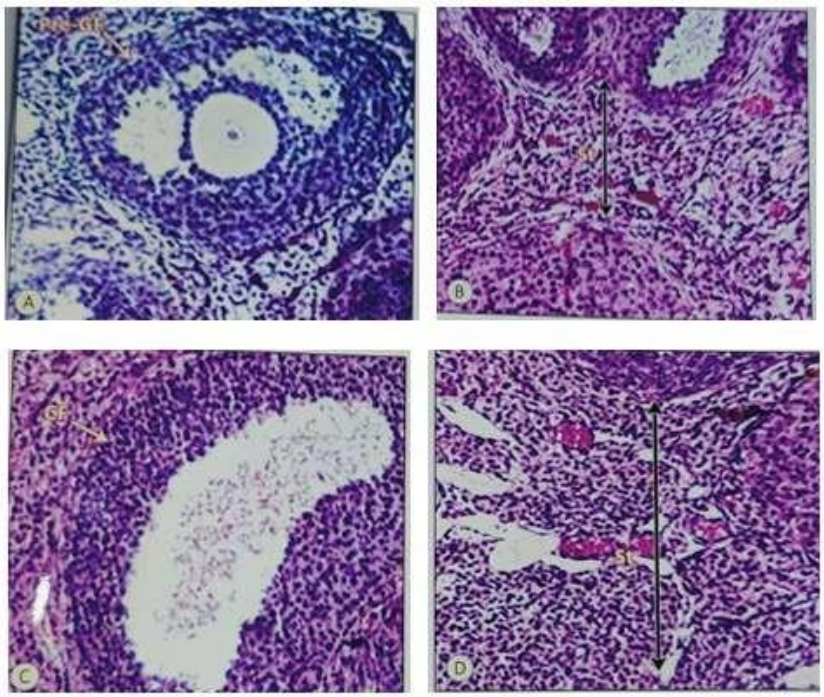

Figure 3: A, B: Control ovary; C, D: Treated ovary

Table 3: Estrous cycle in control and treated mice.

Stage of estrous cycle	Group I	Group II	Group III
Proestrous	14	4	4
Estrous	8	12	22

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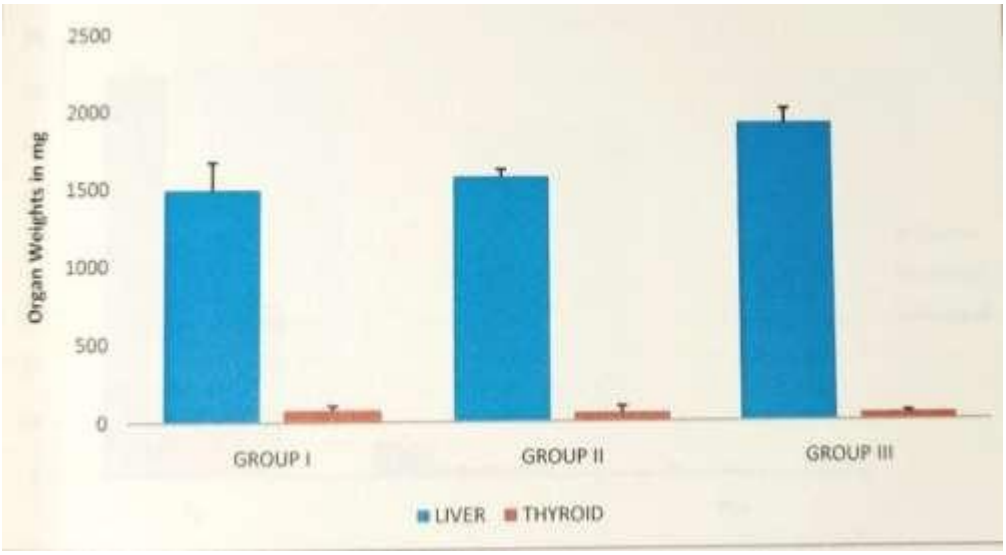
Metestrous	12	24	21
Diestrous	36	30	23

Group I- Control, Group II- 500mg treated, Group III-700mg treated.

Liver:

In the control group, liver had normal hepatic lobules and hepatocytes without any histopathologic lesion. The liver is divided into hepatic lobules formed of radially arranged strands of hepatocytes that extend from central vein to periphery of the lobule. The hepatocytes are separated from each other by blood sinusoids that are lined with the endothelial cells and Kupffercells (Fig 4, A). In the treated liver, increased liver weight (Table 4, Table 5 and Graph 2, C) hepatic lesions were observed. Disrupted hepatocytic plates were noticed. Cytoplasmic vacuolations and severe congestion in the sinusoids was observed. Pyknotic nuclei were distributed throughout the liver. The histopathologic examination revealed hepatocellular degeneration and necrosis (Fig 4, B).



Graph 2 (c): Liver and Thyroid weights of control and Oxyfluorfen treated mice

Table 4. Average body weight of control and treated mice.

Group I		Group II		Group III	
Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**
29.5±2.27	29.9±2.89	25.8±3.76	28.0±4.06	28.8±5.21	33.2±1.09

Group I- Control, Group II- 500mg treated, Group III-700mg treated.

Table 5. Weight of different body organs of control and treated mice.

Organs	Group I	Group II	Group III
Ovary	11.62±2.108	10.64±2.57	13.56±2.322
Liver	1485.06±180.69	1574.9±48.84	1927.9±95.32
Adrenal	7.22±3.107	7.06±2.075	6.44±1.408
Thyroid	74.8±31.29	52.14±46.21	45.36±15.04

Group I- Control, Group II- 500mg treated, Group III-700mg treated.

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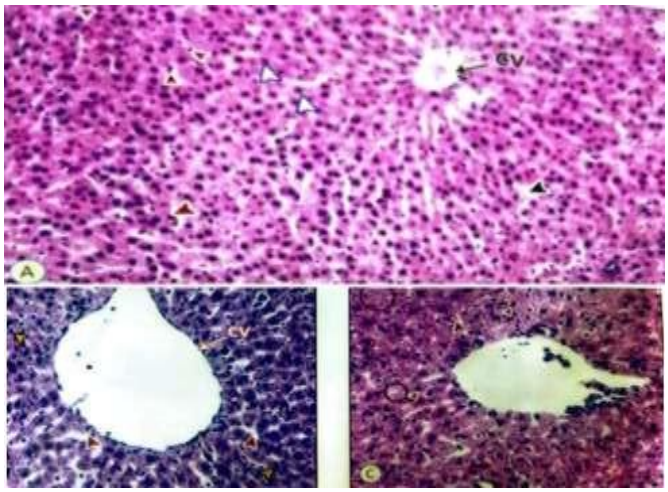
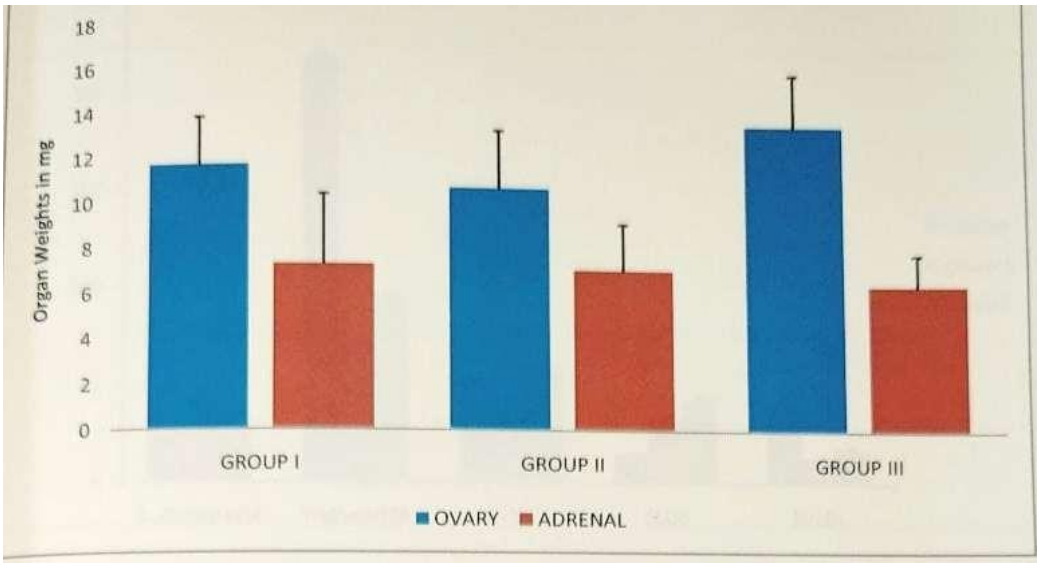


Figure 4: A: Control liver; B, C: Treated liver.

Adrenal:

This gland is a compound structure consisting of an outer cortex and inner medulla. The harmone of the cortex is steroids and that of medulla are amines. The location of medulla and cortex is very much clear structurally and functionally. The medulla is central surrounded by cortex tissue. The cortex is again divided into three layers. They are outer zona glomerulosa, middle zona fasciculate and inner zona reticularis. The medulla has distinctly clumped cell groups. The chromaffin tissue and steroidegenic tissue is always intermingled (Fig 5, A and B).

The adrenal treated with oxyfluorfen showed mild lesions throughout the cortical regions of zona glomerulosa and zona reticularis of adrenal cortex and also changes in the medullary region. Most if the sinusoids present in the zona glomerulosa and zona reticularis were filled with red blood cells, which is the indicative sign of congestion (Fig 5, C). Vascularisation (fig 5, D) was observed in the medulla. There was increase in the size of chromaffin cells. Adrenal weight decreased (Table 5 and Graph 2, b) and vacoulation in zona reticularis was noticed.



Graph 2 (b): Ovary and adrenal weights of control and Oxyfluorfen treated mice.

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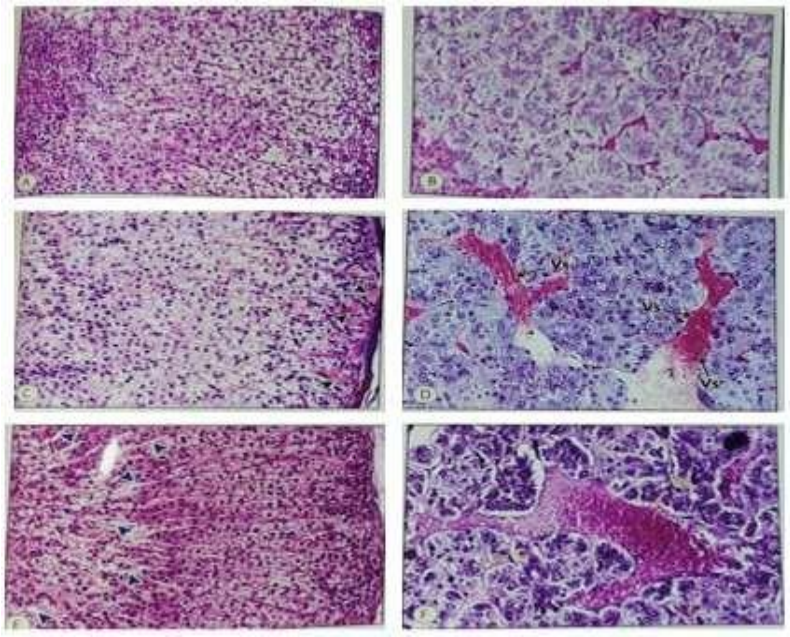

Figure 5: Control; A: Cortex, B: Medulla; Treated: C: Cortex, D: Medulla

BIO ASSAYS:

Harmonal Assays:

The decrease in the serum level of T_3, T_4 and total thyroid level was noticed (Table 7 and Graph 3). The alterations in the E_2 and T serum level were noticed in the treated groups (Table 6 and Graph 4).

Table 6. Testosterone and Estradiol in control and treated mice.

Element	Group I	Group II	Group III
Testosterone	0.646±0.03	0.536±0.01	0.631±0.02
Estradiol	0.570±0.06	1.157±0.01	1.081±0.01

Group I- Control, Group II- 500mg treated, Group III-700mg treated.

Table 7. Thyroid hormone profile in female mice treated with different doses of Oxyfluorfen.

Element	Group I	Group II	Group III	FGP Value
T_3	72.0±1.41	43.9±0.81	31.20±1.77	225.07 P<0.001
T_4	6.26±0.25	1.32±0.14	1.40±0.17	207.024 P<0.001
TSH	0.35±0.02	0.35±0.01	0.13±0.01	66.665 P<0.001

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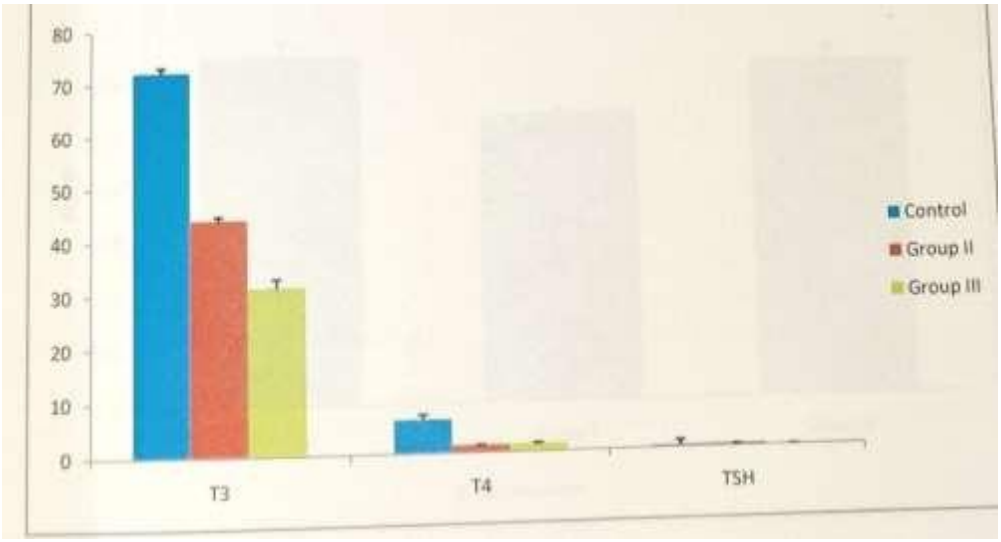
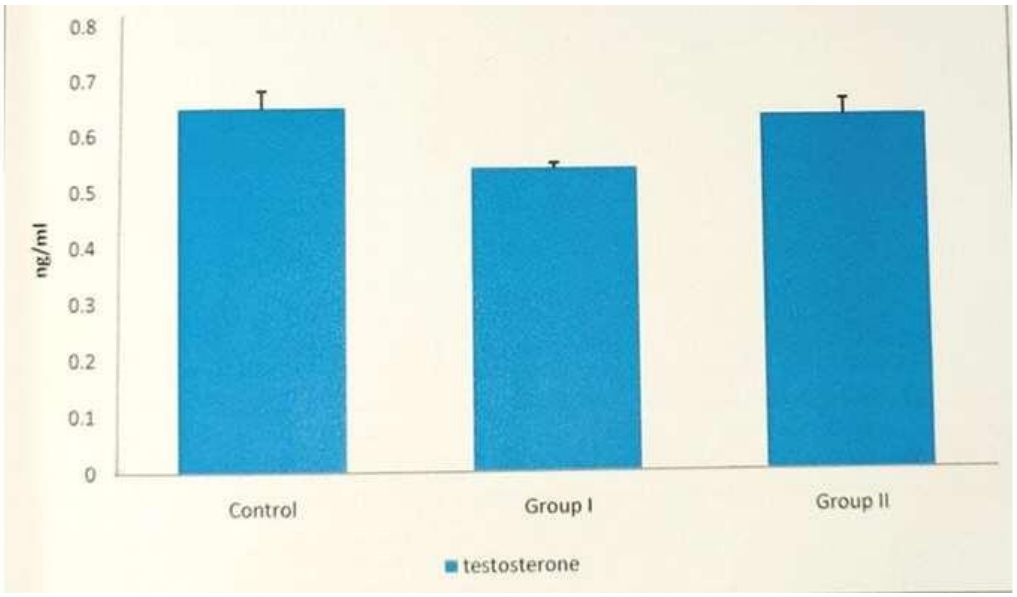


Graph 3: Thyroid hormone profile of control and Oxyfluorfen treated mice



Graph 4: Testosterone levels in control and Oxyfluorfen treated mice

Biochemical Assays:

The increase in the Serum Glutamic Oxaloacetic Transaminase (SGOT) , Serum Glutamic Pyruvic Transaminase (SGPT) activates has been observed in treated groups which indicates liver damage (Table 9 and Graph 5) The alterations in the HDL-c and LDL-c levels were noticed in the treated groups (Table 8).

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Table 8: Serum lipid prfile levels (serum chlestrol, triglycerides, HDL-c, LDL-c) in female mice treated with different doses of Oxyfluorfen. Duration – 14 days

Element	Group I	Group II	Group III	FGP Value
Serum cholestrol	131.0±2.16	42.50±1.08	33.72±0.20	1468.0
Triglyceride	216.0±2.35	71.14±1.05	94.72±0.19	2724.0
HDL-c	51.2±2.57	41.42±1.50	58.58±0.40	24.691
LDL-c	13.9±1.81	33.00±0.74	44.42±0.18	183.29
VLDL-c	40.6±2.31	12.26±0.70	17.58±0.38	113.21

n=5, values are expressed as mean ± SE

Group I- Control, Group II- 500mg treated, Group III-700mg treated.

Table 9. Liver enzyme profile (Bilirubin T, Bilirubin D, SGOT, SGPT, Alakaline phosphate, total protein, Albumin, Globulin, A:G ratio) in female mice treated with different doses of Oxyfluorfen.

Element	Group I	Group II	Group III	FGP Value
Bilirubin T	8.04±0.08	0.42±0.04	1.26±0.11	4553 P<0.001
Bilirubin D	4.34±0.19	0.16±0.02	0.73±0.03	388.0 P<0.001
SGOT	67.2±5.83	96.3±0.14	114.5±0.48	2853 P<0.001
SGPT	80.0±0.37	35.4±0.17	49.12±0.48	20.40 P<0.001
Alakaline phosphate	51.6±0.74	34.8.±0.28	38.22±0.08	360.8 P<0.001
Total protein	10.3±0.06	8.22±0.07	8.46±0.16	115.5 P<0.001
Albumin	6.40±0.83	5.04±0.15	5.18±0.18	25.82 P<0.001
Globulin	3.38±0.13	3.44±0.16	3.32±0.16	0.145 P>0.05
A:G ratio	2.22±0.06	1.44±0.12	1.30±0.04	32.05

n=5, values are expressed as mean±SE

Group I- Control, Group II- 500mg treated, Group III-700mg treated.

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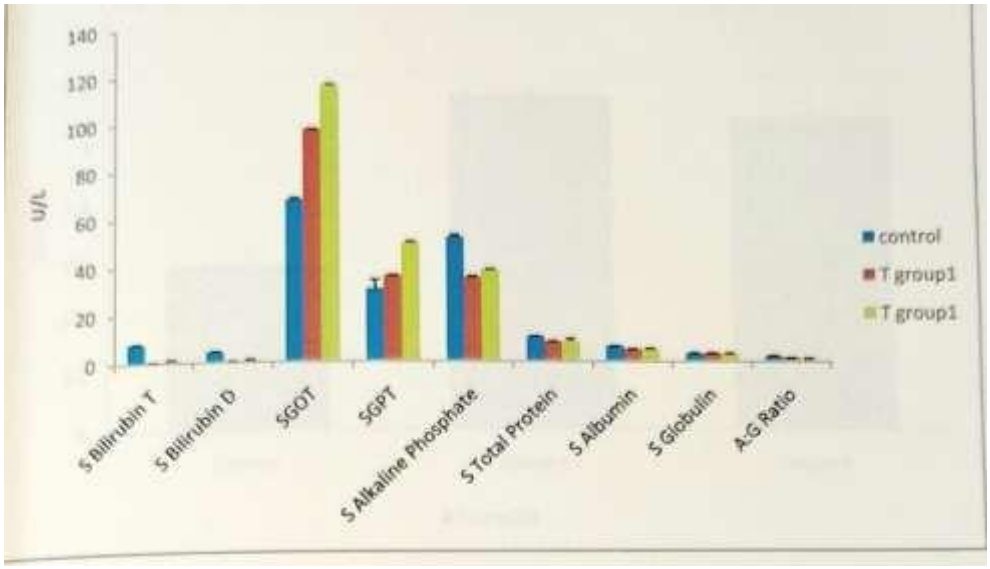

Graph 5: Liver enzyme profile of control and Oxyfluorfen treated mice

DISCUSSION:

During the last decades, the extensive use of different pesticides in agriculture and for public health purposes, has lead to drastic effects especially in animals and humans. Herbicide and insecticides are the most commonly used formulations in the world's pesticide market. Most of these chemicals are not highly selective but generally the proved to be toxic to many non-target species including man and other desirable forms of life that co-inhabit the environment. One of the herbicide Oxyfluorfen is widely used to control broad spectrum pre and post emergent annual broadleaf and grassy weeds in a variety of field crops. The present study was projected out to find out the effect of different dosages of oxyfluorfen on ovary, liver and adrenal mice.

The intraperitoneal administration of Oxyflourfen elicits measurable and distinct effect on estrous cycle and body weight. Oxyfluorfen treated mice showed irregularity in the estrous cycle when compared to normal mice. Similarly, it was observed that the treatment of atrazie, mancozeb, endosulfan, monocotophos and carbofuran drugs disrupts the estrous cycle and initiates a premature reproductive senescence (Parimala and kaliwal, 2005; Azarnia et al, 2008; Baligar and Kaliwal, 2004) respectively.

Oxyflourfen treated female mice revealed decreased thyroid weight and decreased serum level of T_3 and T_4 as compared to control. The similar results were reported in rat treated with chlorpyrifos F1 female and male rat treated with chlorpyrifos (HeeJeong et al., 2006) also mice treated with herbicide 2, 4-dichlorophenyl-P-Nitrophenyl ether (NIT), (Gray et a.l, 1983). A significant suppression of TSH levels in rat were observed when treated with 2,4-dichloro-4-nitrodiphenyl ether.

In the present study Oxyfluorfen produced significant changes in the ovarian histoarchitecture and ovulation. A reduction in the number of healthy follicles were seen after Oxyfluorfen treatment, with concomitant significant increase in the number of atretic follicles in the ovary. The present investigation is comparable to the case of follicular toxicity caused by organophosphate pesticide (Endosulfan) which increased follicular atresia (Azarnia et al, 2008). In another experiment reported that cyclophosphamide inhibited the antral follicular

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development in rats and increased atretic follicles reducing estradiol oncentration.the reduction in the number of healthy follicles in the ovary following the treatment of several pesticides is reported in the rats and mice, methyl parathion; (Baligar and Kaliwal, 2002), mancozeb; (Nishi et al., 2013); (Parimala and Kaliwal 2005); monocotrophos. Theoxyfluorfen treatment also leads to the formation of cyctic follicles, increased ovarian stroma, similarly herbicide paraquat lead to the disruption of oogenesis and ovarian structure of Wistar rat. The catecholmine are inhibitory to pancreatic beta cells (insulin) probably the compound might have hyperglycaemic condition. This herbicide compound has induced stress in the mice, which may lead to alteration in heart. Epinephrine increases both the force and rate of heart beat through stimulation of cardiac muscles beta-Ars (adrenoreceptor). The adrenal medullary cells are generally regarded as modified post ganglion neurons and functional activity seems to control largely by nervous mechanism. The hypertrophy of chromaffin cells may effect on regulation and maintenance of nervous system. It is reported that Oxyfluorfen in the diet decreases adrenal weight and some hypertrophy of chromaffin cells as also observed by Hashin et al, 2009. Drastic alterations of cortex and medulla region were observed in Wistar rat treated with Atrazine which were similar to the results observed in this experiment.

CONCLUSION:

The result shows that the treatment of Oxyfluorfen compound disrupts estrous cyclicity. It has an impact on ovulation. Significant alteration in liver enzymes reveals the hepatotoxicity in the liver following the administration of Oxyfluorfen. This herbicide has affected the latr staged follicles specially Graffian follicle. It also alters the serum lipid, thyroid and steroid harmone profile. The overall results indicate that the compound Oxyfluorfen interferes with the metabolic pathways and it affects the HPGA axis.

Conflict of interest:

None

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