Original Research Article

Biochemical And Histological Evaluation Of Penoxsulam Herbicide On An Animal Model

ABSTRACT:

Aims: Many herbicides react to the body system and might effect the activity of hormones in the human body. The present work aims to determine the potential impact of 90 days of repeated exposure to Penoxsulam herbicide by oral gavage on the Liver, Kidney, Thyroid endocrine profile and biochemical stress in the wistar rat model.

Study design: The primary study was performed to Wistar rats grouped into 6 groups. Four groups were picked for low dose, middle dose, high dose, and high recovery dose, respectively. They were administered the Penoxsulam at dose levels of 100, 300, 500 mg/kg body weight. Similarly, 2 recovery groups were classified as control and recovery control groups, and doses were administered to them only through the corn oil (vehicle) via the oral route with the help of a suitable cannula for 90 days.

Place and Duration of Study: Toxicology department, Shriram Institute for Industrial Research, Delhi (INDIA), July 2020 and June 2021.

Methodology: In this study, healthy 60 male and 60 female Wistar rats aged 6-8 weeks, weighing 130-190 gm, were used. Before commencing the study, permission from IAEC (Institutional animal ethics committee) was taken for this experiment (CPCSEA).

Results: This study evaluates significant changes in the body weight of rats; moreover, Penoxsulam elevated the significance level of SGOT, SGPT, BUN, Urea, and Creatinine. No alterations were seen in Hematology parameters and Ophthalmology examination. Also, physiological changes were examined after exposure to penoxsulam in rats.

Conclusion: Therefore, Penoxsulam showed harmful toxic effects on the Kidney and Liver. However, no alteration has been seen in the thyroid profile (T3 triiodothyronine, T4 thyroxine, TSH thyroid-stimulating hormone) of Wistar rats during the experimentation period.

Keywords: Penoxsulam, Oral toxicity, Wistar rat, Thyroid profile, Liver, Kidney

1. INTRODUCTION

Penoxsulam (2-(2,2-difluoroethoxy)-N-(5,8-dimethoxy1,2,4] triazolo [1,5-c] pyrimidin-2-yl)-6-(trifluoromethy) benze-nesulfonamide)) .[24] is a new triazolopyrimidine sulfonamide herbicide used in California for post-emergence, broad-spectrum weed control in rice (*Oryza sativa L.*). Penoxsulam is a systemic herbicide that internally disrupts growing weeds is absorbed by the phloem and xylem tissues, leading to death. Penoxsulam is used to control broadleaf, sedge, and grass weeds in transplanted, dry-seeded, and water-seeded rice. [5] Adverse effects on plants by Penoxsulam depend on the defoliant metabolic process rate in plants and not only on their inhibitory strength upon the target site. [26] This systemic herbicide is absorbed primarily via leaves and secondarily via roots translocated in both xylem and phloem. Weeds treated with Penoxsulam will stop growing almost immediately. The signs show rapid growth obstruction and a chlorotic growing site with the death of the terminal bud, resulting in at the end of sensitive weeds in two to four weeks. Its primary use would be in a post-emergence implementation in parched seeded, water-seeded, and transplanted rice .[6-18] Rice is an essential food in one's daily life, and 90% of the world's population depends on it. Nowadays, herbicide sprays are widely used for weed control, and they are very harmful to humans and animals.
Farmers working with Penoxsulam can also be exposed to the herbicide in the course of maintenance, sampling, trials, or any other process .[27] Normally farmers are exposed to herbicides via inhalation, ingestion or topically, and these herbicides are mainly absorbed in the blood stream to damage the organs. The hepato-renal are major target organs which plays an important role in purifying the organisms, via absorption and elimination of the chemicals and its consumption. These two organs are more sensitive for enhance toxic effects by chemicals as herbicides.[22-2] Hepato-renal changes after exposure of herbicides in farmers have been published from palestine and India .[13-29] On the other side at highest dose, severe renal toxicity has also been reported after 6-mp treatment. [9]

In recent years, the use of such kinds of herbicide has increased. There is a need to study the adverse effects likely to arise from repeated exposure to Penoxsulam. In addition, these experiments are carried out to establish safety criteria concerning to human exposure as many pesticides are toxic for the liver and kidneys. Thyroid gland also plays a significant role in keeping the body functioning normally. But a very few studies have been carried out to see the exposure of herbicide on the thyroid. Therefore, the present study discusses the comparative effects of Penoxsulam herbicide on the liver and kidney and thyroid profile. The present study has been carried out to evaluate the physiological impact of repeated exposure to Penoxsulam herbicide on the Liver, Kidney, Thyroid endocrine profile and biochemical and histological stress in the Wistar rat model.

2. MATERIAL AND METHODS

2.1 Animals and experimental design

In this study, healthy Wistar rats aged 6-8 weeks, weighing 130-190 gm, and bred at the animal house facility in Shriram Institute for Industrial Research, Delhi, were used. Before commencing the study, permission from IAEC (Institutional animal ethics committee) was taken for this experiment (CPCSEA). Temperature, humidity, light intensity, and were recorded daily in the experimental room. The room temperature was maintained at 20-24°C with 50-60% relative humidity. The room was ventilated at the rate of approximately 15 air changes per hour with 12 hours of artificial light (8 am - 8 pm) each day and the lighting was controlled. Three rats were kept in polypropylene cages (size 430×290×150 mm) with wire mesh tops and each of these cages was tagged with a unique identification number using tail marking. Krishna Valley Pvt. Ltd. provided the standard feeds, and the filtered water was given *ad-libitum* to the Wistar rats. Feed and water quality was monitored at the NABL-accredited laboratory at Shriram Institute for Industrial Research, Delhi. The floor of the experimental room was swept on a routine basis with a disinfectant solution comprising D-125. The rat cages were rotated every week till the end of the experiment to provide uniform artificial light to the animals. During the experimental period, acclimatization was done for 5 days before administering the doses. Rat cages were tagged with study number, animal number, sex, dose, experiment start date, date of dosing, experiment completion date, and cage number. [16]

2.2. Grouping of animals and dose level

Animals were randomized according to their body weight and divided into six groups of 10 animals belonging to each sex. All the doses were freshly prepared in a volumetric flask using corn oil as a vehicle. A recovery group was taken to observed the reversibility of the toxic effects. All groups are as follows: (OECD 408, 2018)
G1: The control group (only corn oil was administered to the rats)
G2: Penoxsulam (Low dose) 100 mg/kg body weight
G3: Penoxsulam (Middle dose) 300 mg/kg body weight
G4: Penoxsulam (High dose) 500 mg/kg body weight
G5: The Recovery control group (only corn oil was administered to the rats)

G6: Penoxsulam (Recovery High dose) 500 mg/kg body weight

2.3. Acute oral exposure study

In the judgment, the toxic features of a test item is the resolution of the acute oral toxicity in Wistar rats which is usually a step-wise procedure. Hence, this study was carried out to evaluate the acute oral toxicity of Penoxsulam in Wistar rats. A study at the dose of 2000 mg/kg body weight was performed in three nonpregnant female rats as per OECD guideline No. 423. A single oral dose was administered to the animal with the help of a cannula attached to syringes. The animals were fasted overnight before dosing and after 4 hours of post-dosage. In the acute study, animals exposed to Penoxsulam via the oral route did not demonstrate any toxic effect or mortality at the dose level of 2000 mg/kg body weight. Hence, the LD_{50} range is categorized as per the GHS (Globally Harmonized Classification system) *i.e.* 2000 mg/kg < LD_{50} <5000 mg/kg body weight .[17]

2.4.Repeated oral exposure 90 days

Based on the acute study and dose range-finding study and existing literature on Penoxsulam, dose levels of 100, 300, 500 mg/kg bodyweight were selected for the main study. The primary study was performed with 60 male and 60 female Wistar rats grouped into 6 (G1, G2, G3, G4, G5, G6). Four groups (*i.e* G2, G3, G4, and G6) were picked for low dose, middle dose, high dose, and high recovery dose, respectively. They were administered the test compound at dose levels of 100, 300, 500 mg/kg body weight. Similarly, G1 and G5 were classified as control and recovery control groups, and doses were administered to them only through the corn oil vehicle via the oral route with the help of a suitable cannula for 90 days. The purpose of the recovery group is to determine the process of becoming well again after an illness or toxic effect. The animals were observed for toxic signs, haematology and biochemical parameters, body weight, histopathology analysis, and ophthalmology examination. All the rats were anesthetized by CO_2 exposure and blood was drawn from the orbital sinus. The whole blood was collected in a gel tube containing K2EDTA anticoagulant, and this blood was studied using AU14091 Beckman Coulter Hematology analyzer. Clinical, biochemical parameters like SGPT, SGOT, BUN, UREA, and Creatinine were studied by Beckman Coulter AU480 Clinical chemistry analyser system. The thyroid profile (T3, T4, TSH) was examined by an immunology analyser system COBAS-E-411 ROCHE. At the end of the experiment, the animals were sacrificed and tissues were preserved in 10% neutral buffered formalin for histopathological evaluation (OECD-408).

2.5.Statistical Analysis

All data were expressed as mean ± S.D using GraphPad prism software (version 9.2 for windows). The data of body weight gain, clinical chemistry were compared by one-way ANOVA. A 95% confidence level was used to determine statistically significant differences that are dependent on the p-value.
If p value<0.05=significant
If p value>0.05=non significant

3. RESULTS AND DISCUSSION

3.1. Clinical signs

Animal were observed daily for toxic signs, and this study found no mortality or toxic signs and symptoms in any of the dose groups (G1, G2, G3, G5). However, in the high dose (G4) group and recovery high dose (G6) group (at the dose level of 500 mg/kg body weight), all the animals demonstrated ruffled fur, lethargy, anorexia, discoloration of faeces, emaciation, hunched posture, and polyuria. (Table 1)

Table 1. Clinical signs and symptoms

Group & Dose level	Toxic signs and symptoms
G1- 0 mg/kg body weight	No toxic sign and symptoms were noticed
G2- 100 mg/kg body weight	No treatment-related toxic sign and symptoms were noticed
G3- 300 mg/kg body weight	No treatment-related toxic sign and symptoms were noticed

G4- 500 mg/kg body weight	ruffled fur, lethargy, anorexia, discoloration of faeces, emaciation, hunched posture and polyuria.
G5- 0 mg/kgbody weight	No treatment-related toxic sign and symptoms were noticed
G6- 500 mg/kg body weight	ruffled fur, lethargy, anorexia, discoloration of faeces, emaciation, hunched posture and polyuria.

3.2. Mean body weight

Body weight gain of the low dose (G2) and middle dose (G3) group animals was comparable to that of control group (G1) animals. However, bodyweight gain in the high dose (G4) group of animals was decreased in males and females when compared to that of the control group (G1) of animals after administration of the dose; though the decrease was statistically significant in the high dose group (G4) (figure:1), and body weight gain of the high recovery dose (G6) group animals was comparable to the body weight gain of the recovery control (G5) group animals (Table 2)

Table 2. Effect of dose level on body weight in Wistar rats

Group & Dose level	Male		Female	
	Day 1	Day 91	Day 1	Day 91
G1- 0 mg/kg body weight	121.80±4.92	262.70±5.76	123.00±6.04	239.10±5.97
G2- 100 mg/kg body weight	122.70±3.62	261.60±3.31	122.60±4.12	239.50±4.48
G3- 300 mg/kg body weight	124.40±4.33	257.00±5.77	121.90±4.31	238.30±3.20
G4- 500 mg/kg body weight	122.1±3.93	219.00±5.23	121.10±4.33	216.60±3.50
G5- 0 mg/kg body weight	122.70±2.50	281.50±6.13	119.20±2.90	258.70±5.54
G6- 500 mg/kg body weight	122.60±6.38	281.20±1.03	121.20±3.19	257.50±3.10

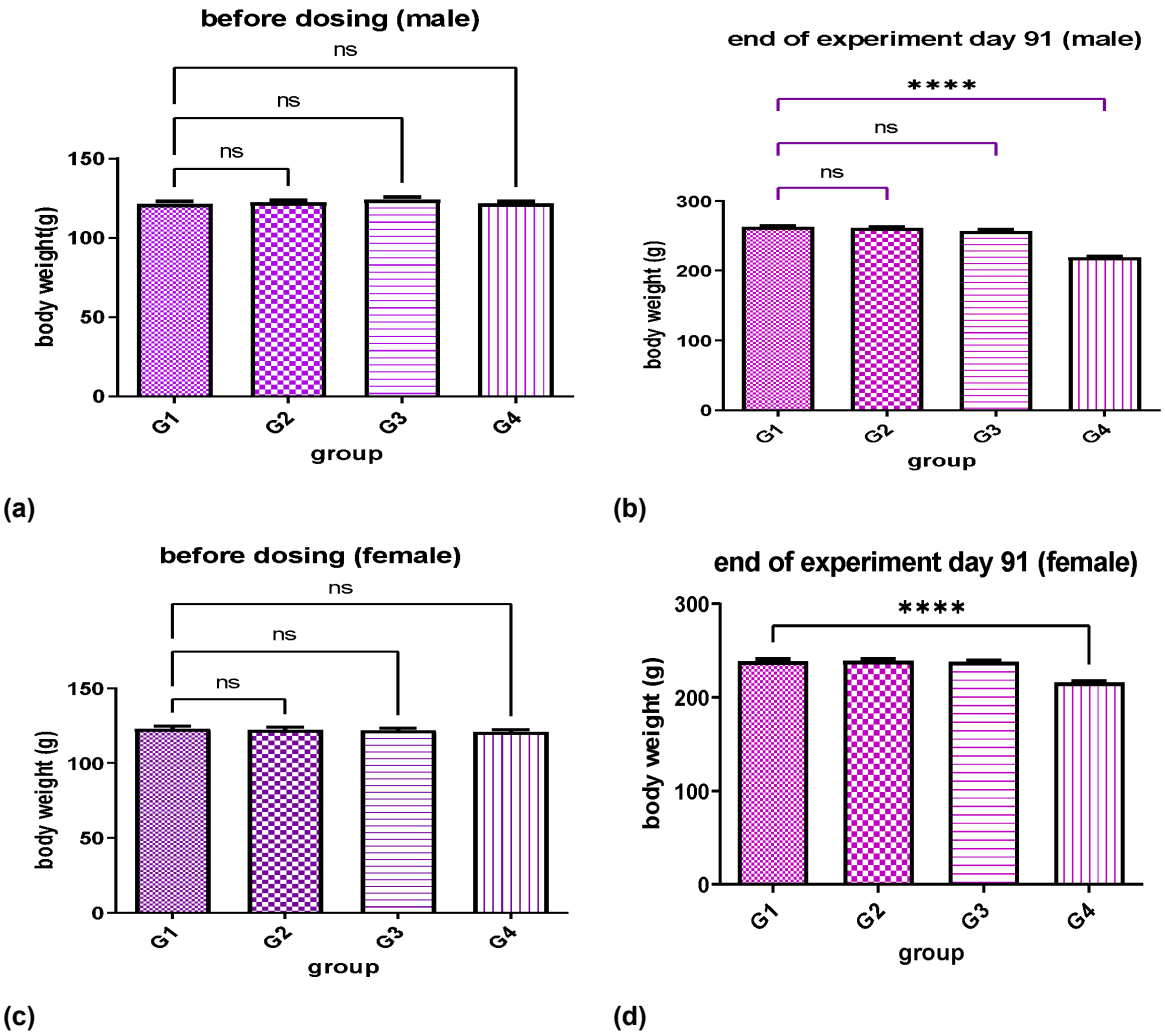


Fig. 1. Growth curve of male (a and b) and female rats (c and d) during the course of the life-phase experiment, the curves are based on mean body weight for each sex group (ns = non-significant, *** = significant).**

3.3. Haematology Evaluation

The hematological blood parameters of all the treated groups (G1, G2, G3, G4) as well as the control group of animals in male and female rats were found to be normal when examined at terminal sacrifice. Similarly, the blood parameters of animals in the recovery high dose (G6) group (500 mg/kg b.wt.) were noticed to be normal (p value>0.05= nonsignificant) to their control counterparts (G5) as the parameters fell within the accepted limits (data not given) in both male and female Wistar rats.

3.4. Biochemical evaluation

The biochemical parameters examined in the serum sample showed that there was no significant change in the low (G2) and middle dose (G3) group concerning the control (G1) group; However, a slight increase in SGOT (Serum Glutamate Oxaloacetate Transferase), SGPT (Serum Glutamate Pyruvate Transferase), BUN (Blood Urea Nitrogen), Urea, and Creatinine was noticed in animals belonging to the high dose (G4) group that was observed at terminal sacrifice i.e the 91st day, and the parameters of recovery high dose (G6) group animals showed no significant change concerning the recovery control group (G5). Thyroid profile T3, T4, and TSH examined in the serum sample revealed no modifications in any of the dose groups and the control group of animals (Table 3).

Figure 2 reflects that p value<0.05= significant in SGOT, SGPT, BUN, Urea, Creatinine of the high dose group (G4) as compared to the control group (G1) of males and female rats. G2 and G3 groups have a non-significant value with respect to all the parameters. The G6 group represents reversibility in SGOT, SGPT, BUN, Urea, and Creatinine compared to the recovery control group (G5) in male and female rats. Changes in the serum liver function test (LFT) and Kidney function test (KFT) suggest an increases incidence of lesions to the hepatocytes and renal cells.

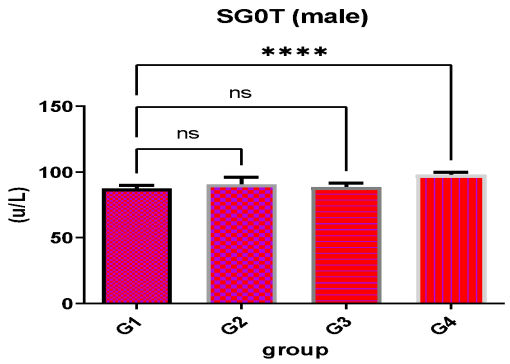
The thyroid profile showed no alteration values (p value>0.05=non significant) in any of the treated groups and the control group of male and female rats.

Table 3. Effect of Penoxsulam on SGOT, SGPT, BUN, Urea, Creatinine, Thyroid profile in male and female Wistar Rats

Group & Dose level	SGOT U/L		SGPT U/L		BUN mg/dl		UREA mg/dl		CREATININE mg/dl		T3 ng/dl		T4 ng/dl		TSH ng/ml	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
G1-0mg/kg body weight	87.70 ± 2.31	91.76 ± 4.31	51.05 ± 3.18	49.67 ± 3.56	17.99 ± 0.72	18.38 ± 0.86	38.37 ± 1.43	39.15 ± 1.73	0.77 ± 0.07	0.75 ± 0.07	84.30 ± 1.13	83.65 ± 0.95	1.51 ± 0.22	1.59± 0.22	3.58 ± 0.08	3.50 ± 0.62
G2-100mg/kg body weight	90.81 ± 5.56	89.80 ± 3.79	51.08 ± 2.34	49.75 ± 5.71	18.42 ± 0.83	18.45 ± 0.79	39.23 ± 1.65	39.29 ± 1.58	0.77 ± 0.05	0.76 ± 0.05	83.93 ± 0.94	83.80 ± 0.76	1.70 ± 0.17	1.70± 0.18	3.74± 0.53	3.79 ± 0.33
G3-300mg/kg body weight	88.74 ± 2.89	90.44 ± 2.57	50.79 ± 2.05	49.89 ± 2.05	18.16 ± 1.22	18.25 ± 0.95	38.71 ± 2.44	38.90 ± 1.90	0.76 ± 0.03	0.75 ± 0.06	83.90 ± 0.86	83.11 ± 0.61	1.67 ± 0.23	1.73± 0.13	3.75± 0.48	3.77 ± 0.43
G4-500mg/kg body weight	97.91 ± 2.08	97.39 ± 3.67	63.65 ± 2.05	59.26 ± 2.10	22.44 ± 2.11	21.13 ± 1.38	47.27 ± 4.21	44.65 ± 2.76	1.29 ± 0.28	1.04 ± 0.18	83.48 ± 1.25	83.86 ± 1.23	1.69 ± 0.10	1.75± 0.12	3.43± 0.49	3.66 ± 0.56
G5-0mg/kgbody weight	90.31 ± 5.26	91.35 ± 4.54	48.96 ± 1.48	49.75 ± 1.93	18.22 ± 0.55	18.42 ± 0.75	38.83 ± 1.10	39.24 ± 1.50	0.76 ± 0.05	0.76 ± 0.02	83.34 ± 0.61	83.17 ± 0.66	1.74 ± 0.13	1.77± 0.12	3.68± 0.14	3.64 ± 0.17

G6-500mg/kg body weight	90.29 ± 7.46	91.42 ± 4.34	48.07 ± 2.95	47.93 ± 2.10	18.26 ± 0.65	18.12 ± 0.71	38.91 ± 1.31	38.64 ± 1.43	0.76 ± 0.02	0.75 ± 0.05	83.62 ± 0.78	84.23 ± 1.25	1.70 ± 0.12	1.72± 0.15	3.56± 0.11	3.70 ± 0.15

Note: M: Male, F:Female, SGPT: Serum glutamic-pyruvic transaminase u/l, SGOT: Serum glutamine-oxaloacetic transaminase u/l, BUN: Blood Urea Nitrogen mg/dl, T3: Triiodothyronine ug/dl,T4:Thyroxine ng/dl, TSH:Thyroid stimulating hormones ng/dl



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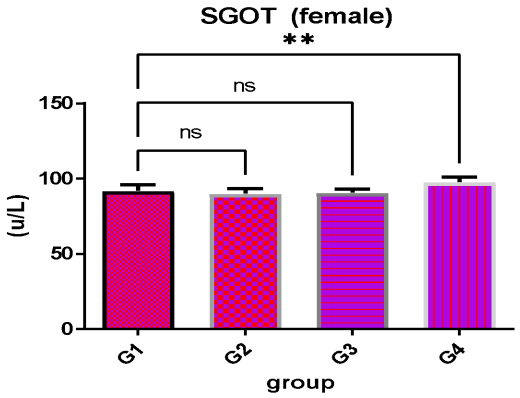

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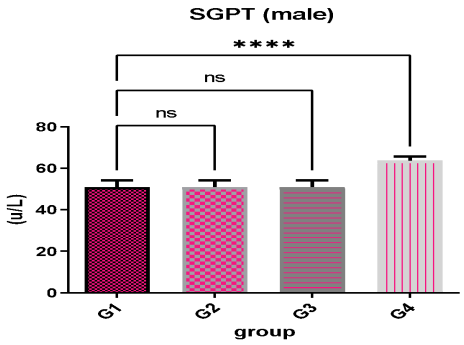

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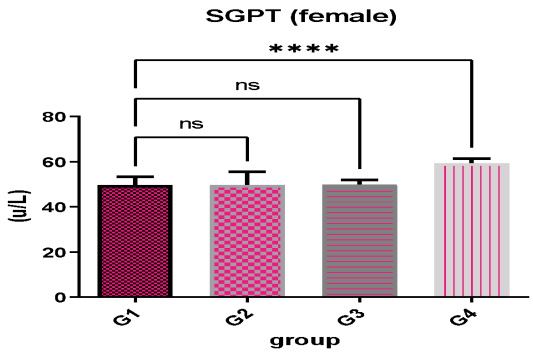

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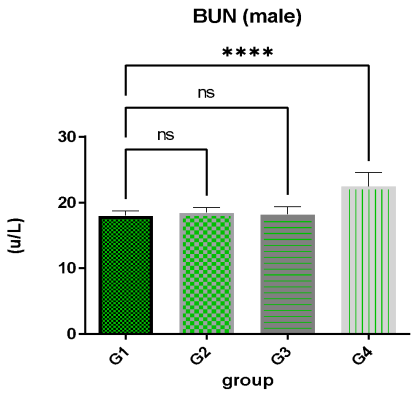

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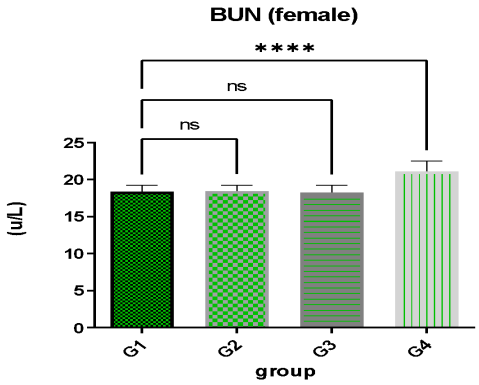

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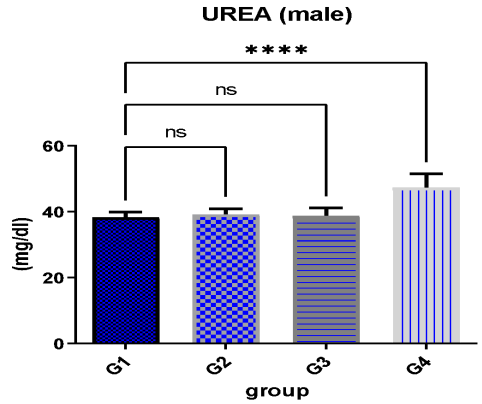

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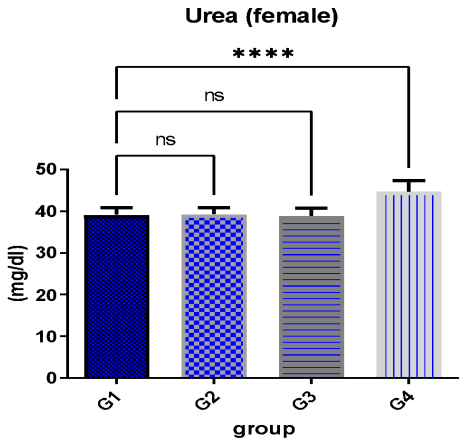

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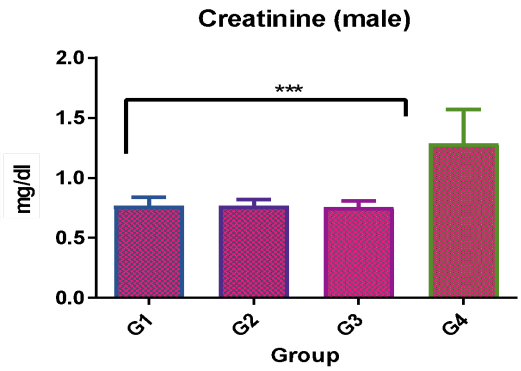

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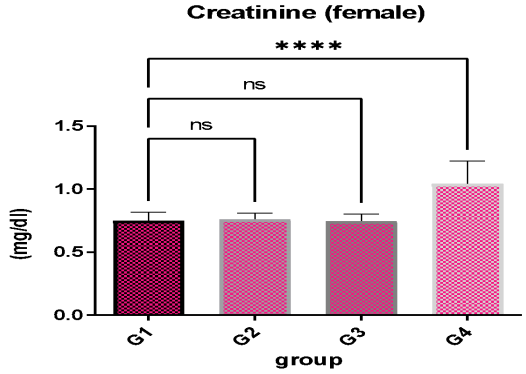


(j)

Fig.2. Graphical representation of Male (a,c,e,g,i) and Female(b,d,f,h,j) rats were showed p value<0.05= highly significant in SGOT, SGPT, BUN, Urea, Creatinine of (G4) as compared with the (G1).

3.5. Ophthalmoscopic Examination

Eye examination was done before initiation of the experiment and at the end of the experiment using Ophthalmoscopic equipment in animals belonging to control (G1), low dose (G2), middle dose (G3), high dose (G4), recovery control (G5), and recovery high dose groups (G6); no changes were observed in the eyes of both male and female rats during the whole experiment.

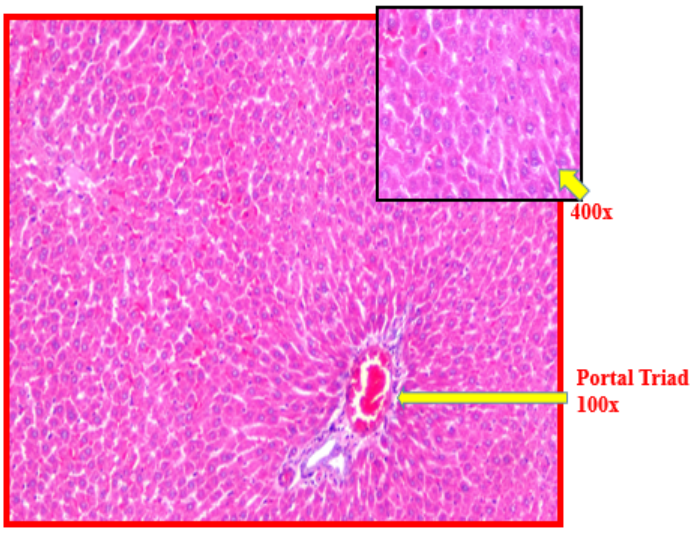
3.6. Histopathology Evaluation

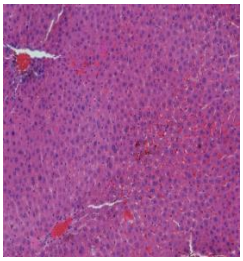
The liver, kidney, and thyroid were subjected to a histopathology study, and all organs that were examined under the microscope showed the following profiles.

***3.6.1.Liver**:* In control dose (G1) level i.e., 0 mg/kg bodyweight section from most rats showed normal hepatocytes (fig 3 a). However, sinusoidal congestion in the liver was observed at a high dose level, i.e. 500 mg/kg body weight (fig 3 b) No significant changes in the microscopic liver profile could be noticed at low and middle dose levels, i.e. 100 mg/kg bodyweight and 300 mg/kg body weight.
After 28 days of post-dosage recovery, the liver was seen in the recovery high dose group of animals compared to their recovery control counterparts.

***3.6.2.Kidney**:* In high dose level (500mg/kg b.wt), shrunken and degenerated glomeruli with loss of bowman's space (Fig 3 c) occurred in kidneys when compared to kidneys of male and female Wistar rats in the control group showing normal architecture of glomeruli and Bowman's Capsule (Fig 3 d). No significant changes in the kidney's microscopic profile could be noticed at low and middle dose levels i.e. 100 mg/kg body weight and 300 mg/kg body weight, respectively. In the remaining two groups i.e. recovery control and recovery high dose level, normal cytoarchitecture with a good arrangement was observed in both male and female Wistar rats.

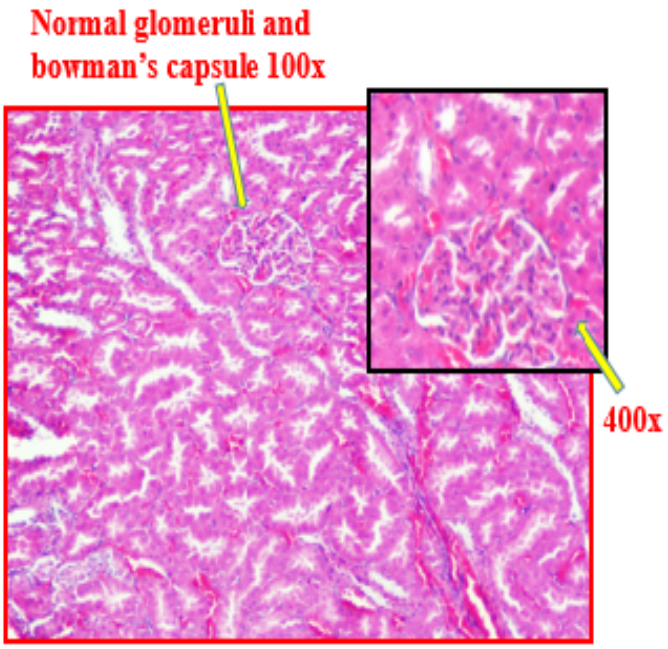
***3.6.3.Thyroid*:** There have not been any alterations in the serum sample of the thyroid profile. A microscopic examination was done, and it did not find any lesions in any of the dose groups and the control group (fig 3 e,f).
The histopathological alterations in the present study can be summarized as follows:





Sinusoidal congestion
100x
400x

(a) (b)



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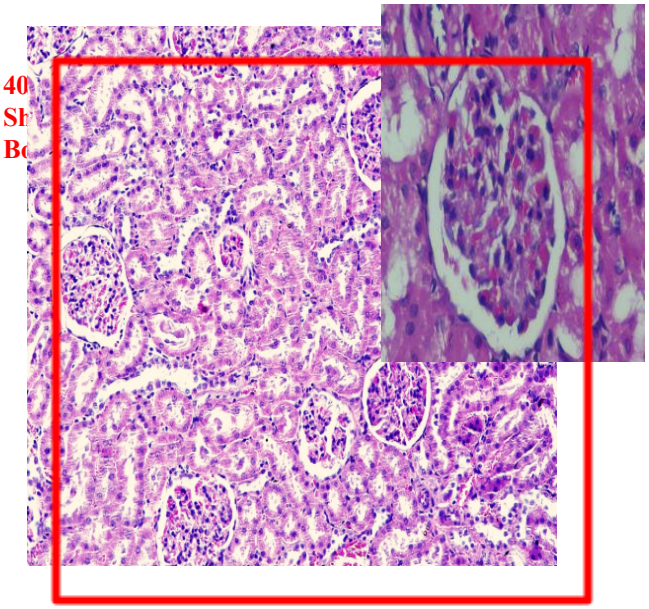

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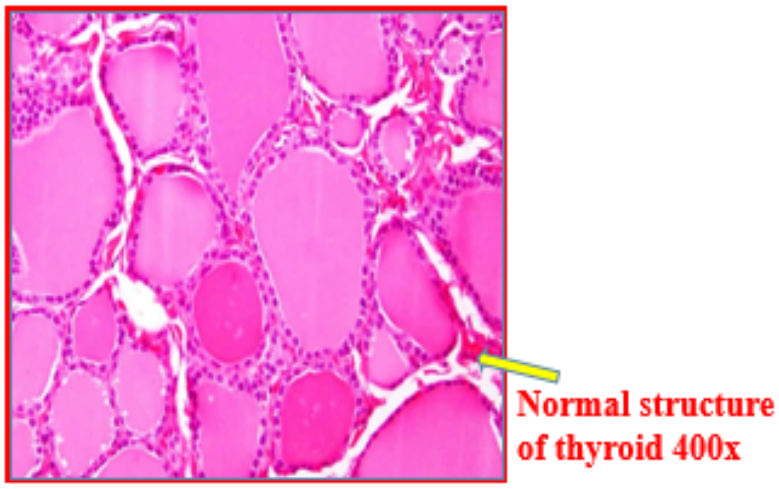

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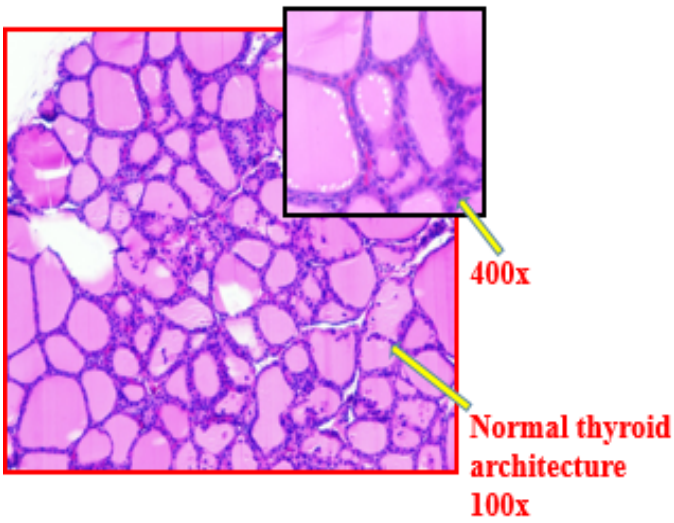

(f)

Fig. 3. Histopathology section: Light microscopy of Liver (a) Liver of (G1) group showing normal hepatocyte with portal triad (b) Liver in Wistar rat treated with Penoxsulam, G4 group showing sinusoidal congestion in hepatocytes. (H & E) (c) Histological view of Kidney showing normal architecture of glomeruli and Bowman's Capsule in (G1) group of animals (d) Photomicrograph of Kidney of Wistar rat treated with Penoxsulam G4 group showing shrunken and degenerated glomeruli with loss of bowman's space (H & E). (e) G1 group of animals showing normal thyroid architecture (H & E) (f) Photomicrograph of thyroid of Wistar rat treated with Penoxsulam (G4) group showing normal architecture.

Researchers utilize animals as replica to help acknowledge and anticipate responses in human beings. Animal representation has delivered the necessary units for exploration and benefit as the origin that has allowed the combustible growing of concern in this area with assembly welfare to both humans and animal species. The utilization of animals in

experimental medicine, the science of drugs, drug evolution, well-being safety assessment, and toxicological assessment have become a well-accepted and essential exercise. Animals have been used as a replica for hundreds of years to forecast what chemicals and environmental factors could do to Man . [21]

- There are several advantages in using rats; as is evident, humans cannot be used for this research purpose. Therefore, there is no option of an appropriate (non-rodent) species for pre-clinical toxicology studies based on a contrast of the pharmacokinetics and metabolism of the particular chemical in different laboratory species. [12] The rat species have become a frequent choice because of their metabolic resemblance, small size, relatively willing nature, short-agedness, i.e. 24 to 30 months, and short maturation (gestation). The ample use of rats in research has led to the development of tremendous historical details based on their nutrition, diseases, and general biology. This awareness, along with facts about the species' metabolic reaction and response to toxicants, has proved rats to be a good, known example for predicting human response to foreign compounds. Rats have a short lifespan which is suitable for long-term toxicity studies in which animals need to be exposed for most of their lifetime. [3] Exposure to chemicals has led to increasingly harmful effects on the numerous organs of the body. Within these organs, inadequate responses from cells and tissues is an insult caused by a drug, and its metabolites are determined by the interplay of many factors. [19] The poison acts in small doses, affecting the whole body. Today, it is widely acknowledged that poisonous substances affect the organism by affecting its enzyme system .[1]

Due to the accumulation of chemicals in the body, exposure for short periods may not produce immediate effects but repeated exposures can induce delayed effects. Repeated exposure is the adverse or deleterious general toxicological effects that can be both local and systemic. A substance was considered safe in the past if it did not cause immediate death or acute injury to a living organism. Nowadays, a substance is considered relatively harmless if it does not elicit any adverse effects from the biological system, either from organs or single links of the metabolic system.[25] Due to the large taxonomic diversity, life cycles vary greatly .[22]

On the other hand, the excessive use of herbicides has become a topic of concern as it has finally led to different issues about the ecosystem. [23] Agricultural farmers might be exposed to pesticides while mixing or applying herbicide in the field.[4] Thus, in every agricultural facility, they must have a thorough training assessment for employees inappropriate work processes and also provide them with the required safety equipment to limit unnecessary exposure because they are not able to understand the instructions or follow label precautions including wearing personal protective equipment (PPE kit) appropriate to the application method. Consumers could be exposed to residues in rice or drinking water, home-lawn applications, contact with treated turf at golf courses and athletic fields, or while swimming in treated lakes. Researchers use animals in their studies to define the potential of a pesticide to cause notorious effects to the internal health of humans.[11] It is relevant to know that these tests are carried out using doses high enough to cause toxicity (poisoning). Effects seen in animals given toxic doses, are unlikely to occur after one-time exposure or low-level exposure in humans. The level of exposure must be considered to estimate the high risks of harmful effects. [3]

The unsustainable and unregulated use of pesticides (herbicides) has increased on a daily basis and may cause adverse effects to human beings, including farmers' health. Most farmers who work in such agricultural fields are not aware of these harmful effects. This study has outlined much of the process required for assessing the impact on hormones after chronic exposure to pesticides. There are many known examples of beneficial and potentially dangerous interactions with pesticides resulting from the modern practice of multi-drug therapy in human clinical pharmacology. [7] For close to an era, rats have been used profitably or conveniently in toxicology pre-clinical animal research studies. They will continue to be used for research in the near future.

4. Conclusion

In view of the current study, all biochemical and histopathological alterations correlate with one another. In this study, the data in the high dose group were statistically significant concerning the control group of animals. However, physiological changes were also observed during the experimentation period along with depression of liver and kidney functions after administration of penoxsulam at highest concentration; since no toxic changes has been noticed in any recorded parameters of group-2 and group-3 . Hence, it is concluded that the condition of this study had a toxic effect on the liver and kidney at 500mg/kg body weight. Moreover, the herbicide does not imbalances the activity of thyroid hormones (t3, t4, tsh) at the maximum concentration. The findings of this study suggest that the use of Penoxsulam (herbicide) was found to be safe at the lowest concentration.

Ethical approval

Institutional Animal Ethics Committee approval was taken before initiation the experiment.

References

1. Bartik M, Piskac A.Veterinary toxicology developments in animal and veterinary science. *Elsevier scientific publishing company*, The Netherlands.1928. ISBN 0-444-41703-1 (Vol.7), pp 13-26.
2. Brzóska M, Moniuszko-Jakoniuk J, Piłat-Marcinkiewicz B, Sawicki B. Liver and Kidney function and histology in rats exposed to cadmium and ethanol, *alcohol and alcoholism*, 2003. volume 38, isssue 1, Januray 2003, pages 2-10, https://doi.org/10.1093/alcalc/agg006
3. Chambers JE, Yarbrough JD. Effect of chronic exposure to pesticides on Animal systems. Ravan press, New york..1982. ISBN 0-89004-756-1
4. Christopher JT, Powles SB, Liljegren DR, Holtum JAM. Cross-resistance to herbicide in annual ryegrass (LoliumRigidum). II. Chlorsulfuron resistance involves a wheat-like detoxification system. *Plant Physiol*. 1991, 95, 1036- 1043.https://doi: 10.1104/pp.95.4.1036
5. De Boer GJ, Thornburgh S, Ehr RJ. Uptake , translocation and metabolism of the herbicide florasulam in wheat and broadleaf weeds. *Pest Management Science*. 2006. 62, 316- 324. https://doi: 10.1002/ps.1163 PMID: 16506146
6. Fischer AJ, Eckert JW, Boddy L, Marchesi C, Merotto A, Osuna-Ruiz MD, et al. Weed Control in Rice; Annual Report Comprehensive Rice Research (Jan 1-Dec 31, 2007); available at http://www.carrb.com/07rpt/2007%20Fischer%20RP-1.pdf, 2007, accessed Aug 28, 2008.
7. Gupta RC. Veterinary toxicology. seconded. Basic and clinical principles Academic press. 2012. ISBN 9780123859273
8. Gerwick BC, De Boer GJ, Schmitzer PR. Mechanisms of tolerance to triazolopyrimidinesulfonanilide herbicides. In Chemistry of Plant Protection; Herbicide Inhibiting Branched Amino Acid Biosynthesis; Statter, J., Ebing, W., Eds., Springer-Verlag: Berlin, Germany, 1994. Vol. 10, pp 145-160.
9. Govindappa K, Joladarashi D, Hallur RLS, et al. Toxicity evaluation of 6-mercaptopurine-Chitosan nanoparticles in rats. *Saudi Pharmaceutical journal.* https://doi.org/10.1016/j.jsps.2019.11.018
10. Hodges C, De Boer GJ, Avalos J. Uptake and metabolism as mechanisms of selective herbicidal activity of the 1,2,4-triazolo[1,5-a] pyrimidines. *Pestic. Sci*. 1990. 29, 365–378. https://doi.org/10.1002/ps.2780290311
11. Hidayat I, Preston C. Cross-resistance to imazethapyr in a fluazifop-P-butyl- resistant population of Digitariasanguinalis. *Pestic. Biochem. Physiol*. 2001, 71, 190–195.
12. Hayes AW. Principles and methods of Toxicology. fifth ed. Taylor & Francis group, New York. 2008.1359-1392
13. Jamal F, Haque SQ, Singh S. The Influence of Pesticides on Hepatic and Renal Functions in Occupational Sprayers of Rural Malihabad, Lucknow (India). *Toxicol open access.*2016.1:106. doi:10.4172/2476-2067.1000106
14. Mohammad K Parvez, Mohammed S Al-Dosari, Ahmed H Arbab, Sakina Niyazi. CYP450 modulating potential of Cyperus rotundus, Saudi Pharmaceutical Journal, Volume 27, Issue 4, 2019, Pages 558-564, ISSN 1319-0164, https://doi.org/10.1016/j.jsps.2019.02.003.
15. Mallory-Smith CA, Thill DC, Dial MJ. Identification of sulfonylurea herbicide-resistant prickly lettuce (Lactucaserriola). *Weed Technol*. 1990, 4, 163–168.
16. OECD, 2018. OECD Guideline for testing of chemicals: Repeated dose 90 Days Oral toxicity Study in Rodents Guideline No. 408. Organization for Economic Co-operation and Development, June 25, pp. 1-14

17. OECD, 2001. OECD Guideline for testing of chemicals: Acute Oral Toxicity- Acute Toxic Class Method. Guideline No. 423. Organization for Economic Co-operation and Development, December 17, pp. 1-14
18. Primiani M, Cotterman J, Saari L. 1990. Resistance of kochia (Kochiascoparia) to sulfonylurea and imidazolinone herbicides. *Weed Technol*. 1990, 4, 169–172. https://doi.org/10.1017/S0890037X00025185
19. Riddell RH. Pathology of drug induced and toxic disease. Churchill livingstone. Newyork. 1982. ISBN 0-443-08083-6
20. Ray TB. Site of action of chlorsulfuron: inhibition of valine and isoleucine biosynthesis in plants. *Plant Physiol*. 1984 Jul;75(3):827-31. https://doi: 10.1104/pp.75.3.827.
21. Shayne C Gad.. Animals model in Toxicology. Second ed.2007. CRC Taylor & Francis
22. Sharma RK, Goyal AK. "AGRO-PESTICIDES AND ANDROLOGY. *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 6, no. 10, Oct. 2014, pp. 12-19, https://innovareacademics.in/journals/index.php/ijpps/article/view/2315
23. Torrents A, Jayasundera S, Schmidt WJ. Influence of the polarity of organic matter on the sorption of acetamide pesticides. *J. Agric. Food Chem*. 1997. 45: 3320-3325. https://doi.org/10.1021/jf970112i
24. Turner JA. 2015. The Pesticide Manual seventeenth ed. p.851
25. Tonu Pussa. 2013. Principle of food toxicology. second ed.Taylor & Francis group
26. U.S EPA Registration for Weed Control in Rice," Press Release, Dow AgroSciences LLC, November 2, 2004
27. Van Leeuwen CJ, Vermeire TG. Risk assessment of chemicals an introduction II. Second ed. 2007 Springer, The Netherlands
28. Yasuor H, Osuna-Ruiz MD, Ortiz.A, Saldain EN, Eckert JW, Fischer AJ. Mechanism of resistance to Penoxsulam in late watergrass. *j.Agric,Food chem*, 2009. 57, 3653-3660. https://doi.org/10.1021/jf8039999
29. Yasser. El-Nahhal. Biochemical changes associated with long term exposure to pesticide among farmers in the gaza strip," Occupational Diseases and Environmental Medicine,vol. 4, no. 3, pp. 72–82, https://doi.org/10.4236/odem.2016.43009