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Haematological and biochemical assessment of oxyfluorfen toxicity in female rat *Rattus norvegicus*

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ABSTRACT

'Oxyfluorfen' is diphenyl ether herbicide, used as both pre and post emergent herbicide, with the recurrence of weeds widely used by farmers, uncontrolled and improper use of such chemicals may have toxic effects on exposure, directly or indirectly. In this study the toxic effect of Oxyfluorfen was evaluated by assessing symptomatic behavioral changes, haematological and biochemical parameters in female albino Wistar rat, (*Rattus norvegicus*). Rats were exposed for 120 days, to 1/25 of LD 50 value (100 mg/kg of body weight) of Oxyfluorfen orally. The exposed rat shows lethargy, blackening of tongue, red nasal and ocular discharge, but no mortality was observed. It has been also observed that Oxyfluorfen induces ovarian atrophy, swelling on intestine and kidney along with slight decrease in weight gain. Haematological analysis of blood shows significant reduction in Hb% (8.37**±0.79), R.B.C. (3.8**±0.627), while there was a slight increase in the T.L.C., with significant increase in neutrophil (40.33**±17.23) where as significant decrease was observed in the lymphocytes (56.67*±16.68) and monocytes (1.5*±0.8). Biochemical estimation of blood shows significant increase in Protein (8.461**±0.3199), glucose (229.33**±36.41), serum alkaline phosphatase level (644.8**±150.07) and potassium (7.86** ±0.967). Sub-chronic exposure of Oxyfluorfen causes toxic effects on blood composition as well as biochemical contents of it. By comparing this toxicity strict regulation for the use of Oxyfluorfen should be highly recommended.

KEY WORDS: WISTAR RAT, OXYFLUORFEN, HAEMATOLOGY, BIOCHEMICAL ANALYSIS.

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INTRODUCTION

Today's modern technologies include the use of herbicides to control weeds. The herbicides act as important part of landscape maintenance. Without deweeding, food production would be further reduced. Herbicides are the more reasonable and efficient tools of weed control, they are the synthetic material used to remove the weeds from farm, field, lawns, parks, golf courses and other areas. Herbicides are used to control aquatic weeds that inhibit irrigation withdrawal. Agricultural herbicides play undoubted beneficial roles in preserving crop yields, although they may pose serious concerns for the environment and humans because of their widespread and intensive use/misuse (careless applications, high and repeated application rate, accidental spillage etc.). It is also evident that potential to injure non-target cultivars and microorganisms, in particular those contributing to soil quality, and to cause adverse side-effects in mammals, including hu-mans (Cabral *et al*., 2003; Cabral *et al*., 2004).

Reduction in crop yield has a direct relation with competition in drought situation and weed thrive better than crop plants, when left uncontrolled weeds can grow taller than crop plants and suppress the growth. Weeds are the plants, which grow where they are not wanted. They grow in the fields where they compete with crops for water, soil, nutrients, light and space and thus reduce crop yields. They also harbor insects, pest and micro-organism. Certain weeds release into the soil the inhibitors or poisonous substances which are harmful to the desire plants, human being and live stocks. They increase the expenditure on labor and equipment, render harvesting difficult, and reduce the quality and marketability of agriculture produce. The traditional method to remove weeds is hands weeding but now a day farmer are facing the problem of labor, so they moved towards the use of herbicides.

Oxyfluorfen (2-chloro-1- (3-ethoxy-4-nitrophenoxy) -4- (triflurомethyl) benzene) is diphenyl ether herbicide, used as both pre and post emergent herbicide in the farm, residential site, squares, forestry, etc. Oxyfluorfen is structurally related to other diphenyl ethers including lactofen, acifluorfen, and fomesafen. This herbicide was first registered in the United States 1979 and Manufactured by Dow Agro Sciences and Makhteshim-Agan under the trade names Goal and Galigan (Poletika *et al*., 2001). The field half-life of oxyfluorfen is about 30-40 days. Exposure may occur with this compound at work places through inhalation and dermal contact. Oxyfluorfen the diphenyl ether herbicide acts by inhibiting proto porphyrinogen oxidase, which is the second-to-last enzyme in chlorophyll biosynthesis. This enzyme is the second-to-last enzyme in haeme synthesis, as well (Birchfield and Casida, 1997). A high intensity of solar radiation ac¬celerates the efficacy of these herbicides (Dayan and Duke, 1997). Over 428,000 lbs of Oxyfluorfen were applied to crops, primarily to grapes (20.9% of total), almonds (19.6% of total), and cotton (15.9% of total). Other minor uses included apples, pistachios, field and grass seed, olives, onions, plums, and walnuts (USDA, 2005).

Oxyfluorfen can contaminate surface water through spray drift and runoff; however people may be exposed to residues of Oxyfluorfen through their diet. This exposure can leads to the accumulation of this herbicides in the animal body. Herbicides don't kill the animals quickly but the accumulation can leads to the lethal effects. Toxicity studies with Oxyfluorfen and other similar herbicides suggest the same phototoxic compounds may occur in animals as a result of herbicide exposure. (U.S. E.P.A. 2001). Herbicide is synthetic chemical that kills the plants or inhibits their growth. Herbicide accumulation mainly caused by flowing water from fields. These compounds may also pass from surface to ground waters. In this way, ever increasing agriculture has caused contamination of natural water resources.

With the recurrence of weeds farmers are repeatedly using the herbicides, which may not kill the crop plant but may get accumulated in leaves, fruits or grains. Consumption of such products may result in to slow accumulation of these chemicals in our body. Many of the references available are mostly resulting about acute exposure. This study was carried out to observe the subchronic effect of herbicide Oxyfluorfen on the haematological and biochemical parameters of the female albino Wistar rat, as the half-life of this herbicide is short it is been repeatedly used by farmers.

MATERIAL AND METHODS

Oxyfluorfen was procured from local market of agro products, in Amravati (Maharashtra). It has a **Chemical name:** Oxyfluorfen (2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-triflurомethyl)benzene), marketed as trade name 'Goal' in Maharashtra.

Healthy female albino Wistar rats (*Rattus norvegicus*) weighing about 190 ± 10 gm. were used as experimental animals. They all were feed on pellet diet and water *ad libitum*. All the experiments were carried out in a laboratory condition with proper illumination and temperature in the animal house. The animals were divided in two groups. Group 'A' served as control and consisted of six rats. Group 'B' was experimental and consisted of six rats. Each experimental rat was given an oral dose 1/25 of LD 50 value (100 mg/kg of body weight), dissolved in normal tap water for 120 days, whereas each rat in the

control group 'A' was given an equal amount of normal tap water by the same route. The rats of group 'A' were sacrificed on the 120th day while group 'B' rats were dissected on the 121st day after giving 120 days oral dose according to the norms of ethical committee (1060/ac/07/CPCSEA.). Blood was collected for haematological and biochemical estimation from the renal artery.

METHODS FOR HAEMATOLOGICAL ANALYSIS

1) Haemoglobin percentage was analyzed by- **Beckman counter machine**
2) Total R.B.C. count by- CBC **counter machine**
3) Total W.B.C. count by- CBC **counter machine**
4) TLC by- CBC **counter machine**
5) DLC by- **Haemocytometer Neubar counting chamber**

METHODS USED FOR BIOCHEMICAL ANALYSIS

1) Estimation of Serum glucose, protein, cholesterol, and glucose – was done by **Fully automatic analyzers.**

BIOCHEMICAL ANALYZER "SELECTRA –E"

The Haematological and Biochemical tests were conducted in Dr. Panjabrao alise Bhausaheb Deshmukh Medical College, Amravati using Biochemical Analyzer Selectra E Model.

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 AND DISCUSSION

Various types of pollutants affect our life directly and indirectly, possible upcoming threats to human health and wildlife can be caused by residues of pesticides, which may build up in the food chain and cause widespread contamination of the environment (El-Sebae, 1994). In the last 10 years it is found that many of the animal illness and deaths are due to the pesticides exposure mainly herbicides. In the present study female Wistar rats were exposed to Oxyfluorfen by giving a dose 1/25 of LD50 i.e. 100 mg/ kg/ body weight/day, for 120 days. During above exposure period rats shows various behavioral responses like hyperactivity, it was seen just after the administration of dose and the rats moves round in cage for 5-10 minutes. The experimental rats also showed symptoms of lethargy, reduction in feed and water intake with red nose and eye discharge, blackening of tongue and bulging of eyes was also observed during the last 30-35 days of experimental period.

There was swelling on the uterus and slight reduction in the size and weight of the ovary of experimental rats. No mortality was observed.

Significant increase in the liver weight (7.14633*± 0.49.337) was observed. According to (USEPA, 2001) Oxyfluorfen (72%) on exposure to 10 CRJ-CDf rats for 3 months leads to the absolute increase in liver weight in high dose females (+19%/+4%). Weight of the adrenal gland was also increased significantly (0.021667**± 0.00216) (Table 1).

As compared to control rats, variations in haematological parameters were observed in Oxyfluorfen exposed rats, shown in the table 2. There is significant reduction in the Hb% (8.37**±0.79) of the experimental rat, similarly R.B.C. also shows significant reduction (3.8**±0.627), which may be due to the photo toxicity of Oxyfluorfen. Oxyfluorfen inhibits haeme production, which results in a variety of anemias (EPA RED Oxyfluorfen 2002). In the sub- chronic rat study which used the current 98% a.i. (active ingredient) formulation, the red blood cell count was normal, but the red blood cell mass was decreased due to the small size of the red blood cells, presumably because of inhibition of the protoporphyrinogen oxidase enzyme (USEPA.1984.) Where, the slight increase is observed in the total leucocytes count. Neutrophil shows significant increase (40.33**±17.23). Similar result was observed by USDA, (2005), elevated

TABLE 1: Effect of oral administration of Oxyfluorfen (1/25 of LD 50 value (100 mg/kg of body weight, 120 days exposure), on relative organ weight of Wistar female rats.

Parameters	Control	Oxyfluorfen
Kidney	0.6733±0.113304	0.753±0.016923
Adrenal gland	0.038662±0.009459	0.021667**±0.00216
Liver	6.3475±1.364287	7.14633*±0.49.337
Spleen	0.369667±0.05071	0.378167±0.010704

TABLE 2: Haematological profile, effect of Oxyfluorfen (1/25 of LD 50 value (100 mg/kg of body weight, 120 days exposure), on rats (n=6).

Parameters	Control	Oxyfluorfen
Hb%	11.16±2.12	**8.37**±0.79**
R.B.C. Millions/cu mm	6.13±0.686	**3.8**±0.627**
T.L.C. thousand/cumm	6350±1338.28	6766.67±801.669
Neutrophyl %	18.83±6.24	**40.33**±17.23**
lymphocyte%	77.33±5.78	**56.67*±16.68**
Eosiniphyl%	1±0.63	1.5±1.2247
Monocyte%	2.8±1.32	**1.5*±0.8**

TABLE 3: Biochemical profile, effect of Oxyfluorfen (1/25 of LD 50 value (100 mg/kg of body weight, 120 days exposure) on rats (n=6).

Parameters	Control	Oxyfluorfen
Glucose (mg/dl)	149.33±64.83	**229.33**±36.41**
alkaline phosphatase (U/l)	381±252.22	**644.8**±150.07**
Proteins(gm/dl)	8.115±1.618	**8.461**±0.3199**
cholesterol (mg/100ml)	54±17.66	54.17±9.325592
Sodium(mEq/l)	140.8±4.355	143.17±1.169
Potassium(mEq/l)	6.05±1.68	**7.86** ±0.967**
Calcium(mgm/dl)	5.3±0.42	5.748 ±0.4739

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

leukocyte counts were observed in rats exposed by inhalation to aerosols of Goal 2E for up to 20 days; where the significant decrease was observed in the lymphocytes (56.67*±16.68) and monocytes (1.5*±0.8).

Biochemical alterations observed are shown in the table 3. There was significant increase in the glucose level (229.33**±36.41), Hyperglycemia induced by any toxicant might be explained by the inhibition of neuro-effector sites in the adrenal medulla leading to the hyper secretion of the adrenaline, which stimulates the break-down of glycogen to glucose (Gupta, 1974).

Significant increase in serum alkaline phosphatase (644.8**±150.07) was observed, which may be due to the hepatomegaly of liver. Michele antra- cordone et al., (2005) observe the increase in serum alkaline phosphatase and increase in liver weight in dogs fed (Oxyfluorfen 71.4 - 73.8% a.i.) in the diet for 52 weeks at concentrations of 600 ppm or higher.

Where Proteins (8.461**±0.3199), and Potassium (7.86** ±0.967) level also shows significant increase. No any observable changes were found in the level of serum cholesterol, calcium and sodium.

CONCLUSION

Oxyfluorfen on prolonged exposure found haemotoxic and can leads to the various types of anemias. When rats were dissected for blood; lesions, edemas, swelling on some organs were observed. The potential for toxic effects is a serious concern for this herbicide so farmers/ users should make aware about hazardous effects and toxocity as well as it should be avoided in public places, schools and strict regulation of the use of Oxyfluorfen in our environment should be highly recommended.

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