ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 18, Issue 8, 2025

Online - 2455-3891
Print - 0974-2441

Research Article

EVALUATION OF ACUTE AND SUBACUTE ORAL TOXICITY INDUCED BY HYDROALCOHOLIC EXTRACT OF *IPOMOEA ERIOCARPA* IN EXPERIMENTAL RATS

KOMAL MANWANI*, MOHD. SAIFUL ISLAM, MAYUR PORWAL

[1]Department of Pharmacology, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India.

*Corresponding author: Komal Manwani; Email: komalmanwani17@gmail.com

Received: 17 May 2025, Revised and Accepted: 28 June 2025

ABSTRACT

Objectives: The study aimed to investigate the acute and subacute oral toxicity caused by the hydroalcoholic extract of *Ipomoea eriocarpa* in albino Wistar rats.

Methods: The acute toxicity assessment involved administering a limit dose of 2000 mg/kg body weight, followed by daily assessments for an additional 14 days. The rats were weighed and assessed for mortality, behavior, and signs of illness. In the subacute study, four groups of ten rats were administered distilled water and the extract at doses of 200, 1000, and 1800 mg/kg every 24 h for 28 days.

Results: The animals' organ weight, hematological analysis, and biochemical parameters were evaluated and showed no significant difference when compared to the control group. The histopathological examination of the vital organs of the animals was conducted to assess gross findings in comparison to the control group. No significant difference ($p>0.05$) was observed in the relative organs, body weights, hematological (e.g., Haemoglobin: control 150.97±1.88 g/L vs. 1800 mg/kg 145.50±2.31 g/L, $p>0.05$) or biochemical parameters (e.g., alanine aminotransferase: control 85.41±0.02 U/L vs. 1800 mg/kg 33.50±0.02 U/L, $p>0.05$). Histopathological changes were minor and observed only at the highest dose (1800 mg/kg). No instances of mortality were documented. Investigations into toxicity are essential for guaranteeing the safety of chemicals, pharmaceuticals, and other materials. They safeguard human health and the environment, facilitate regulatory decision-making, inform product development, and enhance scientific comprehension of negative impacts.

Conclusion: The study concluded that the short-term and medium-term oral administration of the hydroalcoholic extract *I. eriocarpa* to rats did not induce toxicity. The oral administration of the extract to rats demonstrated a significant margin of safety and holds promise for the development of a novel medicinal agent.

Keywords: Acute toxicity, Biochemical analysis, Hematological parameters, *Ipomoea eriocarpa*, Subacute toxicity, Histopathology.

 DOI: http://dx.doi.org/10.22159/ajpcr.2025v18i8.55097. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

The utilization of medicinal products as a healthcare modality has risen [1]. In developing nations, traditional herbal treatments are a culturally significant approach to healing therapies. These remedies are effective, widely accepted, economically feasible, and often the only available option, making them economically viable [2]. Traditional plant-based medicines are crucial for global health and disease management, extensively utilized in Africa and Asia, particularly in India and China. Due to adverse reactions and resistance to synthetic medications, they are gaining popularity in developed nations [3]. Recent research has revealed that several therapeutic herbs also have adverse consequences [4]. Prolonged use of therapeutic herbs raises concerns about potential harmful effects. Assessing the toxicological consequences of therapeutic plant extracts is crucial for evaluating potential harmful effects.

Acute and subacute toxicity investigations assess the safety and non-harmfulness of chemicals, examining their mechanism of action. Systemic toxicological findings are used for threat identification and risk control in compound manufacture, management, and utilization [5]. Acute toxicity is a single-dose assessment of rodent toxicity, while subacute toxicity, which involves repeated dose exposure, is conducted after acquiring preliminary data from acute toxicity testing.

Ipomoea eriocarpa, popularly referred to as "tiny morning glory," is located in tropical Asia, northern Australia, Madagascar, South Africa, Egypt, and various regions of tropical Africa and southern India [6]. Approximately 8000 polyphenolic substances were effectively discovered among different species of plants [7]. According to a phytochemical investigation, the plant's (methanolic extract) contains phenols, flavonoids, phytosterols, and alkaloids [8,9]. The traditional uses of *I. eriocarpa* (methanolic and petroleum ether extract) include migraines, joint inflammation, seizures, open sores, and high fever. The preclinical study of *I. eriocarpa* has recently verified defensive properties on brain [10], antioxidant abilities [11], prevention of secretions [12], analgesics [13], antipyretic [14], activity contrary to worms [15], antimicrobial properties [15], also aids from arthritis, diabetes as well as kidney stones preclusion [9,15].

Nevertheless, the literature lacks investigations on the toxicity of the hydroalcoholic extract of *I. eriocarpa* (HEIE). Consequently, this analysisaimed to evaluate the toxicity (acute as well as subacute oral toxicology) of HEIE, thereby enhancing assurance in its wellbeing for human use in treating various diseases.

METHODS

Collection and authentication of plant

Fully grown plant specimens of *I. eriocarpa* were gathered from Barkagao, Hazaribag District, Jharkhand, India (23° 85' 31.10" N latitude, 85° 20' 58.51" E longitude, and 610 m altitude). The voucher specimen (KM81123) was prepared, presented, and authenticated by Dr. P. Santhan, a botanist and taxonomist from Jharkhand.

Preparation of plant extract

The entire extraneous substance was meticulously evacuated from the *I. eriocarpa* plant matter following a thorough cleansing process. After undergoing a clean water wash and being dried under shade for 3–4 weeks, the plant material was roughly grounded with the help of a motorized blender. The grounded stuff was securely kept in desiccated, disinfected baggage. Approximately 25 g of powdered material underwent defatting with petroleum ether at a temperature range of 40–60°C. Following the defatting process, the grounded material was utilized for extraction with hydroalcoholic in a ratio of 3:7 for about a day [16]. The extract was kept at the surrounding climate for parching, also the concentrated extract was employed for the acute oral toxicity and sub-acute toxicity assessments.

Experimental animals

The acute and subacute toxicological experiments were carried out in only healthy male and female albino Wistar rats aged 6–8 weeks, weighing 130–180 g, and free from visible signs of illness or abnormality were included. Animals exhibiting signs of infection, congenital anomalies, or abnormal behavior during the acclimatization period were excluded from the study. Rats were randomly assigned to groups using a simple randomization method to avoid bias. The rats were obtained from the animal facility at TMU, Moradabad, India, and were familiarized with laboratory environments for 7 days before the experimentations. The rats were kept at a controlled room temperature of 22–24°C, with a 12-h light/dark phase and moisture of approximately 50±5%. They were randomly assigned to experimental or control groups, were independently accommodated in disinfected polypropylene cages, utilizing sterilized padded husk as bedding material, and were provided with a normal pellet supply and water as needed. The investigational techniques adhered to guidelines set by the "Institutional Animal Ethics Committee" (IAEC) and the "Committee for the Control and Supervision of Experiments on Animals" (CCSEA), receiving acceptance from the "University Ethical Committee," accompanied by an approval number CCSEA/1205/2024/19.

Acute oral toxicity study

An acute oral toxicological assessment was conducted by the "Organization for Economic Co-operation and Development (OECD) guideline 423 for testing of chemicals" [17]. Both male and female rats, aged 6–8 weeks and exposed for 16-h an abstaining period, were utilized. HEIE was dissolved in normal saline and delivered at 5,50,300, and 2000 mg/kg doses, while the control group got just 10% normal saline. They were monitored independently for the initial crucial 4 h and subsequently twice per day throughout the experiment duration (14 days) for lethality, indicators of noxiousness (alterations in the integumentary system, ocular features, mucosal surfaces, breathing distress), and behavioral changes (salivation, watery stools, nap disturbances, coma, exhaustion). In addition, alterations in body mass, nutrition, and drink consumption were documented throughout the research.

Subacute toxicity study

A subacute oral toxicological assessment was conducted by the OECD "Organization for Economic Co-operation and Development guideline 407 for the testing of chemicals" [18] and the "World Health Organization" guideline [19]. 10 rats, including 5 females and 5 males, were utilized for the investigation. The acute toxicological assessment outcomes demonstrated that HEIE was nontoxic at 2000 mg/kg dose. Subsequently, a subacute toxicity study was conducted, wherein HEIE was administered orally to four groups at the dosages of 20, 1000, and 1800 mg/kg body mass every 24 h for 28 days, while the control group was given 10% normal saline as a vehicle in equivalent volume. Multiple hazardous indicators and observations, including mortality and food and water consumption, were tracked. Body mass was measured on the preliminary dose day, as well as on the 7th, 14th, 21st, and 28th days. Following 28 days, allaliverats were subjected to nightlong fasting and anesthesia. The study involved heparinized blood samples for hematological parameters and serum extraction for clinical blood chemistry. Animals were euthanized, and their internal organs (brain, heart, liver, spleen, stomach, intestines, and kidneys) were weighed and examined for total lacerations. The organs remained conserved for histopathological study in a 10% formalin solution [20].

Weekly body weight

The body mass of individual rats was meticulously recorded earlier the initiation of the study, every week throughout the analysis, as well as on the sacrificial date.

Mortality and toxic signs

Daily optical assessments of mortality, fluctuations in bodily attributes, behavior (including sleepiness, the production of saliva, and exhaustion), as well as any signs of wound or ailment, were performed over 28 days, especially after administration and lasting beyond 4 h post-administration [21].

Relative organ weight

On the 29th day, all animals received ketamine injections through i.p route for anesthesia. Blood samples were taken for hematological as well as biochemical investigation. After euthanization, internal organs were extracted, weighed, and examined for total lacerations. The organs remained conserved for histopathological study in a 10% formalin solution for histopathological analysis [20].

Hematological parameters

Blood specimens were obtained through heart perforation into EDTA-comprising vials and assessed at Dr. Lal Pathology Laboratory in Moradabad, UP, India. Hematological assessments including "haemoglobin (Hb), red blood cell count, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total white blood cell (WBC) count, differential WBCs, platelet count, red blood cell distribution width (RDW), platelet distribution width, platelet large cell ratio, mean platelet volume, and procalcitonin" were examined [22].

Biochemical estimations

Blood obtained in non-heparinized tubings was subsequently centrifuged at 3000 revolutions/min for 10 min. The separated serum was analyzed at Dr. Lal Pathology Laboratory in Moradabad, UP, India, for several factors, including "sodium, potassium, chloride, creatinine, urea, uric acid, total protein, albumin, globulin, albumin-globulin ratio, alkaline phosphatase (ALP), and total bilirubin" [20].

Histopathology study

The organs, specifically the brain, liver, heart, spleen, stomach, intestines, and kidneys, were meticulously extracted and weighed. The organs were kept in a 10% buffered formalin fixation medium for histopathological analysis. The organ paraffin slices have been generated, stained with hematoxylin and eosin, and prepared for light microscopy according to established procedures [23].

Statistical analysis

Each of the assessments was presented as the mean±SD standard deviation, and the outcomes were analyzed statistically using one-way analysis of variance followed by Tukey's multiple comparison tests with statistical software called GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA) version 5.0. A $p<0.05$ compared to the control was accepted as statistically significant.

RESULTS

Acute oral toxicity study

HEIE administered at doses of 5,50,300, and 2000 mg/kg did not exhibit any toxic effects on the behavioral patterns of the once-daily-dosed rats, and was monitored over 14 days thereafter. No alterations were detected in the behavioral patterns, skin condition, ocular appearance, salivation levels, or instances of diarrhea in the rats. No instances of mortality or notable weight loss were recorded.

Subacute toxicity study

Weekly body weight

Body mass was assessed once a week, i.e., on the initial day (0), as well as on the 7th, 14th, 21st, and 28th days in the four groups. The initial group serves as the control. Group I consists of a HEIE at a dosage of 200 mg/kg, Group II includes HEIE at 1000 mg/kg, and Group III features HEIE at 1800 mg/kg. No notable alterations in body weight were detected, as illustrated in Table 1.

Clinical observationand mortality

The day-to-day oral treatment of HEIE for 28 days doesn't elicit any toxic indications in rats, even at the maximum dosage of 1000 mg/kg body mass. Neither fatalities nor discernible clinical manifestations were seen in any of the groups during the trial. No rats exhibited indications of toxicity in their integument, pelage, ocular health, sleep patterns, salivation, diarrhea, or behavior. The food and water intake of the treated rats, monitored during the trial, did not differ substantially from that of the control group.

Relative organ weight

The relative organ weights of rats treated for 28 days are presented in Table 2. The relative organ weights of each organ documented at necropsy in both treatment groups did not exhibit a statistically significant change ($p>0.05$) when compared to the control, as illustrated in Table 2.

Haematological parameters

The outcomes of sub-acute treatment of HEIE on hematological analysis are illustrated in Table 3. The majority of haematological measures, including "haemoglobin, total RBC count, RDW, WBC count, neutrophils, lymphocytes, monocytes, and platelet count," in dose-administered rats did not significantly differ from the control, as illustrated in Table 2.

Biochemical analysis

The impact of sub-acute administration of HEIE on biochemical parameters is detailed in Table 4. HEIE doesn't influence serum electrolytes (Na^+, K^+, and Cl^-). The kidney functioning tests, including "urea, creatinine, and uric acid", showed no substantial modifications. No statistically significant variations were noticed in the liver functioning tests, including "alanine aminotransferase (ALT), aspartate aminotransferase (AST), and ALP." Furthermore, there were no significant alterations detected in "total protein, albumin, and globulin" as illustrated in Table 4.

Histopathology study

Light microscopic examinations of histopathological sections of vital organs such as brain (Fig. 1), heart (Fig. 2), liver (Fig. 3), kidney (Fig. 4),

Table 1: The effect of hydroalcoholic extract of *Ipomoea eriocarpa* on body weight of rats (g) at different days

Group	Doses	On initial (0) day	On Day 7th	On Day 14th	On Day 21st	On Day 28th	n
Control	-	168.20±1.13	171.70±1.05**	175.60±1.26**	177.40±1.34**	179.90±1.37**	10
I	*Ipomoea eriocarpa* 200 mg/kg	163.20±1.22	166.40±1.07**	161.70±1.82**	165.40±1.57**	169.40±1.50**	10
II	*Ipomoea eriocarpa* 1000 mg/kg	165.10±1.10	168.90±1.66**	170.30±1.33**	167.20±1.98**	174.60±1.89**	10
III	*Ipomoea eriocarpa* 1800 mg/kg	166.00±1.15	141.50±1.08**	132.50±1.35**	140.20±1.54**	145.10±1.28**	10

Values are expressed as the mean±SD (n=10; male and female rats); $p>0.05$ using one-way analysis of variance followed by Tukey's multiple comparison test; **Not significant

Table 2: The relative organ weight of rats treated with different doses of hydroalcoholic extract of *Ipomoea eriocarpa* for 28 days

Organs	Control	*Ipomoea eriocarpa* 200 mg/kg	*Ipomoea eriocarpa* 1000 mg/kg	*Ipomoea eriocarpa* 1800 mg/kg	n
Heart	0.46±0.01	0.45±0.00**	0.45±0.01**	0.46±0.00**	10
Liver	3.02±0.00	3.06±0.01**	3.14±0.00**	3.19±0.00**	10
Kidney	0.75±0.01	0.78±0.00**	0.76±0.00**	0.78±0.01**	10
Spleen	0.34±0.00	0.31±0.00**	0.29±0.00**	0.27±0.01**	10
Stomach	0.86±0.01	0.88±0.00**	0.83±0.00**	0.82±0.00**	10
Intestines	0.22±0.00	0.18±0.00**	0.19±0.03**	0.21±0.00**	10
Brain	1.64±0.01	1.67±0.00**	1.69±0.01**	1.72±0.01**	10

Values are expressed as the mean±SD (n=10; male and female rats); Relative organ weight was calculated as (organ weight/body weight) × 100; $p>0.05$ using one-way analysis of variance followed by Tukey's multiple comparison test; **Not significant

Table 3: Effect of hydroalcoholic extract of *Ipomoea eriocarpa* on haematological parameters in the sub-acute oral toxicity study

Haematological parameters	Unit	Control	*Ipomoea eriocarpa* 200 mg/kg	*Ipomoea eriocarpa* 1000 mg/kg	*Ipomoea eriocarpa* 1800 mg/kg	n
Haemoglobin	g/L	150.97±1.88	142.44±2.95**	147.82±2.42**	145.50±2.31**	10
Total red blood cells	10^{12}/L	10.21±1.93	9.83±1.64**	8.75±0.02**	8.89±2.37**	10
Packed cell volume	L/L	0.46±0.02	0.44±0.03**	0.44±0.03**	0.43±0.03**	10
Mean corpuscular volume	fL	61.16±2.22	60.91±2.37**	62.62±1.27**	61.45±2.39**	10
Mean corpuscular haemoglobin	pg	17.92±1.67	15.89±1.75**	16.32±0.02**	17.43±1.31**	10
Mean corpuscular haemoglobin concentration	g/L	310.96±2.26	311.44±0.03**	311.89±2.21**	310.17±1.78**	10
Total white blood cells	10^9/L	7.19±1.60	7.83±1.40**	7.48±1.79**	7.22±1.17**	10
Neutrophils	%	26.74±1.66	25.83±2.08**	26.11±0.01**	29.90±1.22**	10
Lymphocytes	%	86.11±2.20	69.95±2.94**	85.73±1.62**	88.89±2.58**	10
Monocytes	%	3.89±2.10	3.31±1.30**	2.96±0.01**	2.19±0.64**	10
Platelet count	10^9/L	845.32±1.84	838.08±2.04**	839.24±0.02**	840.84±2.03**	10
Red blood cell distribution unit	%	14.39±1.47	13.98±1.49**	13.70±1.53**	13.89±1.50**	10
Platelet distribution width	fL	11.52±1.89	9.83±1.40**	11.92±2.14**	9.40±1.32**	10
Platelet large cell ratio	%	14.85±1.70	14.90±1.68**	14.05±1.82**	13.82±2.09**	10
Mean platelet volume	fL	19.77±1.58	18.20±1.3**	17.75±0.02**	17.14±2.14**	10
Procalcitonin	%	0.99±0.31	0.75±0.02**	0.69±0.02**	0.64±0.02**	10

Values are expressed as the mean±SD (n=10; male and female rats); $p>0.05$ using one-way analysis of variance followed by Tukey's multiple comparison test; **Not significant

Table 4: Effect of hydroalcoholic extract of *Ipomoea eriocarpa* on biochemical parameters in the sub-acute oral toxicity study

Biochemical parameters	Unit	Control	*Ipomoea eriocarpa* 200 mg/kg	*Ipomoea eriocarpa* 1000 mg/kg	*Ipomoea eriocarpa* 1800 mg/kg	n
Sodium	mEq/L	185.23±0.03	173.84±0.02**	163.29±0.02**	181.32±0.02**	10
Potassium	mEq/L	6.97±0.01	6.12±0.02**	5.86±0.02**	5.40±0.02**	10
Chloride	mEq/L	110.46±0.03	107.41±0.02**	105.98±0.02**	102.70±0.02**	10
Phosphorus	mg/dL	8.07±0.02	7.03±0.01**	5.21±0.02**	5.40±0.02**	10
Calcium, total	mg/dL	9.56±0.02	9.32±0.02**	9.17±0.02**	9.00±0.02**	10
Urea	mg/dL	49.43±0.03	49.46±0.02**	37.00±0.02**	32.90±0.02**	10
BUN	mg/dL	19.72±0.02	17.52±0.02**	16.23±0.03**	15.00±0.02**	10
Creatinine	mg/dL	0.63±0.03	0.45±0.01**	0.28±0.03**	0.17±0.01**	10
Uric acid	mg/dL	1.80±0.01	1.69±0.02**	1.47±0.02**	2.48±0.02**	10
Alkaline phosphatase	U/L	500.91±0.02	396.70±0.02**	239.00±0.02**	146.90±0.02**	10
Total protein	g/dL	7.01±0.01	6.92±0.03**	6.41±0.02**	6.27±0.01**	10
Albumin	g/dL	4.01±0.01	3.93±0.03**	3.51±0.02**	3.11±0.01**	10
Globulin	g/dL	3.20±0.02	3.19±0.02**	3.18±0.02**	3.16±0.01**	10
A: G Ratio	g/dL	1.00±0.02	0.99±0.02**	0.98±0.02**	0.98±0.02**	10
AST (SGOT)	U/L	182.87±0.02	176.31±0.02**	157.62±0.02**	148.60±0.02**	10
ALT (SGPT)	U/L	85.41±0.02	80.57±0.02**	71.28±0.02**	33.50±0.02**	10
GGTP	U/L	0.41±0.01	0.36±0.02**	0.34±0.02**	0.30±0.02**	10
Bilirubin total	mg/dL	0.65±0.02	0.57±0.02**	0.32±0.02**	0.10±0.02**	10

Values are expressed as the mean±SD (n=10; male and female rats); p>0.05 using one-way analysis of variance followed by Tukey's multiple comparison test; **Not significant, BUN: Blood urea nitrogen, AST: Aspartate aminotransferase, SGOT: Serum glutamic oxaloacetic transaminase, ALT: Alanine aminotransferase, SGPT: Serum glutamate pyruvate transaminase, GGTP: Gamma-glutamyl transpeptidase

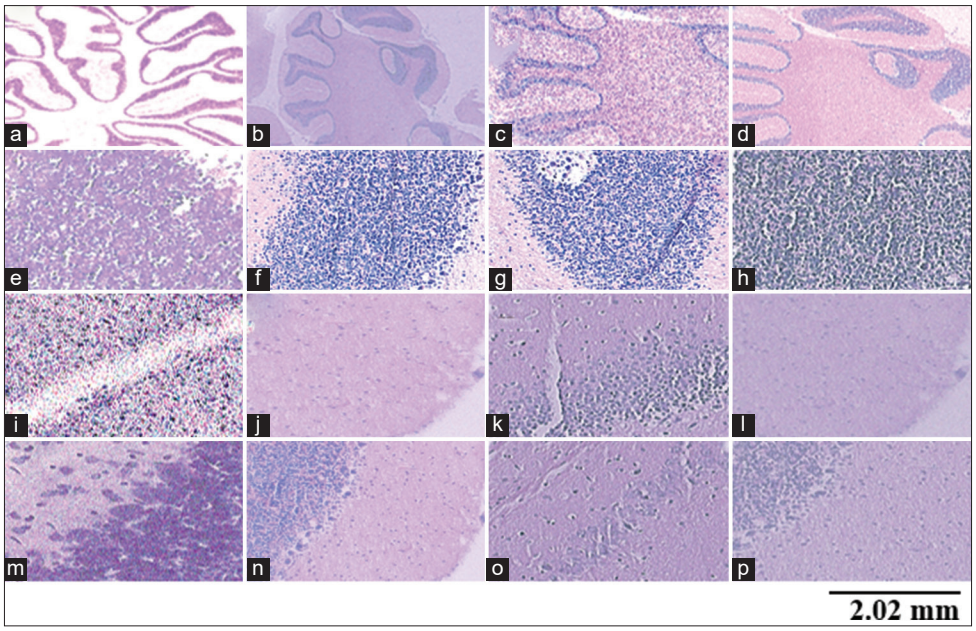

Fig. 1: Histopathology of the brain. Body organs of control (expressed as a, e, i, m), test 200 mg/kg dose (expressed as b, f, j, n), test 1000 mg/kg dose (expressed as c, g, k, o), test 1800 mg/kg (expressed as d, h, l, p). (a-d) are the Cerebellum of control and test animals showing no signs of toxicity, (e-h) are the Inner nuclei of control and test animals showing no signs of toxicity, (i-l) are the Higher nuclei of control and test animals showing no signs of toxicity, (m-p) are the Purkinje cell of control and test animals showing no signs of toxicity. H&E stain was used to view histological sections, which were then examined at ×10 and ×40 magnification using a light microscope

stomach (Fig. 5), spleen (Fig. 6), and intestines (Fig. 7) in test and control group rats have been examined.

Histopathology of brain

Body organs of control (expressed as Fig. 1a, e, i, m), test 200 mg/kg dose (expressed as Fig. 1b, f, j, n), test 1000 mg/kg dose (expressed as Fig. 1c, g, k, o), test 1800 mg/kg (expressed as Fig. 1d, h, l, p). Fig. 1a-d are the Cerebellum of control and test animals showing no signs of toxicity, Fig. 1e-h are the Inner nuclei of control and test animals showing no signs of toxicity, Fig. 1i-l are the Higher nuclei of control and test animals showing no signs of toxicity, Fig. 1m-p are the Purkinje cell of control and test animals showing no signs of toxicity as illustrated in Fig. 1.

Histopathology of heart

Body organs of control (expressed as Fig. 2a, e, i), test 200 mg/kg dose (expressed as Fig. 2b, f, j), test 1000 mg/kg dose (expressed as Fig. 2c, g, k), test 1800 mg/kg (expressed as Fig. 2d, h, l). Fig. 2a-d are the Cardiac muscles of control and test animals, control group and test 200 mg/kg do not produce any toxicity whereas test group in 1000 mg/kg shows acute inflammation with a predominance of lymphocytes and macrophages in the interstitial spaces between cardiac myocytes and 1800 mg/kg shows myocyte degeneration resulting in the loss of the normal striated pattern, Fig. 2e-h are Coronary artery of control and test animals showing no signs of toxicity, Fig. 2i-l are the Muscle cells of control and test animals showing no signs of toxicity as illustrated in Fig. 2.

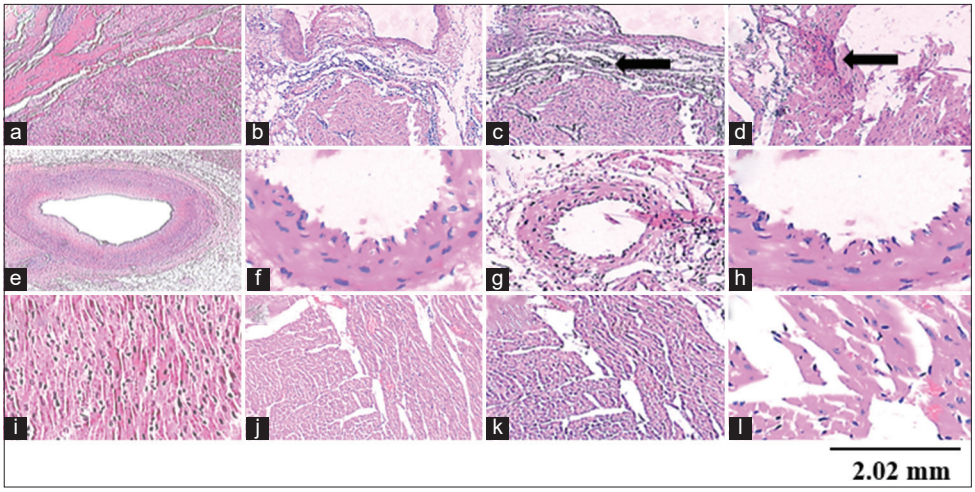

Fig. 2: Histopathology of the heart. Body organs of control (expressed as a, e, i), test 200 mg/kg dose (expressed as b, f, j), test 1000 mg/kg dose (expressed as c, g, k), test 1800 mg/kg (expressed as d, h, l). (a-d) are the Cardiac muscles of control and test animals, The control group and test at 200 mg/kg do not produce any toxicity whereas the test group in 1000 mg/kg shows acute inflammation with a predominance of lymphocytes and macrophages in the interstitial spaces between cardiac myocytes and 1800 mg/kg shows myocyte degeneration resulting in the loss of the normal striated pattern, (e-h) are Coronary artery of control and test animals showing no signs of toxicity, (i-l) are the Muscle cells of control and test animals showing no signs of toxicity. H&E stain was used to view histological sections, which were then examined at ×10 and ×40 magnification using a light microscope

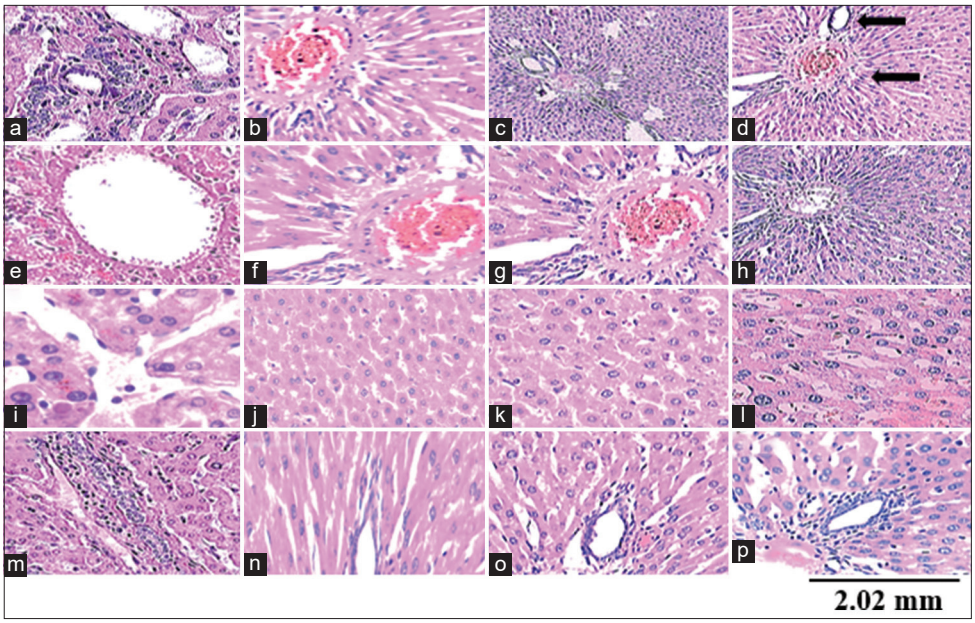

Fig. 3: Histopathology of the liver. Body organs of control (expressed as a, e, i, m), test 200mg/kg dose (expressed as b, f, j, n), test 1000mg/kg dose (expressed as c, g, k, o), test 1800mg/kg (expressed as d, h, l, p). (a-d) are the Portal Traids of control and test animals, control group, test 200 mg/kg and 1000 mg/kg do not produce any toxicity whereas test group of 1800 mg/kg shows that the portal areas have infiltrate of lymphocytes and plasma cells, consistent with chronic inflammation, (e-h) are the Central vein of control and test animals showing no signs of toxicity, (i-l) are the Hepatocytes of control and test animals Showing no signs of toxicity, (m-p) are Bile duct of control and test animals showing no signs of toxicity. H&E stain was used to view histological sections, which were then examined at ×10 and ×40 magnification using a light microscope

Histopathology of liver

Body organs of control (expressed as Fig. 3a, e, i, m), test 200 mg/kg dose (expressed as Fig. 3b, f, j, n), test 1000 mg/kg dose (expressed as Fig. 3c, g, k, o), test 1800 mg/kg (expressed as Fig. 3d, h, l, p). Fig. 3a-d are the Portal Traids of control and test animals, control group, test 200 mg/kg and 1000 mg/kg do not produce any toxicity whereas the test group of 1800 mg/kg shows that the portal areas have infiltrate of lymphocytes and plasma cells, consistent with chronic inflammation. Fig. 3e-h are the central vein of control and test animals showing no signs of toxicity, Fig. 3i-l are the hepatocytes of control and test animals Showing no signs of toxicity, Fig. 3m-p are Bile duct of control and test animals showing no signs of toxicity as illustrated in Fig. 3.

Histopathology of kidney

Body organs of control (expressed as Fig. 4a, e, i, m, q), test 200 mg/kg dose (expressed as Fig. 4b, f, j, n, r), test 1000 mg/kg dose (expressed as Fig. 4c, g, k, o, s), test 1800 mg/kg (expressed as Fig. 4d, h, l, p, t). Fig. 4a-d are the Cortex of control and test animals Showing no signs of toxicity, Fig. 4e-h are Medulla of control and test animals showing no signs of toxicity, Fig. 4i-l are the Hilum of control and test animals

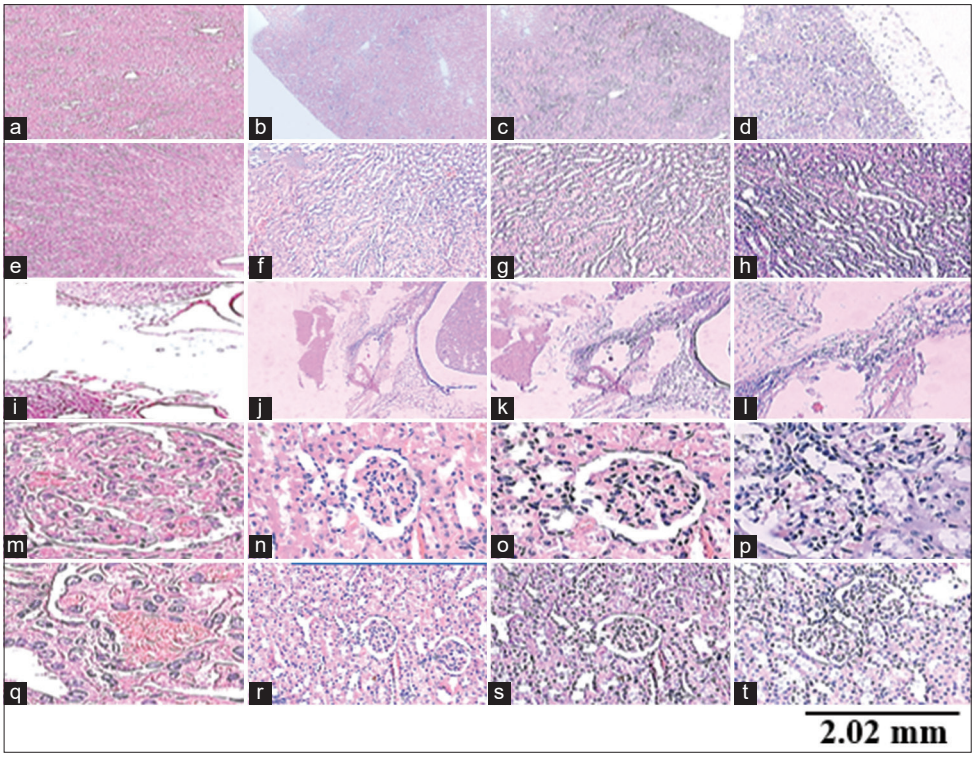

Fig. 4: Histopathology of the kidney. Body organs of control (expressed as a, e, i, m, q), test 200 mg/kg dose (expressed as b, f, j, n, r), test 1000 mg/kg dose (expressed as c, g, k, o, s), test 1800 mg/kg (expressed as d, h, l, p, t). (a-d) are the cortex of control and test animals Showing no signs of toxicity, (e-h) are Medulla of control and test animals showing no signs of toxicity, (i-l) are the Hilum of control and test animals showing no signs of toxicity, (m-p) are the Glomerulus of control and test animals showing no signs of toxicity, (q-t) are the Vascular poles of control and test animals showing no signs of toxicity. H&E stain was used to view histological sections, which were then examined at ×10 and ×40 magnification using a light microscope

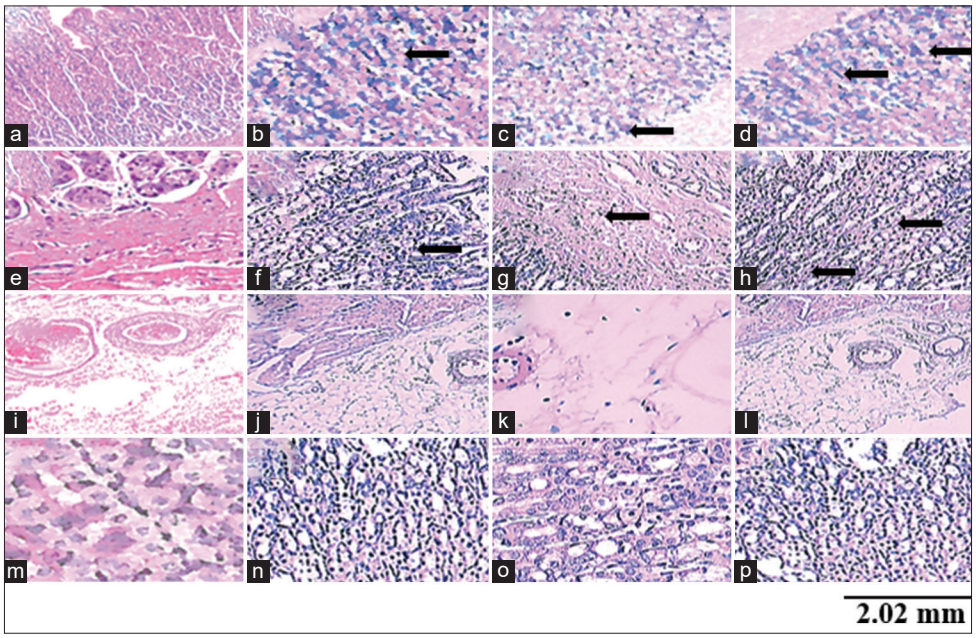

Fig. 5: Histopathology of the stomach. Body organs of control (expressed as a, e, i, m), test 200 mg/kg dose (expressed as b, f, j, n), test 1000 mg/kg dose (expressed as c, g, k, o), test 1800 mg/kg (expressed as d, h, l, p). (a-d) are the Stomach mucosa of the control and test animals, The control group does not produce any toxicity whereas the test group shows acute gastritis due to the presence of lymphocytes, (e-h) are the Muscularis mucosae of control and test animals, control group do not produce any toxicity whereas test group shows neutrophils infiltrating deeper layers of the mucosa indicating more severe or acute gastritis, (i-l) are the Adventitia of control and test animals showing no signs of toxicity, (m-p) are the Parietal and chief cells of control and test animals showing no signs of toxicity. H&E stain was used to view histological sections, which were then examined at ×10 and ×40 magnification using a light microscope

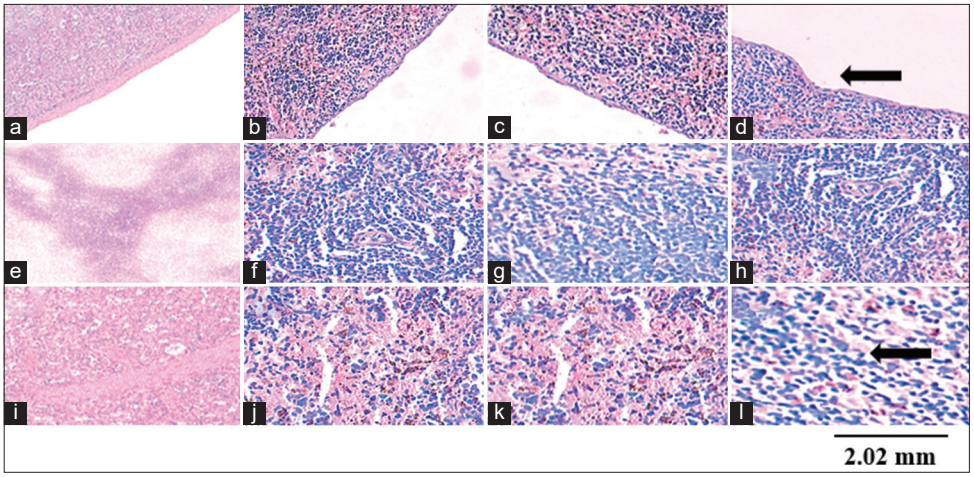

Fig. 6: Histopathology of the spleen. Body organs of control (expressed as a, e, i), test 200 mg/kg dose (expressed as b, f, j), test 1000 mg/kg dose (expressed as c, g, k), test 1800 mg/kg (expressed as d, h, l). (a-d) are the Capsule of control and test animals, control group, tests 200 mg/kg as well as 1000 mg/kg have no toxicological indications whereas the test group in 1800 mg/kg shows hematoma seen due to less severe trauma causing the capsule to deform due to internal bleeding, (e-h) are White pulp of control and test animals showing no signs of toxicity, (i-l) are the Red pulp of control and test animals, control group, test 200 mg/kg as well as 1000 mg/kg have no toxicological indications whereas test group in 1800 mg/kg shows atrophy due to hypersplenism. H&E stain was used to view histological sections, which were then examined at ×10 and ×40 magnification using a light microscope

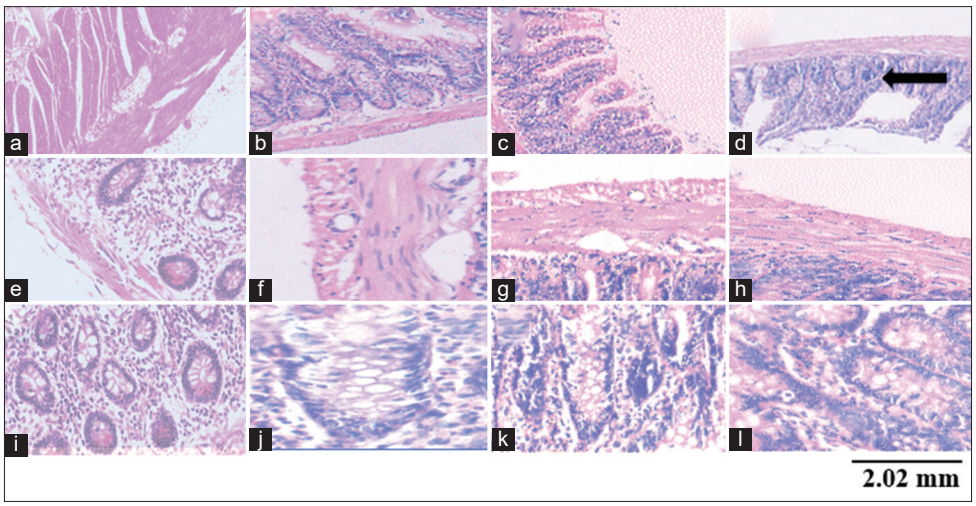

Fig. 7: Histopathology of the intestines. Body organs of control (expressed as a, e, i), test 200 mg/kg dose (expressed as b, f, j), test 1000 mg/kg dose (expressed as c, g, k), test 1800 mg/kg (expressed as d, h, l). (a-d) are the Muscularis externa of control and test animals, control group, test 200 mg/kg as well as 1000 mg/kg have no toxicological indications whereas the test group in 1800 mg/kg shows a higher number of neutrophils indicating acute infection, (e-h) are Muscularis mucosae of control and test animals showing no signs of toxicity, (i-l) are the Crypts of control and test animals showing no signs of toxicity. H&E stain was used to view histological sections, which were then examined at ×10 and ×40 magnification using a light microscope

showing no signs of toxicity, Fig. 4m-p are the Glomerulus of control and test animals showing no signs of toxicity, Fig. 4q-t are the Vascular poles of control and test animals showing no signs of toxicity as illustrated in Fig. 4.

Histopathology of stomach

Body organs of control (expressed as Fig. 5a, e, i, m), test 200 mg/kg dose (expressed as Fig. 5b, f, j, n), test 1000 mg/kg dose (expressed as Fig. 5c, g, k, o), test 1800 mg/kg (expressed as Fig. 5d, h, l, p). Fig. 5a-d are the stomach mucosa of the control and test animals. The control group does not produce any toxicity whereas the test group shows acute gastritis due to the presence of lymphocytes, Fig. 5e-h are the muscularis mucosae of the control and test animals, control group do not produce any toxicity whereas test group shows neutrophils infiltrating deeper layers of the mucosa indicating more severe or acute gastritis, Fig. 5i-l are the Adventitia of control and test animals showing no signs of toxicity, Fig. 5m-p are the parietal and chief cells of control and test animals showing no signs of toxicity as illustrated in Fig. 5.

Histopathology of spleen

Body organs of control (expressed as Fig. 6a, e, i), test 200 mg/kg dose (expressed as Fig. 6b, f, j), test 1000 mg/kg dose (expressed as Fig. 6c, g, k), test 1800 mg/kg (expressed as Fig. 6d, h, l). Fig. 6a-d are the Capsule of control and test animals, control group, test 200 as well as 1000 mg/kg have no toxicological indications whereas the test group at 1800 mg/kg shows hematoma seen due to less severe trauma causing the capsule to deform due to internal bleeding, Fig. 6e-h are White pulp of control and test animals showing no signs of toxicity, Fig. 6i-l are the Red pulp of control and test animals, control group, test 200 as well as 1000 mg/kg have no toxicological indications whereas test group in 1800 mg/kg shows atrophy due to hypersplenism as illustrated in Fig. 6.

Histopathology of intestines

Body organs of control (expressed as Fig. 7a, e, i), test 200 mg/kg dose (expressed as Fig. 7b, f, j), test 1000 mg/kg dose (expressed as Fig. 7c, g, k), test 1800 mg/kg (expressed as Fig. 7d, h, l). Fig. 7a-d are the Muscularis externa of control and test animals, control group, test 200 as well as 1000 mg/kg have no toxicological indications whereas the test group in 1800 mg/kg shows a higher number of neutrophils indicating acute infection, Fig. 7e-h are Muscularis mucosae of control and test animals showing no signs of toxicity, Fig. 7i-l are the Crypts of control and test animals showing no signs of toxicity as illustrated in Fig. 7.

DISCUSSION

At present, medicinal plants are recognized for their pharmacological properties. Nonetheless, the potential toxicity of these biologically active compounds remains little understood [24,25]. An investigation into acute toxicity evaluates the adverse consequences that arise in a short period following the administration of a single dose of a tested product. Testing procedures are typically performed on rodents and are conducted at the initial stages of developing an entirely novel material to gather information regarding its toxicity [26]. According to the prior research conducted on petroleum ether extract of *I. eriocarpa* (PIE), it was found to be non-toxic in rats when administered orally in doses up to 2000 mg/kg, p.o. in an acute toxicity study. In a 6-week chronic toxicity study, PIE at doses of 100, 200, and 400 mg/kg did not affect body weight or behavior, and caused no treatment-related changes in haematological parameters between control and treated groups. This indicates that PIE was not toxic to circulating red cells or interfered with their production [27]. The present study showed HEIE did not cause death or behavioral changes in rats administered 1800 mg/kg of dose. The OECD classification indicates that with an LD_{50} >2 g/kg, this plant has been determined to present no potential threat of acute or sub-acute toxicity.

The assessment of the toxic properties of medicinal products, including extracts, isolated compounds, and formulations, is typically an initial phase in the screening of natural products for pharmacological activity. The LD_{50} measurement is a preliminary procedure. The acute toxicity study (OECD 423) provides preliminary insights into a substance's toxic action, serves as a basis for classification and labeling, and aids in determining the dosage of new compounds in animal studies [28]. The study found that HEIE, administered at doses of 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg, did not cause any adverse effects on rats over 14 days. No changes were observed in behavior, skin condition, ocular appearance, salivation levels, diarrhea, mortality, or weight loss. The extract did not show acute toxicity effects at the tested dose, with LD_{50} values exceeding 2000 mg/kg. Further research was conducted to assess the subacute toxicity of the extract over 28 days, aiming to gather comprehensive toxicological data on the plant aiming to compile comprehensive toxicological data on this plant.

The sub-acute oral toxicity study (OECD 407) assesses the toxicity of a substance in its target organs and physiological and metabolic outcomes from low doses. This study found that HEIE, administered at doses of 200 mg/kg, 1000 mg/kg, and 1800 mg/kg, did not cause any adverse effects in rats over 28 days. However, animals administered 1800 mg/kg showed a minor reduction in body weight. HEIE is used to enhance metabolic rate in animals.

In terms of pathological and physiological health, organ weights serve as indicators, as stated by Raina *et al*. [29]. Consuming natural products can potentially harm essential organs like the brain, heart, stomach, kidneys, liver, spleen, and intestines due to their diverse functions. However, organ weight findings showed no significant changes in these organs compared to the control group.

Blood serves as the primary medium for the transportation of vital nutrients and foreign materials throughout the body. Consequently, the components of blood, specifically erythrocytes, leukocytes, platelets, and Hb, are subjected to elevated levels of toxins [30,31]. WBCs are fundamental to the immune system, disseminating throughout the body via blood vessels [32]. The HEIE did not cause significant changes in blood and tissue levels, suggesting that the extract is not likely to have any toxic substances that could lead to anaemia or additional defects. However, animals administered 1800 mg/kg of dose showed increased lymphocyte and neutrophil levels, suggesting the body may be responding to infection, stress, or an autoimmune condition.

HEIE showed a diminution in ALT (serum glutamate pyruvate transaminase) readings in rats administered 1800 mg/kg of dose compared to the control group, but this was not statistically significant. The study also found no changes in biochemical parameters such as AST, gamma-glutamyl transpeptidase, albumin, globulin, and total bilirubin at the high dosage [33]. This verifies that HEIE possesses hepatoprotective properties.

Renal function is assessed through changes in creatinine, urea, and glucose levels, with elevated levels indicating renal damage and filtration process dysfunction [34]. The study found that rats treated with 1800 mg/kg of HEIE showed marginal reductions in creatinine and ALP levels, but no significant changes in uric acid levels. The results suggest that HEIE does not cause adverse effects, but prolonged use may cause toxicity in essential organs.

Histopathological examinations provide corroborative data for hematological and biochemical assessments [35]. Photomicrographs of rats' brain, heart, liver, kidney, stomach, spleen, and intestinal sections showed significant histological alterations following HEIE administration at a dosage of 1800 mg/kg over 28 days. Neutrophils infiltrated deeper mucosa, indicating severe gastritis in the stomach. Chronic inflammation in the liver was observed, with the systema lymphaticum along with cells of plasma in the portal areas. Heart inflammation was characterized by the systema lymphaticum as well as macrophages in the space between cells. Hematoma in the spleen was caused by internal bleeding, and a higher number of neutrophils indicated an acute infection in the intestines. Further chronic analysis is needed to support the toxicity of *I. eriocarpa*.

CONCLUSION AND FUTURE PERSPECTIVES

This research presents significant findings regarding the acute and subacute toxicological assessments of HEIE. Given the absence of fatalities or indications of toxicity in the treated rats throughout the acute toxicity study, it can be inferred that the LD_{50} HEIE exceeds 2000 mg/kg body weight when administered orally. The findings from the subacute toxicological assessments indicate that the prolonged administration (28 days) of HEIE at the evaluated dose levels, together with the therapeutic dosage, does not result in any toxic effects in the treated rats when compared to the control group rats. The oral administration of HEIE to rats demonstrates a significant improvement in well-being and holds promise for the advancement of a novel medicinal agent. Toxicological assessments are performed to examine the safety of compounds, such as chemicals, pharmaceuticals, or food additives, and to detect possible adverse effects on humans, animals, and the environment. They facilitate regulatory compliance, conduct risk assessments, understand the impact of toxins on biological systems, and protect public health and ecosystems from hazardous exposures. Future studies should include assessments of the toxicity of bioactive compounds, as well as assessments of neurotoxicity, reprotoxicity, and genotoxicity associated with this plant. HEIE can be investigated in a broadened way to do clinical studies to treat numerous ailments for the imminent perception of the development of the international herbal market, also assessing their toxicity and ADRs. It is essential to evaluate the effectiveness and toxicologic considerations of all other conventionally utilized therapeutic plants.

AUTHOR CONTRIBUTION

Komal Manwani: Conceptualization, Methodology, Writing – Original Draft, Review and Editing. Mohd. Saiful Islam: Conceptualization,

Methodology. Mayur Porwal: Investigation, Data curation, Formal analysis, Supervision.

ACKNOWLEDGMENT

The authors convey their sincere appreciation and indebtedness to Professor Phool Chandra and Professor Mukesh Singh Sikarwar of Teerthanker Mahaveer University, Moradabad (U.P.), India, for their unwavering encouragement and assistance at the College of Pharmacy laboratories.

CONFLICTOF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

FUNDING

Not applicable.

REFERENCES

1. Monisha A, Bhuvaneshwari S, Velarul S, Sathiya Vinotha AT, Umamageswari MS, Vijayamathy A. Acute toxicity study of Arsenicum album in Wistar albino rats. Int J Pharm Pharm Sci. 2024;16(12):37-41.
2. Porwal M, Kumar A, Maheshwari KK, Khan NA. Phytochemical, pharmacological screening and importance of *Marsdenia tenacissima*: A review. IDMA. 2021 Apr 1;58(4):7-22.
3. Porwal M, Chandra P, Kumar V, Verma N, Monika K. Evaluation of the oral toxicity of *Litchi chinensis* pericarp extract in experimental rats. Orient J Chem. 2023 Dec 27;39(6):1495-509. doi: 10.13005/ojc/390609
4. Arika W, Kibiti CM, Njagi JM, Ngugi MP. *In vitro* antioxidant properties of Dichloromethanolic leaf extract of *Gnidia glauca* (Fresen.) as a promising antiobesity drug. J Evid Based Integr Med. 2019;24:1-17. doi: 10.1177/2515690X19883258, PMID 31766874
5. Li Y, Zhuang Y, Tian W, Sun L. *In vivo* acute and subacute toxicities of phenolic extract from rambutan (*Nephelium lappaceum*) peels by oral administration. Food Chem. 2020;320:126618. doi: 10.1016/j.foodchem.2020.126618, PMID 32229397
6. Burkill HM. The Useful Plants of West Tropical Africa. Vol. 2. London: Royal Botanic Gardens; 1994.
7. Akmal T, Julianti AI, Syamsudin SS. Polyherbal formulation optimization from *Clitoria ternatea*, *Rosmarinus officinalis* and *Aquilaria malaccensis* using simplex lattice design. Int J Appl Pharm. 2023;15(2):79-84. doi: 10.22159/ijap.2023.v15s2.15
8. Khalifa AA, Mohamed AA, Ibrheim ZZ, Hamoda AM. Macro- and micromorphology of the leaves, stems, seeds and fruits of *Ipomoea eriocarpa* (R. Br.) growing in Egypt. Bull Pharm Sci Assiut Univ. 2017;40:9-31.
9. Das M, Malipeddi H. Antiurolithiatic activity of ethanol leaf extract of *Ipomoea eriocarpa* against ethylene glycol-induced urolithiasis in male Wistar rats. Indian J Pharmacol. 2016 May 1;48(3):270-4. doi: 10.4103/0253-7613.182886, PMID 27298496
10. Subash V, Dhanapal R. Cerebroprotective effect of *Ipomoea eriocarpa* against cerebral ischemia in rats. Int J Exp Pharmacol. 2012;2(1):13-8.
11. Thamizhvanan K, Kumuda P, Babu S. Evaluation of antioxidant activity of whole plant of *Ipomoea eriocarpa* extract. Innov Drug Discov. 2013;2(1):2012-3.
12. Kumar KK. Evaluation of antisecretory activity of *Ipomoea eriocarpa* in albino Wistar rats. Int J Exp Pharmacol. 2012;2(1):19-25.
13. Shiva PP, Sarojini S. Antinociceptive activity of plant extracts. Int J Res Pharm Pharm. 2012;1(1):85-8.
14. Naik ND, Reddy RG, Naadella BC. A study on antipyretic activity of petroleum ether extract of *Ipomoea eriocarpa* whole Plant. Int J Pharmacol Screen Meth. 2012;2(2):58-60.
15. Das M, Malipeddi H. Phytochemical screening, GC-MS analysis and biological activitiys of *Ipomoea eriocarpa* Leaf extracts. Int J Pharm Pharm Sci. 2014;6(4):592-4.
16. Nyero A, Anywar GU, Achaye I, Malinga GM. Phytochemical composition and antioxidant activities of some wild edible plants locally consumed by rural communities in northern Uganda. Front Nutr. 2023 Apr 4;10:1070031. doi: 10.3389/fnut.2023.1070031, PMID 37081916
17. OECD. Test No. 423: Acute Oral Toxicity - Acute Toxic Class Method. OECD Guideline for Testing of Chemicals; 2002. p. 1-14.
18. OECD. Test No. 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents; 2008.
19. World Health Organization. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. Vol. 35. Switzerland: World Health Organization; 2000.
20. Das N, Goshwami D, Hasan MS, Raihan SZ. Evaluation of acute and subacute toxicity induced by methanol extract of *Terminalia citrina* leaves in Sprague Dawley rats. J Acute Dis. 2015 Oct 1;4(4):316-21. doi: 10.1016/j.joad.2015.05.001
21. Olaniyan JM, Muhammad HL, Makun HA, Busari MB, Abdullah AS. Acute and sub-acute toxicity studies of aqueous and methanol extracts of *Nelsonia campestris* in rats. J Acute Dis. 2016 Jan;5(1):62-70. doi: 10.1016/j.joad.2015.08.006
22. Yuet Ping K, Darah I, Chen Y, Sreeramanan S, Sasidharan S. Acute and subchronic toxicity study of *Euphorbia hirta* L. methanol extract in rats. BioMed Res Int. 2013;2013:182064. doi: 10.1155/2013/182064, PMID 24386634
23. Bigoniya P, Sahu T, Tiwari V. Hematological and biochemical effects of sub-chronic artesunate exposure in rats. Toxicol Rep. 2015 Jan 1;2:280-8. doi: 10.1016/j.toxrep.2015.01.007, PMID 28962361
24. Ouahchia C, Saida CH, Hamaidi-Chergui F, Marzen L, Deradji S, Hemma R, *et al*. Toxicité aiguë et subaiguë des extraits méthanoliques d'Inula viscosa L. *(Dittrichia viscos*a L.). Agrobiol. 2017 Dec 30;7(2):562-73. Available from: www.agrobiologia.net. [Last accessed:on 2025 Jul 31].
25. Benrahou K, Doudach L, El Guourrami O, Mrabti HN, Zengin G, Bouyahya A, *et al*. Acute toxicity, phenol content, antioxidant and postprandial anti-diabetic activity of *Echinops spinosus* extracts. Int J Second Metab. 2022 Mar 10;9(1):91-102.
26. Vahalia MK, Thakur KS, Nadkarni S, Sangle VD. Chronic toxicity study for Tamra Bhasma (A generic ayurvedic mineral formulation) in laboratory animals. Recent Res Sci Technol. 2011 Oct 7;3(11):2076-5061.
27. Harish BB. Toxicity studies on Ipomoea eriocarpa extract in experimental animals. Indian J Pharm Sci Res. 2011;2(1):2012-9.
28. Porwal M, Khan NA, Maheshwari KK. Evaluation of acute and subacute oral toxicity induced by ethanolic extract of marsdeniatenacissima leaves in experimental rats. Sci Pharm. 2017 Aug 21;85(3):29. doi: 10.3390/scipharm85030029, PMID 28825665
29. Raina P, Chandrasekaran CV, Deepak M, Agarwal A, Ruchika KG. Evaluation of subacute toxicity of methanolic/aqueous preparation of aerial parts of *O. sanctum* in Wistar rats: Clinical, haematological, biochemical and histopathological studies. J Ethnopharmacol. 2015 Dec 4;175:509-17. doi: 10.1016/j.jep.2015.10.015, PMID 26456329
30. Mukinda JT, Syce JA. Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. J Ethnopharmacol. 2007 May 30;112(1):138-44. doi: 10.1016/j.jep.2007.02.011, PMID 17367969
31. Li X, Luo Y, Wang L, Li Y, Shi Y, Cui Y, *et al*. Acute and subacute toxicity of ethanol extracts from *Salvia przewalskii* Maxim. in rodents. J Ethnopharmacol. 2010 Aug;131(1):110-5. doi: 10.1016/j.jep.2010.06.012, PMID 20561576
32. Dillasamola D, Aldi Y, Wahyuni FS, Rita RS, Alen Y, Dachriyanus. Study of active isolated compounds from Sungkai leaf (*Peronema canescens* jack) as immunostimulant from exposure of the SARS-COV-2 virus antigen to natural killer cells. Int J Appl Pharm. 2024;16(1):11-6. doi: 10.22159/ijap.2024.v16s1.02
33. Mrabti HN, Doudach L, Kachmar DM, Ed-Dra A, Zineb AD, Khalil Z, *et al*. Phenolic content, antibacterial, antioxidant, and toxicological investigations of *Erodium guttatum* (*Geraniaceae*) collected from the Northeast of Morocco. Turk J Botany. 2021 Jan 1;45(8):739-49.
34. Taghizadeh M, Ostad SN, Asemi Z, Mahboubi M, Hejazi S, Sharafati-Chaleshtori R, *et al*. Sub-chronic oral toxicity of *Cuminum cyminum* L.'s essential oil in female Wistar rats. Regul Toxicol Pharmacol. 2017 Aug 1;88:138-43. doi: 10.1016/j.yrtph.2017.06.007, PMID 28634146
35. Traesel GK, Menegati SE, Dos Santos AC, Carvalho Souza RI, Villas Boas GR, Justi PN, *et al*. Oral acute and subchronic toxicity studies of the oil extracted from pequi (*Caryocar brasiliense*, Camb.) pulp in rats. Food Chem Toxicol. 2016 Nov 1;97:224-31. doi: 10.1016/j.fct.2016.09.018, PMID 27639543