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ORAL AND DERMAL TOXICITY PROFILING OF ETHYL ACETATE FRACTION OF PTEROSPERMUM LANCEIFOLIUM ROXB LEAF IN EXPERIMENTAL RATS

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ABSTRACT

The species of the Pterospermum genus have been traditionally used to cure inflammation, blood troubles, leprosy, tumors, hemostatic, ear pain, stomachache, smallpox, leucorrhea, and ulcer. Despite its historical and widespread use, there is a significant gap in our understanding of the plant's toxicological profile. As such, this study aims to comprehensively evaluate the acute, sub-acute, and cutaneous toxicity assessments of the ethyl acetate fraction of *P. lanceifolium* (EAPL) leaf in experimental rats. The present study involved an array of toxicity assessments, including an acute oral toxicity study by administering a single dose of 2000 mg/kg EAPL to rats as per OECD guidelines no-423. Additionally, a 28-day sub-acute toxicity trial was conducted by administering daily doses of EAPL at 200 and 400 mg/kg p.o. as per OECD 407. To assess cutaneous toxicity, rats were exposed to topically applied concentrations of 1% and 5% EAPL over a fourteen-day period, as per OECD 402. Acute oral toxicity testing revealed no lethal effects or behavioral indicators of toxicity at the tested doses, indicating that the LD50 should be greater than 2000 mg/kg. The sub-acute study demonstrated a EAPL at 200, 400 mg/kg did not impart any significant change in any parameter. Notably, acute dermal toxicity analysis indicated no mortality in rats and no significant change in biochemical parameter when exposed to 1% and 5% EAPL. This study establishes that the oral LD50 (lethal dose, 50%) of the EAPL exceeds 2000 mg/kg. The findings further potentiate the safety of EAPL at the tested ethno-medicinal doses and further pave the path for its potential use in traditional and modern therapeutic applications.

Keywords: Pterospermum Lanceifolium, Oral Toxicity, Sub-Acute Toxicity, Cutaneous Toxicity.

1. INTRODUCTION

As per World Health Organization, phytonutrients are used for health care by about 80% of the global total. The traditional medical system and Indian culture both rely primarily on the utilization of medicinal herbs. According to the policy, only the drugs that are biologically defined and scientifically analyzed had value [1]. Therefore, the contemporary study is an effort to utilize the herbal flora for the treatment of incurable diseases like diabetes, ulcer, pain, inflammation, wound stress/fatigue, etc [1]. Also, for their respective therapeutic effect without any side effects with scientifically proven toxicity profiling for better acceptance on human disease either oral or topical application. These are also available at low cost and are comparatively safe. It is also clear that the time-tested herbal remedies used for health care are the best and safest than instant relief giving allopathic drugs [2, 3]. Pharmacological and toxicological assessment of phytoconstituents of medicinal plants for their potential toxic exposure becomes essential due to the blind faith of masses on herbs as well as traditional or folk preparations of the plants. Medicinal plants have been essential for holistic welfare to maintain and prevent diseases for the well-being of people's health [4]. This Pterospermum genus has great ethnopharmacological value, including antioxidant and anti-inflammatory [5], anti-cancer [6], osteogenic [7] anti- nociceptive [8], anti-ulcer, and Anthelmintic activity[9]. P. lanceifolium is native of the Tropical and Temperate region of Asia. In India, it is distributed in Bihar, Kerala, Madhya Pradesh, Andaman & Nicobar Island, Assam, Maharashtra, Odisha, Punjab, and Uttar Pradesh and Rajasthan. The pharmacological value and Phytochemical Constituent of P. lanceifolium is un- explored. In this study, we have analyzed the ethyl acetate fraction of P. lanceifolium plant extracts rich in phenolic and flavonoid compounds that exhibit the potent wound healing effect against STZ induced in rat model with significant antioxidant activity. Therefore, considering the prevalence and emergence of herbal drugs based on Traditional knowledge scientifically validated herbal combination for wound management in diabetes condition.

P. lanceifolium toxicity profiling has not yet been determined. So, this study examines the acute, sub-acute, and cutaneous toxicity of P. lanceifolium leaf ethyl acetate fraction in rats.

2. MATERIALS AND METHODS

2.1 Reagents

EDTA, Biochemical Reagents Kit (Transania Bio- Medicals Ltd.), and all the chemicals and reagents were of analytical grade, from Merk and Himedia Lab Pvt. Ltd. in Mumbai, India.

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2.2 Plant material identification Extraction and fractionation

The leaf of P. lanceifolium was collected from National Botanical Research Institute, and the plant specimen was submitted to the institutional herbarium (specimen no. 1098). We extracted the shade-dried leaves using 70% ethanol as solvent, concentrated them using a rotary evaporator, and then freeze-dried them in a lyophilizer. Using a separating funnel, the bioactive ethyl acetate fraction was separated from the crude extract following fractionation with hexane, chloroform, and ethyl acetate. The ethyl acetate fraction was further concentrated and freeze dried in order to obtain dried EAPL.

2.3 Experimental animals

In this study, we used healthy male and female Wistar rats that weighed between 180 and 240 g at the same age. Sourced from CSIR, Central Drug Research Institute. Rats were independently housed in cages under standard laboratory settings (light/dark cycle of 12 h/12 h at 25 ± 3 °C with 50–70% humidity). The animals were given a standard pellet meal and free access to water during the experimental periods. The experimental procedures used were those authorised by the institutional animal ethics committee (IAEC) of the national botanical research institute (NBRI) in Lucknow, India (Reg. No. 1732/GO/Re/S/13/CPCSEA).

2.4 Acute oral toxicity studies

The Organization for Economic Co-operation and Development (OECD) guidelines under testing of chemicals 423, were followed for the acute oral toxicity experiment [10]. Rats of both sexes that were almost 6 to 8 weeks old were used. The control animal received only 1% CMC solution (which was used as the vehicle). The ethyl acetate fraction of P. lanceifolium (EAPL) was used at a dose of 2000 mg/kg. Rats were observed for 24 hours after ingesting EAPL, with close observation during the first 4 hours and at least once per day for the following two weeks (14 days). Throughout the trial period, the rats were physically examined for body weight, feed and water intake, mortality, behavioral changes (salivation, fur health, lethargy, and sleep), alteration in physical appearance, damage or irritation, pain, and sickness symptoms. Following the experiment, blood was collected for biochemical and hematological analysis.

2.5 Sub-Acute oral toxicity studies

Subacute toxicity determination was conducted in both male and female wistar rats according to OECD guideline number 407 [11]. were randomly separated into three groups of six identically sized rats each. The group-I animal received normal saline, while the group- II and group-III animals received EAPL at a dose of 200 and 400 mg/kg body weight, once daily for 28 consecutive days. Rats were not given food overnight after the final treatment. On day 28, urethane (1 g/kg) was injected intraperitoneally to put them to sleep.

2.6 Body weight, feed and water intake measurements

Daily weight checks and checks for convulsions, excitation, posture, piloerection, breathing issues, drowsiness, anorexia, diarrhea, bleeding, and death were performed on all animals. Both before and up to four hours after the dosage, observations were made. Using a Mettler PE 1600 analytical balance (+0.01 g, Switzerland), the weights of the control and experimental rats were measured on the first day of the study (before the administration of test extracts), and 1st week, 2nd week, 3rd week, 4th week in sub-acute toxicity study.

2.7 Estimation of biochemical markers

An auto-chemistry analyzer was used to perform biochemical analysis on serum samples (Csense 100). Glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Total protein, triglyceride, cholesterol, bilirubin, urea, creatinine. Each biochemical analysis was conducted according to the manufacturer's instructions.

2.8 Hematological studies

An automatic hematological analyzer was used for the hematological analysis (EUROCOUNTTS). Each rat had a glass capillary tube puncture to get approximately 1 mL of blood, which was then collected in ethylene-diamine tetra-acetic acid (EDTA) anticoagulant tubes from a retro-orbital vein. Measured hematological parameters were mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean platelet volume, white blood cell count (WBC), red blood cell count (RBC), platelets (PLT), and hemoglobin (Hb), neutrophils, eosinophils, basophils, lymphocytes, monocytes were evaluated.

2.9 Urine analysis

Urine examination was done via a chemistry auto analyzer (csence100) to evaluate various parameters, including volume, specific gravity, ketone, protein, and blood sugar.

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2. 10 Acute dermal toxicity studies

The acute dermal toxicity test was conducted in accordance with OECD guideline No. 402 for chemical testing [12]. Before application, the animals' dorsal skin surface hairs (about 6–8 cm2) were neatly shaven using a razor 24 hours in advance. A minimum of 10% of the body surface area should be free for the application of the test substance, according to OECD Rule 402. EAPL topical preparations in concentrations of 1% and 5% were applied to patches of skin with a diameter of L inches in the interscapular region. Group I functioned as the normal control, and the other two groups were EAPL 1% and EAPL 5%. The following day, mice were killed, their body weight measured, their treated skins removed, and they underwent the same processing for light microscopy as before. Rat skin histopathological abnormalities were evaluated on a scale of 0 to 4, with 0 denoting no lesions, 1 denoting a minimal lesion, 2 denoting mild lesions, 3 denoting moderate lesions, and 4 denoting severe lesions. A serum sample was also used to assess lactate dehydrogenase (LDH).

2. 11 Statical analysis

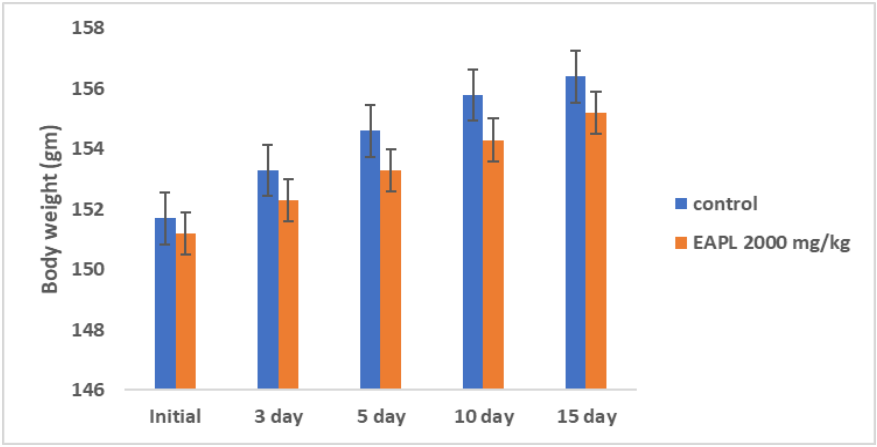
The results of the study are expressed as the mean ± SEM. The values were statistically tested using student t-test in Microsoft excel. and the analysis of variance (ANOVA) test was utilized to identify statistical difference “between the groups”. P value <0.05 were considered significant.

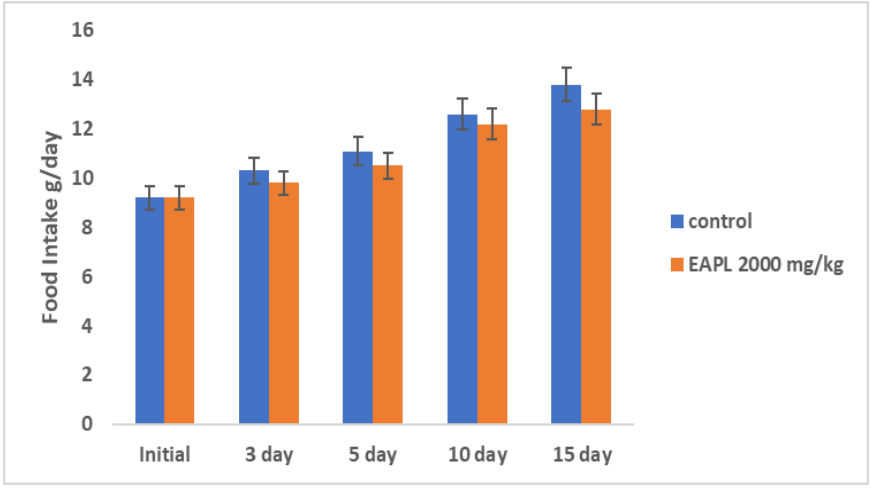
3. RESULTS

3.1 Acute oral toxicity study

The animals in the control and EAPL 2000 mg/kg treated groups were both normal and exhibited no signs of aggression, salivation, rising furs, or writhing as well. There were no significant changes in behavior, skin effects, breathing, postural abnormalities, or hair loss throughout the 14-day study period. The EAPL oral LD50 was considered to be more than 2000 mg/kg in rats.

All animals were found to be normal without any significant change in body weight, food and water intake (fig. 1). The biochemical parameter revealed that there is no significant change in any parameter except ALT was slight increase in treated dose 2000 mg/kg compare to control group (table 1). Different hematological parameter showed there are no significant change in any parameter except platelet count was significant ($p<0.05$) increased in treated group (table 2)





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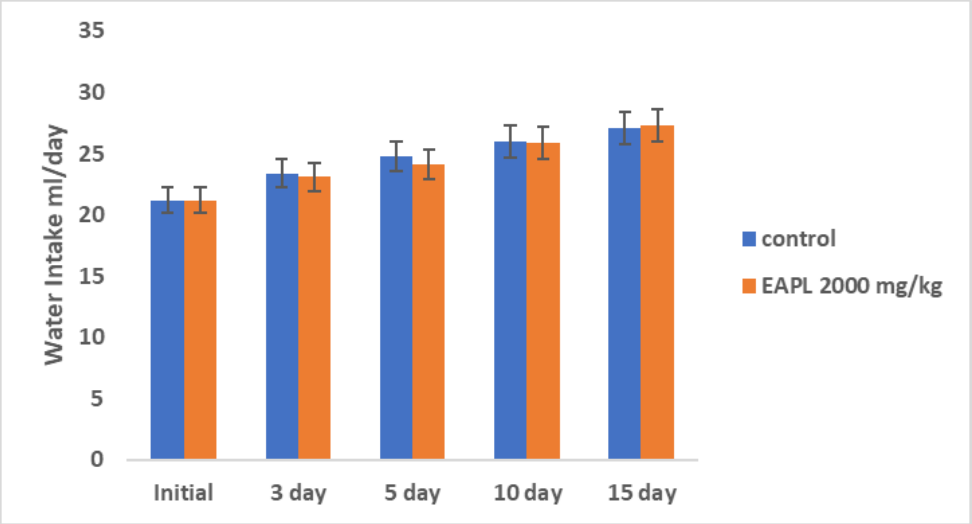

Figure 1: Values expressed as mean ± SEM, n=6 (one-way ANOVA).

Table 1: The influence of EAPL on the biochemical parameter.

Parameters	Control	EAPL 2000 mg/kg
Glucose (mg/dl)	105±8.92	104.2±10.90
ALT (IU/L)	50.81±2.10	56.33±4.96
AST (IU/L)	128.8±10.1	127.2±9.35
ALP (IU/L)	310.6±80.60	308.7±78.05
Total Protein (g/dl)	5.75±0.34	5.35±0.13
Triglycerides (mg/dl)	110.9±12.4	110.5±12.72
Cholesterol (mg/dl)	97.80±5.40	96.08±3.16
Bilirubin (mg/dl)	0.160±0.03	0.162±0.22
Urea (mg/dl)	35.20±3.10	36.92±2.26
Creatinine (mg/dl)	0.55±0.02	0.54±0.04

Values are presented as mean ± S.E.M., n = 6, significant in relation to control at *p < 0.05, one-way. A student t test was used to test for significance.

Table 2: Effect of EAPL on hematological parameters

Parameters	Control	EAPL 2000 mg/kg
WBC (103/uL)	13.35±1.90	13.40±1.70
RBC (106/uL)	7.50±0.20	7.20±0.042
Hb (g/dL)	13.30±0.60	13.10±0.10
HCT (%)	40.64±1.48	40.3±0.62
MCV (fL)	53.05±0.82	56.30±1.22
MCH (pq)	16.70±0.39	17.70±0.20
MCHC(g/dL)	30.8±0.56	31.75±0.20
PLT (103/uL)	806±77.53	950±80.04*

Values are presented as mean ± S.E.M., n = 6, significant in relation to control at *p < 0.05, one-way. A student t test was used to test for significance.

3.2 Subacute oral toxicity study

All the treated rats at doses of 200 and 400 mg/kg survived throughout the 28 days of treatment. However, at all doses, the treated rats' fur did appear smoother than that of the control rats. No observable toxicity signs were noticed in the treated rats compared to the control. the

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weight decrease was not significant at dose of 200 mg/kg when compared to the control group, which was frequent and considerable. A significant ($p > 0.05$) increase in the quantity of feed at 200 mg/kg and water ($p > 0.05$) at 200 and 400 mg/kg was observed when compared with the control (Fig. 2).

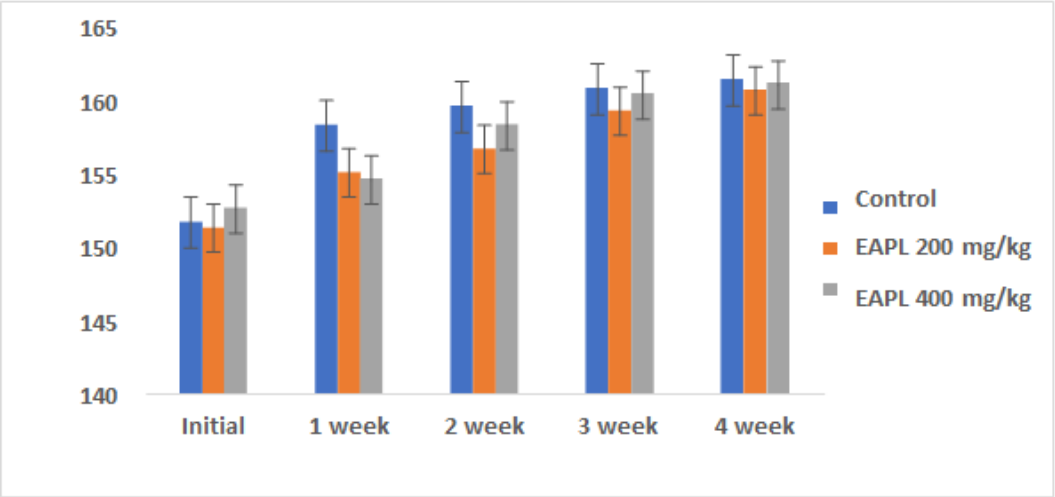

Figure 2: Values expressed as mean ± SEM, n=6 (one-way ANOVA).

3.2.1 Effect of EAPL on biochemical and hematological parameter.

The biochemical parameters among the treated and control groups did not vary substantially. Table 3 displays the values of the biochemical limits, respectively. Except for elevated ALT and AST markers when compared to the control group, majority of the hematological indicators were still within the normal range ($P > 0.05$), as shown in Table 4, except for PLT, which was significantly increased at a dose of 200 mg/kg when compared to the control group. There was no significant change in volume, protein, glucose, ketone, or blood after urine analysis when compared to control animals (Table 5).

Table 3: Effect of EAPL on biochemical parameters.

Parameters	Control	EAPL	
		200 mg/kg	400 mg/kg
Glucose (mg/dl)	101±8.92	104.2±10.90	99.2±10.90
ALT (IU/L)	49.81±2.10	56.33±4.96*	52.33±4.96
AST (IU/L)	124.8±10.1	127.2±9.35	125.2±9.35
ALP (IU/L)	302.6±80.50	308.7±78.05	305.7±78.05
Total Protein (g/dl)	5.65±0.34	5.35±0.13	5.32±0.13
Triglycerides (mg/dl)	105.9±12.4	110.5±12.72	110.4±12.72
Cholesterol (mg/dl)	97.80±5.40	96.08±3.16	95.08±3.16
Bilirubin (mg/dl)	0.160±0.02	0.162±0.22	0.159±0.22
Urea (mg/dl)	35.10±3.10	35.92±2.26	36.32±2.26
Creatinine (mg/dl)	0.54±0.02	0.54±0.04	0.51±0.04

Values are presented as mean ± S.E.M., n = 6, significant in relation to control at *$p < 0.05$, one-way. A student t-test was used to test for significance.

Table 4: Effect of EAPL on hematological markers

Parameters	Control	EAPL	
		200 mg/kg	400 mg/kg
RBC (×106/µL)	08.24 ± 0.35	08.92 ± 0.87	09.27 ± 1.13
Hb (g/dL)	12.16 ± 1.02	13.82 ± 0.97	13.78 ± 1.08
Ht (%)	49.41 ± 2.45	51.63 ± 3.34	51.37 ± 3.61

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PLT (×103/µL)	806.2 ± 89.73	833.5 ± 87.81	787.74 ± 76.38
MCV (fL)	51.21± 5.04	50.06 ± 4.32	51.61 ± 5.41
MCH (pg)	17.96 ± 1.23	19.12 ± 2.21	18.15 ± 1.76
MCHC (g/dL)	35.41 ± 3.51	34.36 ± 4.72	36.17 ± 4.12
WBC (×103/µL)	07.27 ± 1.34	07.59 ± 1.37	06.89 ± 0.93
Neutrophils (%)	23.61 ± 2.13	22.93 ± 3.74	24.79 ± 3.64
Eosinophils (%)	01.47 ± 0.55	01.65 ± 0.62	01.23 ± 0.45
Basophils (%)	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
Lymphocyte (%)	69.82 ± 6.53	67.96 ± 6.54	66.34 ± 6.28
Monocyte (%)	02.00 ± 0.48	01.46 ± 0.39	01.44 ± 0.32

Values are presented as mean ± SEM, n = 6; significant in relation to control at *p < 0.05, one-way. A student t-test was used to test for significance.

Table 5: Effect of EAPL on urine analysis

Treatment/dose	Volume	SP. Gravity	Protein	Glucose	Ketone	Blood
Control	5.11 ± 0.81	1.016± 0.004	NIL	NIL	NIL	NIL
EAPL 200 mg/kg	5.18 ± 0.73	1.017± 0.003	NIL	NIL	NIL	NIL
EAPL 400 mg/kg	5.26 ± 0.78	1.015± 0.005	NIL	NIL	NIL	NIL

Effect of EAPL on acute dermal toxicity study. In the skin irritation test, no erythema or oedema was displayed in both the control animals and the test animals after 3 days of experimentation. In determining the dermal toxic effect of EAPL in acute dermal toxicity bioassay, no significant (P > 0.05) clinical change was observed in any of the treated rat groups, except for the initial reaction within the first 30 minutes of patch attachment when the rats tried to tear the patch. The behavioral patterns and general appearance of the rats in the control and test groups were recorded after one hour and twelve hours post-application of test substances. No change meant that the manner in which the animals behaved after acclimatization did not alter when the skin was shaved, and test substances were applied. No erythema or oedema was observed over the 14-day study period in both the control and EAPL-treated animals. (Table. 6-10).

Table 6: Effect of EAPL on behavioral patterns and general appearance

Signs	Control	EAPL (1%)	EAPL (5%)
Skin and fur	N	N	N
Eyes	N	N	N
Mucous membrane	N	N	N
Behavioral patterns	N	N	N
Salivation	N	N	N
Lethargy	N	N	N
Sleep	N	N	N
Diarrhea	N	N	N
Coma	NO	NO	NO
Tremors	NO	NO	NO

Where N=Normal and NO = not found.

Table 7: The score of irritation and edema after application of EAPL with their respective bases in rats.

		The score of skin irritation		
Reaction	**Value**	**Formulation**		
		EAPL 1%		**EAPL 5%**
		24 hr 48 hr 72 hr	**24 hr**	**48 hr 72 hr**

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No Erythema	0	0 0	0	1	0 0
Very slight Erythema	1	0 0	0	0	0 0
Well defined Erythema	2	1 0	0	1	0 0
Moderate to severe Erythema	3	0 0	0	0	0 0
Severe eschar formation Erythema	4	0 1	0	0	0 0
Reaction	**Value**	**Formulation**			
		EAPL 1%		**EAPL 5%**	
		24 hr	**48 hr 72 hr**	**24 hr**	**48 hr 72 hr**
No Edema	0	0 0	0	0	0 0
Very slight edema	1	0 0	0	1	1 0
Well defined edema	2	0 0	0	0	0 0
Moderate to severe Edema	3	0 1	0	1	0 0
Severe to eschar formation Edema	4	0 0	0	0	0 0

Table 8: Effect of EAPL on erythema, eschar, and edema formation in rats

Groups	**Grading and time interval**		
	24 hr	**48 hr**	**72 hr**
Control	0 0	0	
STD	0 0	0	
EAPL 1%	0 0	0	
EAPL 5%	0 0	0	

Table 9: Effect of EAPL on Edema formation at different time intervals in rats

	Grading and time interval		
	24 hr	**48 hr**	**72 hr**
Control	0	0	0
STD	0	0	0
EAPL 1%	0	0	0
EAPL 5%	0	0	0

Table 10: Effect of EAPL 1% and 5% topical application on biochemical markers.

Parameters	**Control**	**STD**	**EAPL 1%**	**EAPL 5%**
AST (U/L)	201.5±62.90	201.76±75.90	202.4±34.56	203.6±71.80
ALT (U/L)	85.87± 9.81	86.89± 10.61	86.04± 26.57	87.91± 10.61
LDH (U/L)	732 ±129.5	733 ±131.6	731.2 ±164.6	730 ±132.5
GGT(U/L)	1.01±0.51	1.02±0.53	1.05±0.25	1.02±0.61
CHE (U/L)	457.5±161.4	458.5±172.3	451.5±114.3	453.5±171.4
Creatinine(mg/dl)	0.51±0.23	0.52±0.34	0.51±0.16	0.51±0.24
Total protein (g/dl)	6.59 ±0.45	6.87 ±0.58	6.13 ±0.71	6.79 ±0.95
Albumin (g/dl)	2.53± 0.99	2.89± 1.09	2.66± 0.54	2.81± 0.57
Globulin (g/dl)	4.06± 1.20	4.05± 1.10	4.48±0.62	4.09± 1.21

Values are presented as mean ± SEM, n=6

Values are presented as mean ± SEM, n = 6. A statistical comparison control versus reated group student t-test was used to test for significance.

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4. DISCUSSION

The purpose of the study was to evaluate ethyl acetate fraction of P. lanceifolium leaf acute and subacute toxicity in Wistar rats. Animal toxicology studies are frequently conducted to determine the possible risks to human health posed by the detrimental effects of chemical compounds and plant extracts. Enzymes and metabolites, as well as normal organ functions and histomorphology, may be significantly altered by these negative impacts [13]. Easy availability and the belief that they are nontoxic or minimal in toxicity are the main reasons medicinal herbs' usage is so popular and widely used to treat diseases. Contrary to what is commonly believed, research data has shown that some herbal bioactive compounds have adverse effects that are linked to toxic plant secondary metabolites. In plant-based therapeutic approaches, it's crucial to treat illnesses without harming other sections of the body. In this study, the ethyl acetate fraction of P. lanceifolium (EAPL) was examined for its in vivo acute, subacute and dermal toxicity profiles. Studies on acute toxicity are helpful in determining the toxic effects that a phytoconstituent has after being administered in a single dose, and they will also be helpful in choosing dosages for studies on long-term toxicity [14, 15]. It was determined that a single oral dose of EAPL at a body weight-based dose of 2000 mg/kg was safe in terms of body weight, food and water intake, and general behaviour patterns. The treated group showed no mortality. It was concluded that the LD50 of EAPL should be greater than 2000 mg/kg body weight. Due to everyday usage in chronic conditions, there is a possibility of toxicity buildup in the body and detrimental effects on tissues and organs (sub-acute toxicity research) [16]. To study the effects of EAPL, daily oral administration of sublethal dosages, such as 200 mg/kg and 400 mg/kg, was used for 28 days. Regardless of therapy, the animals all gained identical amounts of body weight. However, when compared to the control group, weight decreased. Compared to the control group, more food and drink were consumed. Hematological and biochemical markers did not alter noticeably. In the animals given the high dose, both males and females had considerably lower levels of hemoglobin, RBCs, and packed cell volume (PCV). When compared to the vehicle's control, this indicates anemia. Nevertheless, after EAPL treatment, the values of MCV, MCH, and MCHC were unaltered. This demonstrates that the oxygenation of tissues was unaffected by the EAPL treatment [17, 18]. All the stained organ slices from the treated group, when examined microscopically, showed normal tissue architecture. The values noted in the biochemical evaluations are likewise supported by the histopathological results obtained in this investigation. These findings imply that EAPL treatment at the chosen levels had no harmful effects on tissues. It is safe to choose dosages of the ethyl acetate fraction up to 200 mg/kg body weight for subsequent more investigations to explore its intriguing pharmacological potential. Following direct skin damage, dermal toxicity causes localized inflammation, which manifests as erythema and oedema. Single, recurrent, or extended contact with a chemical agent on the skin can result in direct skin damage. Rats were employed in this investigation because they were easily accessible and would make it simple to conduct comparative studies, and they are a well-established model for studies of cutaneous toxicity [19, 20, 21]. Rats are known to respond to treatments and their toxic consequences similarly to humans; as a result, they can be used to predict if an agent will likely be hazardous to humans when administered [22]. Because no erythema or oedema were shown in the test group during any of the times used, a skin irritancy test showed that EAPL was not an irritant. Both the erythema and oedema ratings were zero. In studies on serum biochemistry, when compared to the control group, the treated group showed no significant differences. Also, there were no discernible variations in any other parameters between the control and test groups.

5. CONCLUSION

Single dosage of EAPL at 2000 mg/kg body weight was found to be fairly non-toxic to rats. Subacute toxicity study revealed that 200 mg/kg body weight and 400 mg/kg body weight of EASB does not cause much adverse effects to rats according to the hematological, biochemical parameters, and histopathological analysis even though slight change in some parameter at dose of 200 mg/kg. Thus a dose below 200 mg/kg body weight can be taken as more safe doses for further pharmacological studies. Dermal toxicity profiling also showed there was no significant change in any parameter on topical application of EAPL. Further studies are required to understand major bioactive components of this ethyl acetate fraction of this extract. It is advised to conduct additional research to describe any additional toxicological effects that may manifest after prolonged exposure to P. lanceifolium because it’s often used as a topical therapeutic agent at those dosages, particularly in rural areas where conventional medications are unaffordable due to their high cost.

Informed Consent Statement: N.A.

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