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Safety Evaluation of Acute and Subacute Dermal Toxicity Potential against Penoxsulam Herbicide in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author VC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MLA and MCG managed the analyses of the study. Author MCG managed the literature searches. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aims: The present experiment was conducted (comparative study) to determine the effect of single and repeated exposure of Penoxsulam herbicide by topical route.
Study Design: To assess acute toxicity, rats were topically exposed by Penoxsulam at 2000 mg/kg body weight and all the animals were observed for 14 days experiment period while, in Subacute toxicity , the rats were topically exposed with Penoxsulam at three multiple dose levels; 200, 500, 1000 mg/kg body. weight once daily for 28 days.
Place and Duration of Study: Toxicology department, Shriram Institute for Industrial Research, Delhi (INDIA), June 2018 and June 2019.
Methodology: Acute study was carried out in 10 wistar rats and in subacute study the wistar rats were divided into 4 groups i.e., control group, low dose group, middle group, high dose group; 5 male and 5 female rats/ group at the age of 2-3 kg were exposed over a period of 28 days. After dose application the patch was removed and the test site were cleaned with cotton moistened with distilled water.

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Results: In both toxicity study found that there were no clinical signs of skin reactions (Draize method) and no significant *P*>0.05 changes were observed in Bodyweight, Biochemistry, and Histopathology among the treated as well as in control group of animals. Therefore, data of this study supports that topical exposure of Penoxsulam in rats were shown normal histology of liver, kidney, and skin at the multiple doses besides this; Penoxsulam does not have potential to produce acute and subacute adverse systemic toxic reaction to the animals.

Conclusion: Therefore, data of this study supports that topical exposure of Penoxsulam in rats were shown normal histology of liver, kidney, and skin at the multiple doses besides this; Penoxsulam does not have potential to produce acute and subacute adverse systemic toxic reaction to the animals.

Keywords: Penoxsulam; dermal toxicity; wistar rat; herbicide; draize method.

1. INTRODUCTION

Penoxsulam is a systemic herbicide and member of the triazolopyrimidine sulfonamide (TP) chemical family that is absorbed mainly by leaves as well as by roots [1]. Its mechanism of action is the inhibition of the acetolactate enzyme (ALS), which blocks the biosynthesis of the branched-chain amino acids: valine, leucine, and isoleucine. This particular herbicide was first used in California for the purpose of post emergence, broad-spectrum weed control in rice (Oryza sativa L). Rice is a highly important crop as it is the major food staple for approximately 60% of the world population (FOA 2006).

A majority of herbicides are widely used to boost the production of crops by degenerating the growth of weeds and hence, minimizing the farmer's efforts to accomplish the same [17]. Weeds have a very high persistence level with only a 10-15% depletion observed in yearly production of rice in Asian countries [2]. In Asia, specifically in Philippines, the percentage of rice farmers that apply herbicides rose from 14% in 1966 to 61 % in 1974 [3]. However, currently 96% to 98% of Filipino rice farmers use herbicides [4]. In developing countries, herbicides are being swiftly adopted in place of hand weeding (oldest method of weed control) in order to elevate the yield of a crop [5]. Herbicides have been alleged to cause various health issues ranging from skin irritation to death. The route of attack can arise from irregular application that results in direct or indirect contact with field workers. The application of Penoxsulam causes damage to the rice (Ellis et al. 2005) even though it is said to be a low-dose, high-efficacy, and broad-spectrum herbicide which offers eco-friendly weed control [26]. The use of this herbicide has massively contributed towards increasing food production [15,20]. However, the overutilization of Penoxsulam has presented several environmental concerns and become a serious threat to the ecosystem topic of concern as it has finally led to several issue regarding ecosystem [6].

The unsustainable and reckless use of herbicides in agricultural fields has only increased steadily as farmers are unaware of its harmful effects on human beings [22]. It is important to note that very few studies have been carried out on the highest experimental point where there are no observed side effects on the epidermis layer of the skin and little is known about the chronic effect of pesticide exposure on the skin at its histological level. This study has been designed to investigate the toxicity potential in Penoxsulam by adopting a dermal route where Wistar rats were exposed to Penoxsulam once in 14 days for acute toxicity and daily for a period of 28 days in subacute toxicity . The rat has long been identified as the animal of choice for preclinical safety evaluation of topically applied material. The field of dermal toxicity continues this practice in order to accurately predict dermal and systemic response in humans to topically applied drugs. This study has not done, So, it is necessary to carried out comparative experiment on dermal toxicity.

The objective of this study was to investigate the dermal application of Penoxsulam and its effects on the target organs (liver and kidney) and skin of wistar rats for 14 days and 28 days and obtain the information on health hazards likely to arise from short term as well as long term exposure by the topical route which will be used to establish a optimal dose to the animal.

2. MATERIALS AND METHODS

2.1 Experiment Design

This study used young adult (12 to 14 weeks old) male and female wistar rats (weighing 200-300

grams) that were kept in polypropylene cages fitted with wire mesh tops having autoclaved corncob bedding. All rats were housed individually in separate cages, each with a stainless steel top grill. Sterilized corncobs layered the bottom of the cage which was maintained at a temperature of 20-24^{0}C, a 50-60% humidity, and a 12-hour light and dark cycle, light intensity of 250-300 lux in the animal housing facility at the Toxicology Centre in Shriram Institute for Industrial Research, Delhi (India). Sterilized feed and aqua filtered water was served *ad-libitum* daily to the rats. The laboratory was mopped daily with a disinfectant solution of D-125. [7].

2.2 Preparation of Animals for Skin Exposure

Prior to experimentation, the animals were acclimatized for 5 days. During the acclimatization period, all the animals were observed daily for new developments. The animals were randomized on the last day of acclimatization and the rats were housed individually with a unique identification on each cage mentioning details such as Title of the study, Unique identity number, Dose leve and Group, Gender, experiment initiation date, and experiment termination date. A day prior to the application of Penoxsulam, hair present on the dorsal part covering 10% of the body surface area of each animal was shaved with utmost care in order to avoid abrading the skin area and untreated skin serve as a control (Fig. 1). After 24 hours of the exposure patch was removed in acute toxicity study while, in sub acute toxicity exposure period was 6 hours. After patch removal the test site were examined critically for dermal reaction by using Draize scoring criteria. For behavior, this study noted down signs of appearance and toxicity on a daily basis in both male and female rats [7].

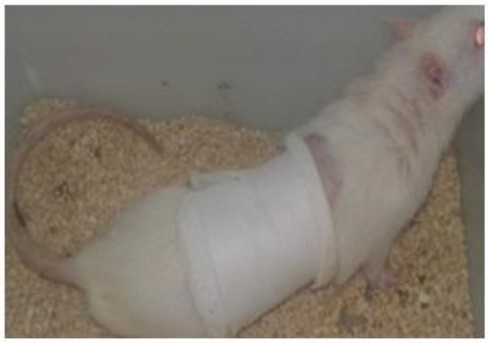
The body surface area was calculated as follows [8]:

Body Surface Area= K (Body weight of the animal in gram) $^{2/3}$

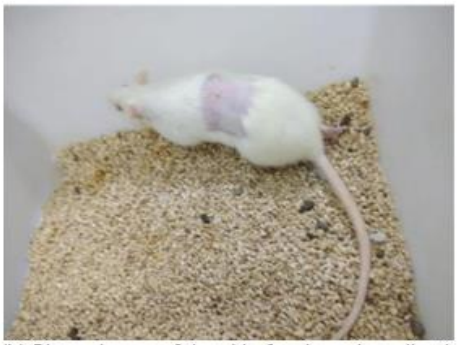
Where; K=Constant for estimating Body Surface Area

2.3 Acute Dermal Toxicity Study

In the assessment of toxic characteristics of a Penoxsulam of acute dermal toxicity in wistar rats is usually an initial step. Before initiating the main study (i.e. repeated dermal exposure of rats to Penoxsulam) the acute study was carried out and a limit test was performed at 2000 mg/kg b.wt in 5 male and 5 female Wistar rats. After 24 hours of exposure period the patch was removed and test site were cleaned with cotton moistened with distilled water. Non-treated site were treated as a control. In animals which were exposed by topical route, this acute study found no treatment-related toxic signs or symptoms, no deaths and no microscopic changes were observed in any of the animals. Each rat was critically examined for skin reaction erythema, edema after removal of patch at 24 hours, 48 hours and once daily for 14 days at a dose level of 2000 mg/kg b.wt. Hence, Globally Harmonized system of classification and labeling of chemicals 'Penoxsulam Technical' comes under category 5. Based on the findings of the acute study, a main study was conducted [9].



(a) Shaved area for dermal application applied by 'Penoxsulam Technical' and the treated area was covered with non-irritating and non-toxic adhesive tape.



(b) Shaved area of the skin for dermal application after the patch removal applied by 'Penoxsulam Technical'

Fig. 1. Showed shaved skin and removal of the test item

2.4 Repeated Dose 28 Days Dermal Toxicity Study

A test for repeated exposure of dermal toxicity was carried out according to the OECD guideline 410 for testing of chemicals. The formulated Penoxsulam was applied directly on the dorsal lateral part of the clean-shaven skin. After applying the chemical, the concerned area was covered with a porous gauze, non-irritative tape, and occlusive dressing for several groups of test animals. After exposure of 6 hours, the dressing was removed and the 'Penoxsulam Technical' was cleaned with cotton moistened in distilled water and only a gauze patch that was moistened in distilled water was applied to the control group of rats. This process was repeated 5 days a week for 4 weeks. Repeated shaving of concerned area was done at weekly intervals. This study administered Penoxsulam in 3 separate doses. The wistar rats were divided into 4 groups i.e., control group, low dose group, middle group, high dose group; 5 male and 5 female rats/ group were exposed over a period of 28 days. The treatment groups received a Penoxsulam dosage of 200, 500, 1000 mg/kg body weight (Table 1) [7].

2.5 Scoring Criteria for Skin Reaction by Draize Method

The resulting skin reactions in both acute toxicity and subacute toxicity were interpreted by using the scoring criteria (based on Draize J.H [18]) that is mentioned below in Table 1.

2.6 Observation and Evaluation

This study carried out on-spot careful observations for skin reactions (i.e. Erythema and Edema) during the whole experiment in acute and subacute toxicity. The body weight of male and female rats was also recorded regularly in every week. Using a light dose of CO_2 anesthesia, all the animals were sacrificed to collect the blood for biochemical examination, and postmortem examinations were carried out as per the standard procedures [7].

2.7 Histopathological Examination

In 10% neutral buffered formalin solution, tissues from the liver, kidney, and skin of the animals were collected and preserved. Tissues collected for histopathology were processed, embedded in paraffin wax, sectioned at 3-5 microns, and stained using the H&E (hematoxylin and eosin) method [10].

2.8 Statistical Analysis

All types of statistical analysis were performed with GraphPad prism software (version 9.2 for windows) and one way Analysis of Variance tests were calculated for the given data between and within the groups value are reported as mean ± standard deviation for various parameters.

3. RESULTS AND DISCUSSION

3.1 Acute Dermal Toxicity

In this study after exposure by Penoxsulam the treatment site of skin was observed at 24 hours, 48 hours , 72 hours and once daily for 14 days after removal of patch in male and female rats. Body weight were recorded promptly on the day of dosing 1 day, 7 day, 14 day and day 15 which was experiment end day. No significant changes $P>0.05$ were found in body weight changes (Table 2), behaviour changes (Table 3) and in Skin reactions (Erythema and Edema) was scored by using Draize method . No mortality were seen among all the animals at the dose level of 2000 mg/kg b.wt. No Histology alterations were found in hepatocytes cells in liver, renal cells of kidney and epidermis layer of the skin at the dose level of 2000 mg/kg b.wt (Fig. 2).

3.2 Subacute Dermal Toxicity

3.2.1 Clinical signs

No dead animals were observed in either the treated group or the control group. Further, no erythema and edema scoring with Draize method [18] was found on the skin of the treated animals when compared to their counterparts in the control group (Table 4).

3.2.2 Body weight evaluation

The body weight of all the rats were recorded on weekly basis and found there were no significant $P>0.05$ differences between the mean of animals from the three dose groups and the control group of animals. (Table 5 and Fig. 3).

3.2.3 Clinical biochemistry evaluation

Serum sample were quantified among all the groups by AU480 Beckman coulter biochemistry auto-analyzer system and did not reveal any significant alteration in Albumin (ALP), Glucose

(GLU), Serum glutamic-pyruvic transaminase (SGPT), Serum glutamine-oxaloacetic transaminase (SGOT), Blood urea nitrogen (BUN), Serum alkaline Phosphatase (SAP) in all the group of animals when compared to their control group (Table 6).

Table 1. Scoring criteria

A.	Erythema and eschar formation	Values
	No erythema	0
	Very slight erythema	1
	Well defined erythema	2
	Moderate to severe erythema	3
	Severe erythema (beef redness) to eschar formation preventing grading of erythema	4
B.	**Edema formation**	**Values**
	No edema	0
	Very slight edema	1
	slight edema	2
	Moderate edema	3
	Severe edema	4

Note: Maximum possible score-4 for Erythema and Edema

Table 2. Observation of behaviour patterns and appearance of wistar rats

Abnormal signs	Male		Female	
	24 hours after patch removal	14 days after patch removal	24 hours after patch removal	14 days after patch removal
Lethargy	No changes	No changes	No changes	No changes
Salivation	No changes	No changes	No changes	No changes
Hunched posture	No changes	No changes	No changes	No changes
Diarrhoea	No changes	No changes	No changes	No changes
Eyes	No changes	No changes	No changes	No changes
Skin / fur	No changes	No changes	No changes	No changes
Tremors	No changes	No changes	No changes	No changes
Convulsions	No changes	No changes	No changes	No changes

Table 3. Changes in Body weight in male and female Wistar rats

Bodyweight recorded time	Male	Female
On the day of dosing (Day 1)	207.74±2.16	206.2±2.61
Day 7	224.66±2.01	221.98±2.53
Day14	241.46±1.72	237.78±4.06
On the day of Sacrifice (Day15)	242.64±1.22	238.92±4.00

Table 4. Evaluation of skin reaction By Draize scoring method

Group/Dose (mg/kg body weight)	Observed signs	
	Erythma	Edema
Control (0mg/kg b.wt)	Not observed	Not observed
Low Dose (200 mg/kg b.wt)	Not observed	Not observed
Middle Dose (500 mg/kg b.wt)	Not observed	Not observed
High Dose (1000mg/kg b.wt)	Not observed	Not observed

Table 5. Mean body weight data of male and female rats

Group & Dose level	Male		Female	
	Day 1	Terminal	Day 1	Terminal
Control (0mg/kg b.wt)	248.20±5.02	268.00±4.30	250.20±3.49	269.20±4.15
Low Dose (200 mg/kg b.wt)	251.40±5.59	271.40±4.93	250.50±5.40	271.80±5.22
Middle Dose (500 mg/kg b.wt)	249.20±5.72	269.80±5.10	247.40±5.59	266.80±5.26
High Dose (1000mg/kg b.wt)	248.60±6.99	270.40±3.36	248.60±6.99	271.00±6.52

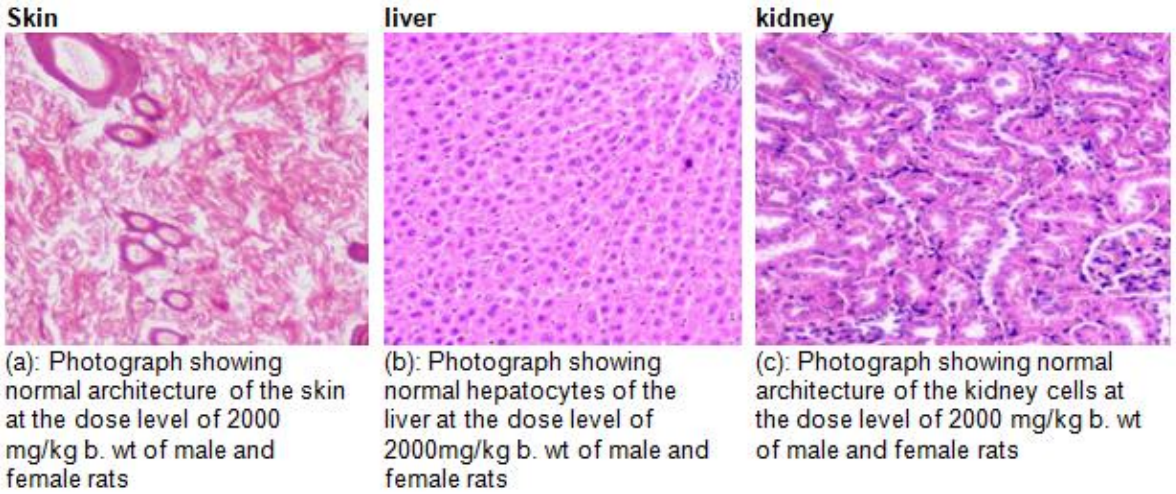


Fig. 2. Microscopic examination for acute toxicity study

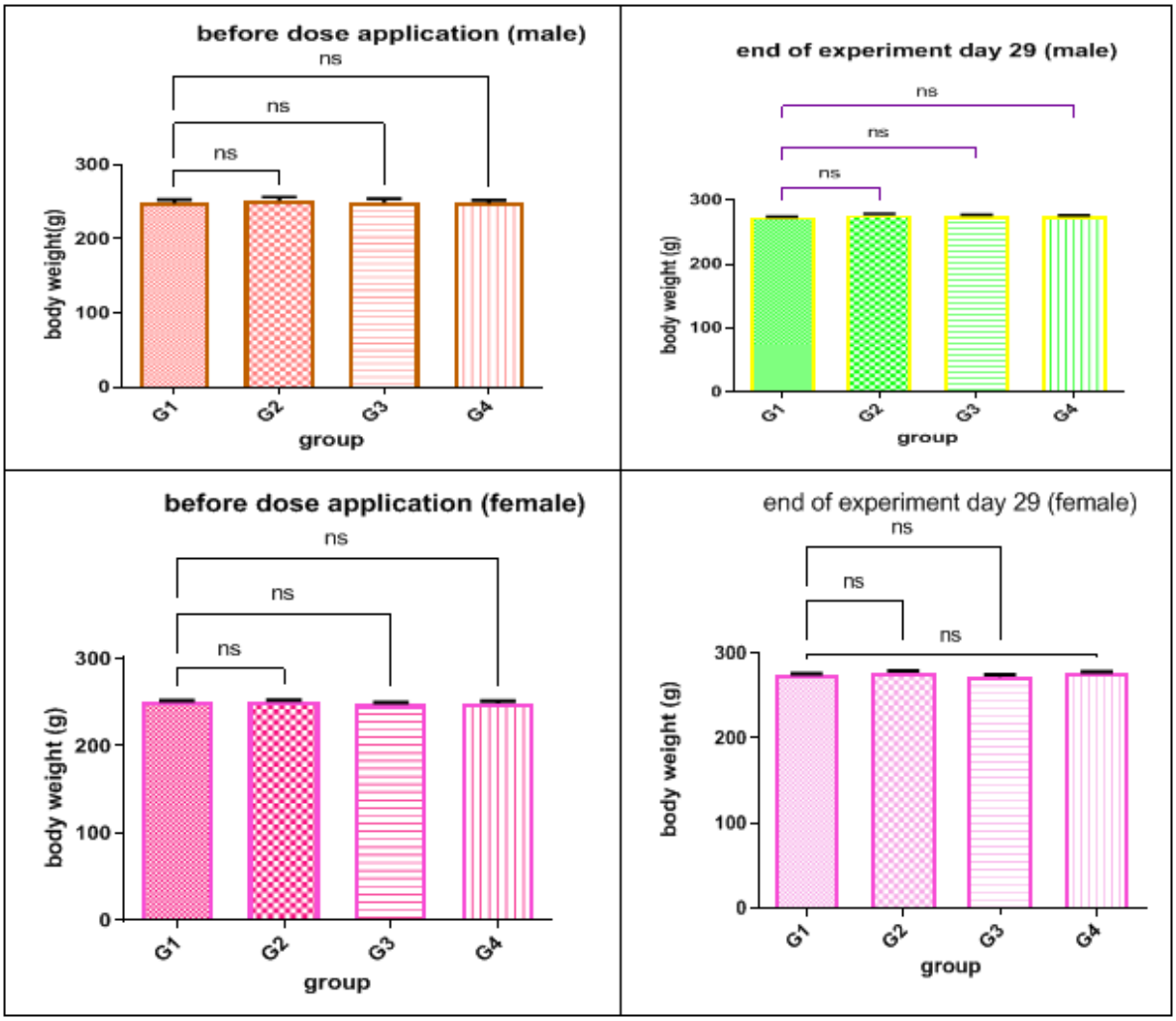
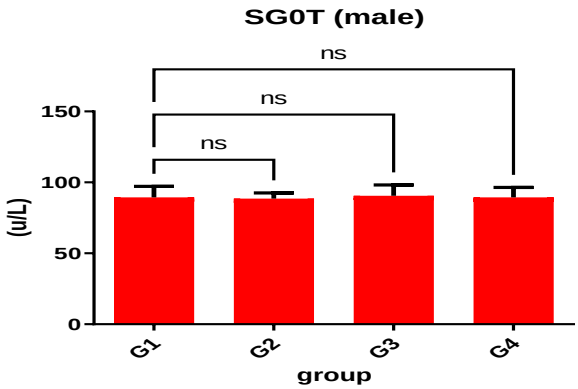


Fig.3. All the equal bars shows no differences in mean±S.D of body weight in all the treated as well as in control group of male and female rats at day 1 (before dosing) and day terminal (before sacrifice)

Table 6. Biochemical evaluation observed at the terminal sacrifice on day 29th

Parametes	ALB (g/dl)		GLU (mg/dl)		SGOT(u/l)		SGPT(u/l)		BUN(mg/dl)		SAP (u/l)	
	M	F	M	F	M	F	M	F	M	F	M	F
Control (0mg/kg b.wt)	4.66±0.51	4.59±0.70	89.60±2.19	88.00±4.53	89.40±7.73	88.80±5.50	48.06±4.25	47.49±0.45	21.92±3.65	21.88±2.37	129.66±3.34	128.40±4.39
Low Dose (200 mg/kg b.wt)	4.88±0.36	4.86±0.38	89.00±3.94	90.40±3.51	88.60±3.91	88.80±7.98	49.58±3.46	48.95±2.29	23.46±2.21	21.94±2.26	128.46±4.12	127.79±1.96
Middle Dose (500 mg/kg b.wt)	4.72±0.31	4.75±0.47	87.80±3.76	88.20±2.17	90.60±6.74	99.00±6.40	48.14±0.98	47.56±1.97	19.54±1.34	20.16±1.60	129.14±8.41	129.58±0.53
High Dose (1000mg/kg b.wt)	4.29±0.12	4.38±0.17	86.60±1.34	88.80±3.56	89.40±7.02	89.80±6.14	50.13±5.83	49.00±3.74	20.74±2.81	20.98±0.90	127.00±2.35	131.70±3.22

Whereas; SGPT: Serum glutamic-pyruvic transaminase u/l, SGOT: Serum glutamine-oxaloacetic transaminase u/l, ALB: Albumin g/dl, GLU:Glucose mg/dl, BUN: Blood Urea Nitrogen mg/dl, SAP: Serumalkaline phosphatase u/l, M: Male, F: Female


SG0T (male)
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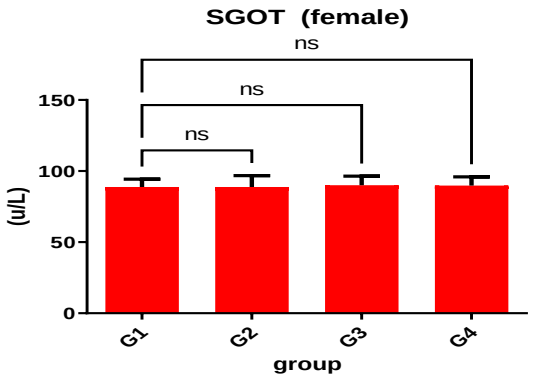
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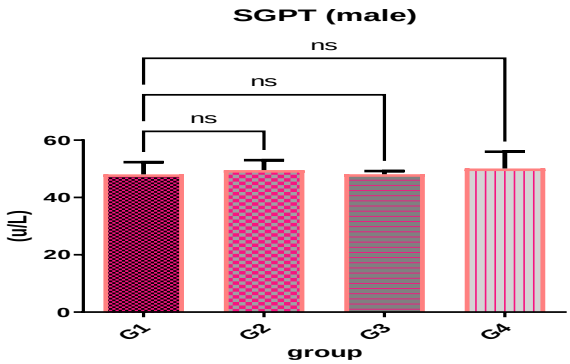
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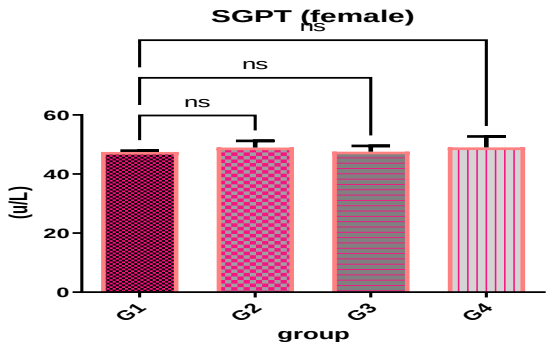
SGPT (female)
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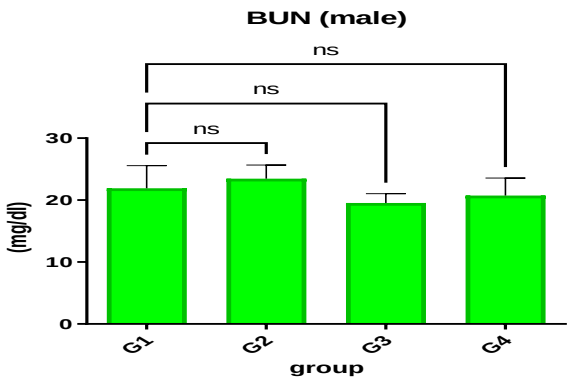
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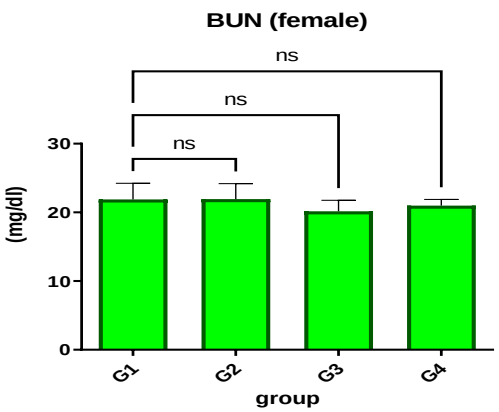
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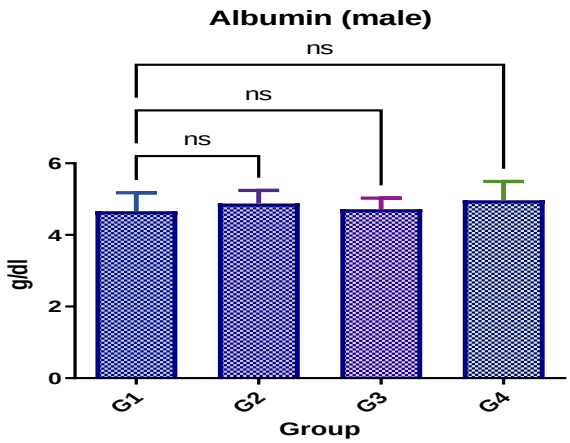
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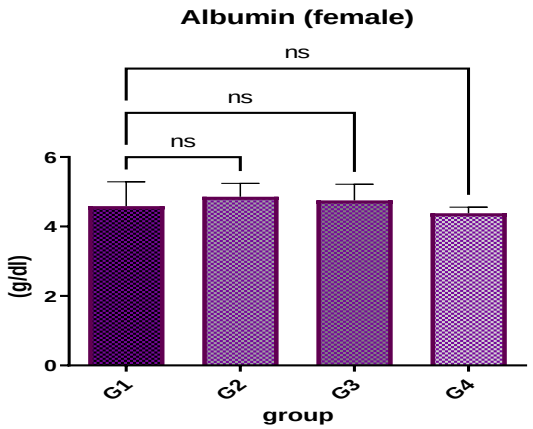
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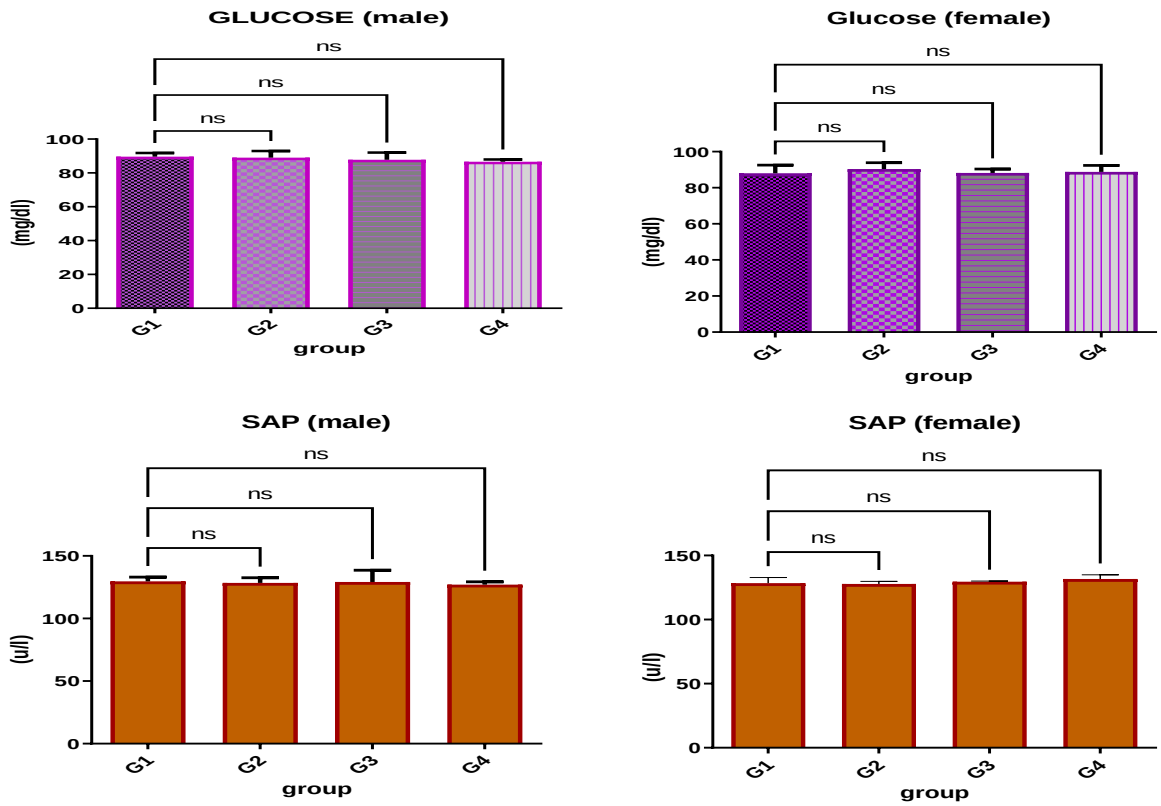


Fig.4. Graph Represent that the data of Biochemical Parameters (SGOT u/l, SGPT u/l, BUN mg/dl, Albumin g/dl, Glucose mg/dl, SAP u/l) shows Non Significant *P*> 0.05 alterations in in all the Treated Groups Compared with Control group of male and female Wistar Rats

3.2.4 Histopathological findings

3.2.4.1 Liver

The histological evaluation of liver tissues treated with Penoxsulam (1000 mg/kg b.wt) showed a normal structure of liver hepatocytes. The levels of liver enzymes in serum sample i.e., Serum glutamic-pyruvic transaminase (SGPT), Serum glutamine-oxaloacetic transaminase (SGOT), and albumin, glucose were normal and no significant alterations were noticed in male and female rats in any of the treated groups as well as in the control group of animals (Fig 5a,b).

3.2.4.2 Kidney

The level of Serumalkaline phosphatase, blood urea nitrogen shows normal in serum. An histological examination of kidney tissues treated with Penoxsulam (1000 mg/kg b.wt) in male and female rats showed a normal structure of glomeruli and Bowman's capsule in Kidney and no significant alterations were observed when compared to their counterparts in the control group (Fig. 5 c,d).

3.2.4.3 Skin

The histological evaluation of the skin of animals belonging to the High dose group (1000 mg/kg b.wt) that was treated with Penoxsulam showed a normal epidermis layer with perfect sequence of cells; no significant P>0.05 changes were seen in male and female rats when compared to the control group of animals (Fig 5 e,f).

In male and female rats, a high dose (1000 mg/kg b.wt.) dermal application of the herbicide did not cause any histopathological implementation in any of the organs i.e., liver, kidney, and skin when compared to the control group.

The histopathological alterations in the present study can be summarized as follows:

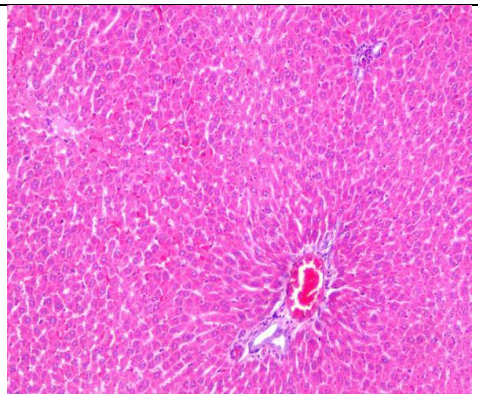
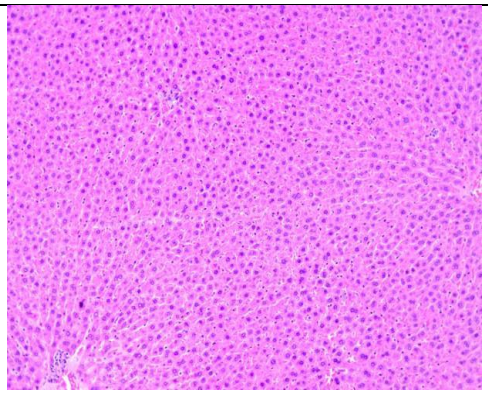
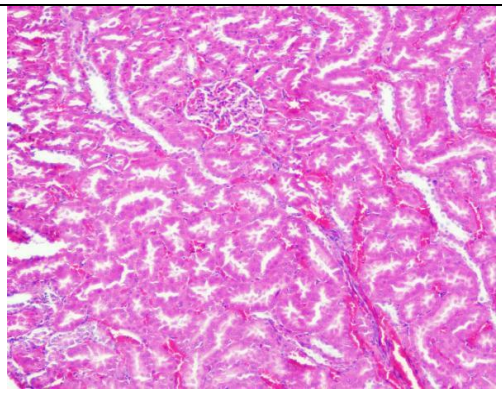
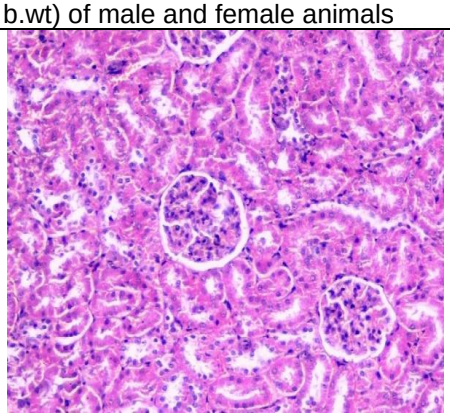
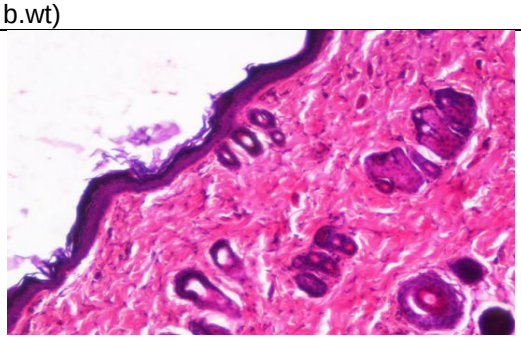
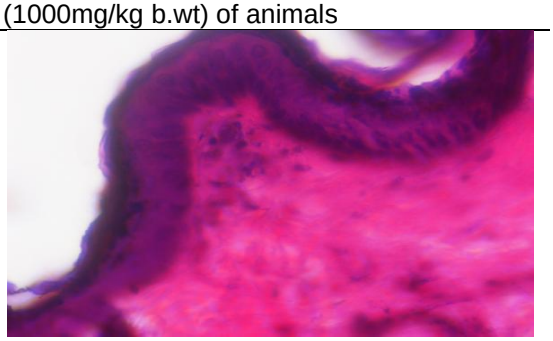
LIVER	 Microscopic photographs of liver (a). shows no changes in hepatic cells and liver enzymes in Control group (0mg/kg b.wt) of male and female animals	 Microscopic photographs of liver (b). shows no changes in hepatic cells and liver enzymes in High dose group (1000 mg/kg b.wt) of male and female animals
KIDNEY	 Microscopic photographs (c). architecture of Kidney shows normal glomeruli and Bowman's capsule in kidney of male and female animals of Control group (0mg/kg b.wt)	 Microscopic photographs (d). architecture of Kidney shows normal glomeruli and Bowman's capsule in kidney of male and female animals of High dose group (1000mg/kg b.wt) of animals
SKIN	 Microscopic photographs (e). male and female rats were showing normal epidermis layer of the skin with perfect sequence in Control group (0mg/kg b.wt) of animals	 Microscopic photographs (f). male and female rats were showing normal epidermis layer of the skin with perfect sequence in High dose group (1000mg/kg b.wt) of animals

Fig. 5. Histopathology section for Subacute toxicity

4. DISCUSSION

Skin is a major part of our body that interacts with the external environment and protects the body against pathogens (infectious agents) [27]. Farmers often apply pesticides by hand in order to protect their farming products from pest infestations [11]. As a result, dermal exposure is one of the most significant routes of exposure to pesticides and is an occupational hazard [24].

There is a lack of appropriate information on the safe handling of pesticides and hence, farmers are unaware of the possible acute and chronic health impacts of pesticides [14,16]. The risk of uptake through the dermal route was shown to be higher than any other method. When a chemical has obtained access to the vulnerable epidermis layer, it may be absorbed into the circulating blood and produce a local or systemic effect [21]. The toxicity assessment of a single topical application is an essential part of the toxicology system for new consumer goods [12]. More than 87 % of deposition was frequently observed on the front and back hands of farmers but on the other side, 19 % deposition was seen on the front of the right upper arms and the back of the right thighs of farmers [13]. The pesticide contaminant was present on the skin due to its release from the mist sprayer which the respondents carried during spraying activities while the presence of pesticide contaminants on the farmer's hands areas were high due to the handling of pesticides without using gloves [25]. Sometimes, they even used their hands to mix the pesticides. Overall, the Draize Scoring [18] System for Erythema and Edema observed no significant correlation with any listed skin sign. (Table 1).

It might not cause direct and acute effects on occupational-related skin indications. However, this aspect was important to consider in order to minimize the long-term health impact through dermal contamination by pesticides [23]. The correct use of Personnel Protective Equipment (PPE) is always helpful in decreasing the amount of substance coming in contact with the exposed surfaces of the skin. In this study, despite the fact that a majority of paddy farmers were aware that herbicides could harm their entire bodies, the use of Personal Protective Equipment (PPE) while applying pesticides was not being practiced in the community. A research study by Chester 1993 showed that the adverse effects of pesticide exposure through the skin might not only lead to acute health conditions, but will result in skin irritation and multiplex systemic disorders that end in death. EPA, 2004 [19] also supported our data related to dermal toxicity; it is safe to animals for maximum doses.

5. CONCLUSION

This study has investigated the effects of herbicides on skin by carrying out a biochemical evaluation, and recording histopathological changes in the skin of rats. The findings in this study suggest that Penoxsulam (Herbicide) does not damage the external layer of the skin at the highest dose. In the summary, the rat model is a key item in forward biological analysis. The prevailing logic is that responses to exercise acquired from rat models imitate human responses. More importantly, rats and humans often fall ill from the same harmful substances because both animals have the same basic anatomy. The results published in this study demonstrate that the herbicide Penoxsulam causes non-toxic effects to acute toxicity and subacute toxicity as designated in wistar rats. This study observed no mortality and no indications of the presence of poison in both the toxicity study among the rats. Hence, the single and repeated dermal exposure of Penoxsulam in male and female Wistar rats at a dosage of (2000 mg/ kg body weight) for single exposure and (1000 mg/ kg body weight) for 28 days had no toxic effects on the outer epidermis layer of the skin.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Prior to initiation of the study the IAEC approval was taken.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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