Applied Biological Research 27(1): 71-79; (2025)
DOI: 10.48165/abr.2025.27.01.7

ACUTE TOXICITY STUDIES OF *Cirsium wallichii* DC. AND *Galium rotundifolium* L. COLLECTED FROM THE HIGHLANDS OF KUMAUN HIMALAYAS (INDIA)

Ankit Kumar[1], Mahendra Rana[1], Satpal Singh Bisht[2], Rashi Miglani[3], Chandrakanta[1] and Nagma Parveen[3]

[1]*Department of Pharmaceutical Sciences, Sir J.C. Bose Technical Campus, Bhimtal - 263 136, Uttarakhand (India)*
[2]*Vice Chancellor, Soban Singh Jeena University, Almora – 263 601, Uttarakhand (India)*
[3]*Department of Forensic Science, D.S.B. Campus Kumaun University, Nainital, Uttarakhand - 263 002 (India)*
*****e-mail: *kumarankit412.ak@gmail.com*

(Received 21 October, 2024; accepted 26 February, 2025)

ABSTRACT

A study was conducted to assess the acute toxicity profile of *Galium rotundifolium* and *Cirsium wallichii* used @ 2000 mg kg^{-1} body weight. These plants, traditionally used in Uttarakhand (India) for liver health, were evaluated for immediate cytotoxic effects in Wistar rats. The randomized controlled trial included five female rats each in control and treatment groups, receiving the doses of 5, 50, 300, and 2000 mg kg^{-1} over a 15-day period. No visible signs of poisoning or fatalities were observed nor any haematological and biochemical parameter did show any signs of toxicity, thereby supporting the safety of these plant extracts. The oral administration of aqueous extracts from *G. rotundifolium* and *C. wallichii* appeared safe and well-tolerated in a single-dose study. However, further research is necessary to assess the potential long-term effects on organs. The extracts may possess hepato-protective, antioxidant, antibacterial, anti-inflammatory, analgesic, and anticancer properties, making them promising candidates for therapeutic applications.

Keywords: Acute toxicity, *Cirsium wallichii*, *Galium rotundifolium*, hepatoprotective, jaundice

INTRODUCTION

Ethnopharmacology provides an essential framework for studying the plant-based therapies, particularly those with historical usage in indigenous medicinal practices (Süntar, 2020; Pirintsos *et al.*, 2022). The Himalayan region, known for its rich biodiversity and diverse ethno-medicinal traditions, harbours numerous plant species with therapeutic potential (Mir *et al.*, 2021). Among them, *Cirsium wallichii* DC. (Himalayan thistle) and *Galiumrotundifolium* L. (round-leaved bedstraw) are widely used in traditional healthcare systems of Uttarakhand (India) and adjacent high-altitude regions (Bhatt *et al.*, 2013; Singh *et al.*, 2014; Tiwari *et al.*, 2020). However, despite their medicinal applications, scientific validation of their safety, particularly their acute toxicity, remains limited.

Cirsium wallichii DC, belonging to the order Asterales and family Asteraceae, is indigenous to the Himalayan region, including Uttarakhand, Nepal, and Bhutan. Traditionally, this species is employed for its purported pharmacological effects, attributed to bioactive compounds such as flavonoids, phenolic acids, sterols, saponins, lignans, and fumaric acid (Boruah *et al*., 2020; Ali *et al.*, 2023; Kozyra *et al.*, 2024). Similarly, *Galium rotundifolium* L., belonging to the order Gentianales and a member of Rubiaceae family, is a perennial herb known for its therapeutic applications in certain regions of Europe and Asia, including the Himalayas. It contains various bioactive compounds, including iridoid glycosides, flavonoids, tannins, coumarins, and possibly anthraquinones and

saponins (Yang *et al.*, 2018; Friščić *et al.*, 2018; Yasin *et al.*, 2020). While the presence of these phytochemicals indicate potential medicinal value, systematic toxicity studies are essential to ensure their safety for therapeutic use. The present study was aimed to evaluate the acute toxicity of hydro-methanolic extract of *C. wallichii* and *G. rotundifolium* in female Wistar rats. The present study provides critical insights into the safety profile of these plants, thereby supports their potential integration into evidence-based medicine. By conducting standardized acute toxicity assessments, this study seeks to bridge the gap between traditional knowledge and scientific validation, ensuring the responsible use of these medicinal plants.

MATERIALS AND METHODS

Plant sample collection and crude extract preparation

The aerial parts of *Cirsium wallichii* and leaves of *Galium rotundifolium* were collected from Pithoragarh and Almora districts in 2024 and the plant identity was authenticated by BSI Dehradun (Uttrakhand). The samples were deposited in BSI Dehradun vide vouchure No. 1376 and 1377, respectively. The plant samples were shade-dried, and coarsely powdered. The powdered material was subjected to cold maceration in 80% methanol for 72 h with occasional stirring. The extract was filtered and concentrated using a rotary evaporator under reduced pressure. The obtained crude extract was stored at 4°C till use (Nerlekar *et al.*, 2024).

Experimental animals and housing conditions

Adult female Wistar rats, each weighing between 240-260 g, were procured from IVRI Bareilly (UP, India) and acclimatized for one week under controlled laboratory conditions (temperature 22±2°C, relative humidity 50–60% and a 12 h light/dark cycle). The animals were housed in standard polypropylene cages with free access to a uniform standard laboratory diet and water *ad libitum*. The evaluation of acute toxicity was performed in compliance with the Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals (Test Guideline 420: Acute Oral Toxicity - Fixed Dose Procedure (Ridgeway, 2002). The study was approved by Institutional Ethical Committee vide No. KU/DOPS/162 dated 23 July, 2022.

Experimental design

For acute toxicity assessment, the animals were orally administered with hydromethanolic extracts of aerial parts of *C. wallichii* and *G. rotundifolium* leaves tested at dosage level (2.0 g kg^{-1} body weight) to separate groups of rats (n = 5 per group). The control group received normal saline 1 mL via oral route. The dosing was conducted after a 12 h fasting period, ensuring that food was reinstated 2 h post-administration.

Observational parameters

Following the administration of test extracts, all animals were closely monitored for immediate toxic effects and behavioural changes at 1, 2, 3 and 4 h post-dosing. Subsequently, daily observations were made for 14 days to detect any signs of delayed toxicity. Key parameters recorded were general behavioural alterations (e.g. alertness, grooming, lethargy, convulsions, or abnormal postures), signs of distress or toxicity (e.g. salivation, tremors, diarrhea, or respiratory distress), body weight measurements on 1st and 14th day to assess any significant changes, food and water intake monitoring throughout the study period and mortality assessment throughout the 14-day study period.

Hematological and biochemical analysis

The blood samples were collected for hematological and serological assessments. Prior to the sample collection, animals were subjected to overnight fasting. Blood was drawn directly from the heart using sterilized disposable syringes equipped with 22-gauge needles following anesthesia induction with

ether. The blood samples were collected in heparinized tubes for hematological analysis and in non-heparinized tubes for serological analysis. The serum was stored in clean vials at -20°C. The biochemical evaluations were done by using automated Medica EasyRA Clinical chemistry analyzer (USA). The biochemical parameters analyzed included total protein, albumin, cholesterol, triglycerides, creatinine, alkaline phosphatase, total bilirubin, direct bilirubin, glucose, serum glutamate pyruvate transaminase (SGPT), serum oxalate oxaloacetate transaminase (SGOT), lactate dehydrogenase (LDH), uric acid, and blood urea nitrogen (BUN) across all the experimental groups. Hematological evaluations comprised of total red blood cell (RBC) and white blood cell (WBC) counts, hemoglobin (Hb) concentration, packed cell volume (PCV), total erythrocyte and leukocyte counts. Estimations were made using standard kits obtained from ERBA and Medica Easy RA for hematological and biochemical analysis using a Nihon Kohden hematology analyzer (Japan) (Kumar *et al.,* 2022).

Statistical analysis

The data was statistically analysed by using analysis of variance (ANOVA) [SPSS version 20] and the results were presented as mean ± standard deviation. ANOVA was used to assess differences between the groups, with significance set at $p < 0.05$ (Hinton, 2014).

RESULTS AND DISCUSSION

Traditional and complementary medicine has long relied on medicinal plants for therapeutic purposes, yet their safety concerns persist due to limited toxicological information and inconsistent research methodologies. The absence of standardized regulations complicates the evaluation of potential risks, emphasizing the need for rigorous toxicity assessments, including acute and subacute evaluations, to establish safe preclinical doses (Kpemissi *et al.,* 2020). In present study, all the test groups animals exhibited body weight gain over 14 day's study period, with the highest increase noted in *C. wallichii* extract fed-group (41.40 ± 5.03 g), followed by control (37.20 ± 9.10 g) and *G. rotundifolium* extract fed group (28.60 ± 2.43 g) [Table 1]. The lower weight gain in *G. rotundifolium* group suggests a potential metabolic influence, warranting further studies on its effects on energy metabolism. Body weight is a crucial parameter in toxicological studies and any significant weight fluctuations indicates systemic toxicity, appetite suppression, or metabolic alterations (Malik *et al.,* 2022; Kumar *et al.,* 2022). The food and water intake remained stable across the groups with minor variations. *C. wallichii*-treated rats consistently showed highest food intake, which suggests a role in promoting feeding behaviour. In contrast, *G. rotundifolium*-treated rats exhibited less food intake comparable to control group indicating possible metabolic modulation. Water intake showed no significant differences supporting the notion that neither plant extract adversely affected hydration status.

Blood glucose levels are critical in evaluating potential anti-hyperglycemic effects of medicinal plants. The *G. rotundifolium* extract fed-group exhibited lowest glucose level (143.20 ± 0.42 mg dL^{-1}) as compared to the control (149.53 ± 0.33 mg dL^{-1}) and *C. wallichii* extract fed- group (151.53 ± 0.42 mg dL^{-1}), suggesting a potential hypoglycemic effect. These findings support the hypothesis that *G. rotundifolium* may have antidiabetic properties, aligning with previous works advocating the need for further pharmacological evaluation of medicinal plants (Kharchoufa *et al.,* 2020).

Importantly, no signs of toxicity were observed in any group. Daily clinical assessments confirmed normal physiological and behavioural activities, with no indications of distress, lethargy, or adverse reactions. These results reinforce the safety profile of *C. wallichii* and *G. rotundifolium* at the administered dose (2000 mg kg^{-1}). Standardized toxicity testing protocols are essential to ensure safety, regulatory approval, and advancement of phytomedicines (Benrahou *et al.,* 2022). Moreover, fluctuations in body and organ weight are critical biomarkers of potential toxicity, particularly in vital organs such as the liver, kidneys, and pancreas (Benrahou *et al*., 2022; Thakur *et al.,* 2024).

Table 1: Effect of *Cirsium wallichii* and *Galium rotundifolium* extracts (@ 2000 mg kg^{-1} body weight) on the body weight and food and water consumption in Wistar rats

Parameters	Control	*C. wallichii* extract	*G. rotundifolium* extract
Body weight (g)			
Day 1	181.20 ± 10.13	179.00 ± 22.07	174.60 ± 22.32
Day 7	194.20 ± 10.28	196.20 ± 19.54	191.80 ± 21.46
Day 14	218.40 ± 19.23	220.40 ± 17.04	203.20 ± 24.75
Body weight gain [day 1-14]	37.20 ± 9.10	41.40 ± 5.03	28.60 ± 2.43
Food intake (g)			
Day 1	21.71 ± 2.46	24.14 ± 0.55	21.99 ± 1.19
Day 7	21.85 ± 1.71	23.36 ± 1.33	20.43 ± 1.88
Day 14	23.84 ± 1.84	24.30 ± 0.31	24.00 ± 1.21
Average food intake	22.47 ± 2.00	23.93 ± 0.73	22.14 ± 1.42
Water intake (mL)			
Day 1	59.17 ± 2.04	59.17 ± 1.33	58.83 ± 1.83
Day 7	57.50 ± 4.18	58.83 ± 1.33	55.67 ± 3.83
Day 14	56.63 ± 4.69	58.50 ± 2.35	56.11 ± 6.86
Average water intake	57.77 ± 3.75	58.83 ± 1.67	56.87 ± 4.17
Average blood sugar level (mg dL^{-1})	149.53 ± 0.33	151.53 ± 0.42	143.20 ± 0.42
Daily observation	No toxicity	No toxicity	No toxicity

The values are average of N = 5 rats per group with standard deviation and significant difference is $p < 0.05$

The administration of *C. wallichii* and *G. rotundifolium* extracts @ 2000 mg kg^{-1} in Wistar rats did not induce any significant adverse impacts on physiological or behavioural parameters. No signs of tremors, convulsions, diarrhea, lethargy, or coma were observed, and sleep patterns remained normal. There were no indications of abnormal salivation, respiratory distress, or autonomic dysfunction. Fur condition and skin integrity were unaffected. CNS assessment showed normal somatomotor activity and behavior, with no neurotoxic effects. Additionally, circulatory function remained stable, confirming the safety of the extracts at this dosage (Table 2).

Table 2: Effect of *Cirsium wallichii* and *Galium rotundifolium* extracts (applied @ 2000 mg kg^{-1}) on general behaviour parameters in Wistar rats

Behavioural parameters	Control	*C. wallichii* extract	*G. rotundifolium* extract
Tremor	Not observed	Not observed	Not observed
Convulsions	Not recorded	Not recorded	Not recorded
Salivations	Normal	Normal	Normal
Diarrhoea	Not observed	Not observed	Not observed
Lethargy	Not observed	Not observed	Not observed
Sleep	Normal	Normal	Normal
Coma	Not observed	Not observed	Not observed
Skin	No Effect	No effect	No effect
Fur	No changes	No changes	Normal
Eyes	No effect	No effect	No effect
Mucous Membrane	No signs	No signs	No signs
Respiratory	No effect	No effect	No effect
Circulatory	Normal	Normal	Normal
Autonomic	Normal	Normal	Normal
CNS	Normal	Normal	Normal
Somatomotor activity	Normal	Normal	Normal
Behaviour pattern	Normal	Normal	Normal

Effect of plant extracts administered @ 2000 mg kg^{-1} on blood and serum parameters

Hematological indices serve as essential biomarkers for assessing physiological and pathological conditions in both humans and animals. The present study showed that total white blood cell (WBC) counts remained relatively unchanged across the test groups indicating that *C. wallichii* and *G. rotundifolium* extracts did not exert significant immunosuppressive or stimulatory effects (Table 3). This suggests that these plant extracts do not induce systemic inflammation or leukopenia, which are commonly observed in toxicological studies. Red blood cell (RBC) counts exhibited slight variations, with *C. wallichii* extract-fed group showing a minor decline in comparison to the control, while *G. rotundifolium* extract fed group remained close to the baseline. This minor decrease in RBC counts for *C. wallichii* suggests a potential mild erythropoietic suppression or transient hemodilution. Further studies including reticulocyte counts and bone marrow analysis are needed to confirm the underlying mechanism. The hemoglobin (HGB) levels followed a similar pattern, with a noticeable reduction in *C. wallichii* extract-fed group over control (Table 3). This slight reduction indicates mild anemia, possibly due to hemolysis or decreased erythropoiesis. In contrast, *G. rotundifolium* fed group showed insignificant variation o control. Hematocrit (HCT) values mirrored almost the same trends. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) did not exhibit any significant differences across the groups, indicating that erythrocyte size and hemoglobin content per cell were largely unaffected. The slight decrease in mean corpuscular hemoglobin concentration (MCHC) in both extract-treated groups suggests subtle changes in hemoglobin synthesis or erythrocyte hydration status. Platelet (PLT) counts exhibited a significant decline in *G. rotundifolium* fed group as compared to the control, suggesting a potential mild thrombocytopenic effect. In contrast, *C. wallichii* fed group remained nearly unchanged, implying that the extract did not impact platelet production or survival. The reduction in platelet count observed in *G. rotundifolium* fed group warrants further studies to explore its impact on megakaryocyte function or increased platelet turnover.

Table 3: Effect of *Cirsium wallichii* and *Galium rotundifolium* extracts (@ 2000 mg kg^{-1} bw) on hematological parameters in wistar rats

Parameters	Control	*C. wallichii* extract	*G. rotundifolium* extract
WBC (10^3 µL^{-1})	14.20 ± 1.41	13.94 ± 2.94	13.74 ± 3.43
RBC (10^6 µL^{-1})	7.47 ± 0.35	7.09 ± 0.68	7.41 ± 0.33
HGB (g dL^{-1})	15.92 ± 0.18	13.56 ± 1.33	15.46 ± 1.03
HCT (%)	42.41 ± 0.84	39.19 ± 3.56	41.47 ± 2.73
MCV (fL)	56.41 ± 2.14	55.31 ± 0.89	56.11 ± 1.51
MCH (pg)	20.14 ± 1.33	19.14 ± 0.51	19.55 ± 0.55
MCHC (g dL^{-1})	35.60 ± 0.96	34.60 ± 0.46	34.87 ± 0.14
PLT (10^3 µL^{-1})	944.70 ± 58.56	943.38 ± 61.02	824.30 ± 51.46

*WBC; white blood cell, RBC; red blood cells, HGB; hemoglobin, HCT; hematocrit, MCV; mean corpuscular volume, MCH; mean cell hemoglobin, MCHC; mean corpuscular hemoglobin concentration; PLT; Platelet counts. The values are mean ± standard deviation, n = 5. Test groups were given single oral dose of 2000 mg *C. wallichii* or *G. rotundifolium* extracts kg^{-1} bw and observed for 14 days.

Exposure to toxic agents can significantly alter hematological indices thereby affect immune function and hematopoiesis. Blood components such as leukocytes, lymphocytes, monocytes, erythrocytes, and granulocytes are highly sensitive to toxins which may lead to immune disturbances and hematological dysfunction (Benrahou *et al.*, 2022). Toxin-induced anemia can arise from hemolysis or the suppression of blood cell production, ultimately reducing overall cell counts (Wu *et al.*, 2018). Since blood plays a central role in transporting nutrients and foreign substances, its cellular elements like red and white blood cells, platelets, and hemoglobin are directly exposed to increased toxin levels, making them susceptible to damage (Kumar *et al.*, 2022; Amoateng *et al.*, 2024; Ameer *et al.*, 2025). Biochemical parameters are crucial markers in toxicology, reflecting the clinical responses to toxicants. Assessing liver and kidney function is essential to determine the toxic effects of extracts and drugs (Loha *et al.*, 2019; Mbugi *et al.*, 2025).

Liver function assessment

No significant changes in total protein and albumin levels were observed across the treated groups (Table 4) suggesting that protein synthesis and liver synthetic capacity remained unaffected. However, mild elevations were noticed in alkaline phosphatase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels following the administration of *C. wallichii* and *G. rotundifolium*. These increases suggest potential hepatic stress but are within non-toxic range, indicating that the extracts did not induce severe hepatocellular damage. The observed elevations could be due to the increased hepatic metabolism or transient liver adaptation to the bioactive compounds in the extracts. Biochemical parameters are critical indicators for assessing the safety and potential toxicity of test compounds, particularly in assessing liver and kidney function. Key biomarkers like total protein, AST, ALT and albumin provide insights into hepatic health. Elevated AST, ALT and alkaline phosphatase levels are often associated with hepatotoxicity, while higher blood urea nitrogen and creatine suggest renal dysfunction. Monitoring these biochemical markers is essential in assessing the physiological impact (Kpemissi *et al.,* 2020; Wang *et al.,* 2021; Malik *et al.,* 2022; Gemeda *et al.,* 2025).

Table 4: Effect of *Cirsium wallichii* and *Galium rotundifolium* extracts (@ 2000 mg kg^{-1} bw) on serum enzyme levels in wistar rats

Parameters	Control	*C. wallichii* extract	*G. rotundifolium* extract
Liver profile			
Total protein (g L^{-1})	157.78 ± 7.17	162.31 ± 4.80	154.51 ± 6.11
Albumin (g L^{-1})	42.48 ± 3.30	42.11 ± 1.49	42.60 ± 4.69
ALP (U L^{-1})	252.91 ± 9.98	257.81 ± 3.99	259.57 ± 5.77
AST (U L^{-1})	155.94 ± 5.07	166.09 ± 6.53	168.15 ± 3.34
ALT (U L^{-1})	46.73 ± 3.32	52.47 ± 3.56	52.55 ± 2.80
Renal profile (m mol L^{-1})			
Urea	5.72 ± 0.23	6.18 ± 0.40	6.54 ± 0.24
Uric acid	162.74 ± 3.25	197.63 ± 3.56	191.46 ± 7.25
Cardiac profile (U L^{-1})			
Creatine kinase	1094.19 ± 7.14	1041.97 ± 33.50	1051.47 ± 37.28
Lactate dehydrogenase	1466.64 ± 30.96	1192.23 ± 41.58	1141.95 ± 48.15
HBDH	132.35 ± 10.06	175.38 ± 9.69	169.00 ± 11.47
Lipid profile (m mol L^{-1})			
HDL-cholesterol	0.67 ± 0.06	0.80 ± 0.05	0.86 ± 0.04
Cholesterol	1.36 ± 0.10	1.56 ± 0.22	1.58 ± 0.07
Triglycerides	0.87 ± 0.06	0.97 ± 0.08	0.95 ± 0.04
Glucose	10.51 ± 1.04	11.17 ± 0.73	11.36 ± 0.68

*ALP: Alkaline phosphatase, ASP: Aspartate aminotransferase, ALT: Alanine transaminase, HBDH: Hydroxybutyrate dehydrogenase; HDL; High density lipoprotein The values are mean ± standard deviation, n = 5. Test groups were given single oral dose of 2000 mg *C. wallichii* or *G. rotundifolium* extracts kg^{-1}bw and observed for 14 days.

Renal function assessment

The administration of both plant extracts resulted in increased urea and uric acid levels, with *G. rotundifolium* exhibiting a more pronounced effect (Table 4). This suggests a possible impact on renal function attributable to the enhanced protein catabolism or altered renal clearance mechanisms. Despite these elevations, creatine levels remained within normal physiological limits, indicating no nephrotoxicity. Renal function is evaluated by measuring urea nitrogen and creatinine levels. An increase in these markers suggests kidney impairment. Excessive doses of extracts may harm mice organs, leading to glomerular swelling, alveolar congestion, liver damage, and partial spleen cell apoptosis (Yang *et al*., 2019; Gemeda *et al.,* 2025).

Cardiac function assessment

A slight decrease in creatine kinase was noted in both the treated groups, suggesting a potential cardio-protective effect. Conversely, lactate dehydrogenase and hydroxybutyrate dehydrogenase levels were elevated, especially in *C. wallichii* treated group. These increases indicate myocardial stress or enhanced metabolic activity in cardiac tissues. Though the elevation was not substantial to confirm cardio-toxicity, yet it emphasizes more studies to confirm any long-term cardiac impact of these extracts.

Lipid and glucose metabolism

Both the extracts induced moderate increase in HDL-cholesterol levels, suggesting a potential improvement in lipid metabolism. Total cholesterol and triglyceride levels exhibited slight increase but remained within normal physiological limits, indicating that the extracts did not significantly disrupt lipid homeostasis. Further, blood glucose levels showed a mild increase in both treated groups, suggesting potential metabolic modulation. Altered LDL and HDL levels suggest disrupted lipid metabolism, possibly affecting lipolysis and the release of free fatty acids from peripheral stores (Obakiro et al., 2021). This study revealed that *C. wallichii* and *G. rotundifolium* extracts may induce some serum biochemical markers leading to specific alterations in liver, renal, cardiac and lipid profiles.

Conclusion: The study demonstrated that *Cirsium wallichii* and *Galium rotundifolium* influence metabolic, hematological, and biochemical parameters in Wistar rats without exhibiting overt toxicity. *C. wallichii* promoted weight gain and increased food intake, suggesting its role in metabolic stimulation; whereas *G. rotundifolium* reduced body weight and lowered blood glucose levels, indicating a potential hypoglycemic effect. Hematological analysis revealed that *C. wallichii* slightly reduced RBC counts, hemoglobin levels, and hematocrit, while *G. rotundifolium* was associated with a decrease in platelet count. Biochemical assessments indicated mild alterations in hepatic, renal, and cardiac profiles, though all changes remained within non-toxic ranges. The absence of adverse physiological and behavioural effects at 2000 mg kg^{-1} supports the safety profile of both extracts. These findings suggest potential metabolic and therapeutic applications for *C. wallichii* and *G. rotundifolium* warranting further investigations to elucidate their mechanisms of action and clinical relevance.

Acknowledgement: The authors express their deep gratitude to Kumaun University and the Department of Pharmaceutical Sciences Sir J.C Bose Technical Campus Bhimtal for facilitating the resources to undertake this research/project.

Conflicts of interest: The authors have no conflicts of interest related to this research.

Authorship contributions: Ankit Kumar analysed the results and drafted the manuscript. Mahendra Rana provided technical reviews. S.S. Bisht contributed to the technical reviewed of the manuscript. Rashi Miglani helped in manuscript drafting, table formatting, and figure setting. Chandrakanta Vishwakarma conducted the experimental work, while Nagma Parveen compiled the data and assisted in manuscript drafting.

Ethical approval: The study protocol was approved by the Animal Care and Use Committee (ACUC) of the Department of Pharmaceutical Sciences, Kumaun University, Nainital (India). The ethical clearance was granted vide No. KUDOPS/162 (23/7/2022) and all the procedures adhered to institutional and international guidelines for the ethical handling of experimental animals.

REFERENCES

Ali, S., Zafar, M., Ahmad, M., Sultana, S., Khan, A., Majeed, S., *et al.*, 2023. Palynotaxonomic approach through light and scanning electron microscopy for the identification of herbaceous

Asteraceae species from Sikaram Mountain Pak-Afghan border. *Microscopy Research and Technique*, **86**(10): 1274-1297.

Ameer, K., Ali, M., Muleta, C.O., Yasothkumar, N., Balakrishnan, S., Geethanjali, K., et al., 2025. *Ziziphus hamur* Engl., a rare medicinal plant modulates cytokine and hematological levels during sub-acute toxicity studies in Wistar rats. *Indian Journal of Pharmaceutical Education and Research*, **59**(1s): s229-s242.

Amoateng, E.O., Amoateng, P., Ossei, P.P.S., Fenteng, E.A., Amponsah, I.K., Ayibor, W.G., *et al.*, 2024. An acute and sub-acute toxicological assessment of *Reissantia indica* plant extract in male Sprague-Dawley rats: Hematological, serum biochemical and histopathology. *Scientific African*, **23**: e02089. [*https://www.sciencedirect.com/science/article/pii/S2468227624000334*].

Benrahou, K., Mrabti, H.N., Assaggaf, H.M., Mortada, S., Salhi, N., Rouas, L., *et al.*, 2022. Acute and subacute toxicity studies of *Erodium guttatum* extracts by oral administration in rodents. *Toxins*, **14**(11): 735. [*https://doi.org/10.3390/toxins14110735*].

Bhatt, D., Kumar, R., Joshi, G. and Tewari, L. 2013. Indigenous uses of medicinal plants by the Vanraji tribes of Kumaun Himalaya, India. *Journal of Medicinal Plant Research*, **7**(37): 2747-2754.

Boruah, B., Kumar, A. and Das, A. 2020. *Cirsium wallichii* DC. (Asteraceae): A key nectar source of butterflies. *Journal of Threatened Taxa*, **12**(14): 17049-17056.

Friščić, M., Štibrić-Baglama, M., Milović, M., Hazler-Pilepić, K. and Maleš, Ž. 2018. Content of bioactive constituents and antioxidant potential of *Galium* L. species. *Croatica Chemica Acta*, **91**(3): 411-417.

Gemeda, H.B., Debella, A., Endale, M., Abebe, A., Mathewos, M., Habtu, W., *et al.*, 2025. Acute and sub-acute toxicity of ethanol extracts of *Hagenia abyssinica* and *Rumex abyssinicus* flowers in Swiss albino mice. *PLOS ONE*, **20**(2): e0319464. [*https://journals.plos.org/plosone/article?Id= 10.1371/journal.pone.0319464*].

Hinton, P.R., McMurray, I. and Brownlow, C. 2014. *SPSS Explained*. Routledge, London, UK. [*https://doi.org/10.4324/9781315797298*].

Kharchoufa, L., Bouhrim, M., Bencheikh, N., El Assri, S., Amirou, A., Yamani, A., *et al.*, 2020. Acute and subacute toxicity studies of the aqueous extract from *Haloxylon scoparium* Pomel (*Hammada scoparia* (Pomel)) by oral administration in rodents. *BioMed Research International*, **2020**(1): [*4020647.10.1155/2020/4020647*].

Kozyra, M., Biernasiuk, A., Gryta, E., Kozyra, P. and Malm, A. 2024. Phytochemical profiling and biological activity of the methanolic extracts of *Cirsium monspessulanum* (L.) Hill. *Chemistry & Biodiversity*, **21**(8): [*e202400944.10.1002/cbdv.202400944*].

Kpemissi, M., Metowogo, K., Melila, M., Veerapur, V.P., Negru, M., Taulescu, M., *et al.*, 2020. Acute and subchronic oral toxicity assessments of *Combretum micranthum* (Combretaceae) in Wistar rats. *Toxicology Reports*, **7**: 162-168.

Kumar, A., Kumar, B., Kumar, R., Kumar, A., Singh, M., Tiwari, V., *et al*., 2022. Acute and subacute toxicity study of ethanolic extract of *Calotropis procera* (Aiton) Dryand flower in Swiss albino mice. *Phytomedicine Plus*, **2**(2): [*100224.http://dx.doi.org/10.32859/era.20.16.1-35*].

Loha, M., Mulu, A., Abay, S.M., Ergete, W. and Geleta, B. 2019. Acute and subacute toxicity of methanol extract of *Syzygiumguineense* leaves on the histology of the liver and kidney and biochemical compositions of blood in rats. *Evidence-Based Complementary and Alternative Medicine*, **2019**(1): [*5702159. 10.1155/2019/5702159*].

Malik, M.K., Bhatt, P., Singh, J., Kaushik, R.D., Sharma, G. and Kumar, V. 2022. Preclinical safety assessment of chemically cross-linked modified mandua starch: Acute and sub-acute oral toxicity studies in Swiss albino mice. *ACS Omega*, **7**(40): 35506-35514.

Mbugi, N.O., Chacha, M., and Mbega, E. 2025. Sub-acute toxicity effects of the methanolic extract from *Searsia longipes* on the hematological, biochemical, and histopathological parameters of Wilstar albino rats. *Natural Product Communications*, **20**(2): [*1934578X251325019. https://journals.sagepub.com/doi/abs/10.1177/1934578X251325019*].

Mir, A.Y., Yaqoob, U., Hassan, M., Bashir, F., Zanit, S.B., Haq, S.M., *et al.*, 2021. Ethnopharmacology and phenology of high-altitude medicinal plants in Kashmir, Northern Himalaya. *Ethnobotany Research and Applications*, **22**: 1-15.

Nerlekar, N., Patil, P., Khot, S., Kulkarni, A., Dandge, P., Berde, A., *et al.*, 2024. Cold maceration extraction of wild fruit *Terminalia bellirica* (Gaertn.) Roxb.: exploring its bioactives for biomedical applications. *Preparative Biochemistry & Biotechnology*, **54**(7): 982-1000.

Obakiro, S.B., Kiprop, A., Kigondu, E., K'owino, I., Kiyimba, K., Drago Kato, C., *et al.*, 2021. Sub-acute toxicity effects of methanolic stem bark extract of *Entada abyssinica* on biochemical, haematological, and histopathological parameters in Wistar albino rats. *Frontiers in Pharmacology*, **12**: [*740305.10.3389/fphar.2021.740305*].

Pirintsos, S., Panagiotopoulos, A., Bariotakis, M., Daskalakis, V., Lionis, C., Sourvinos, G., et al., 2022. From traditional ethnopharmacology to modern natural drug discovery: A methodology discussion and specific examples. *Molecules*, **27**(13): 4060. [*10.3390/molecules27134060*].

Ridgway, P. 2002. Revised fixed dose procedure: OECD test guideline 420. *Rapporti Istisan*, **41**: 27-31. [*https://publ.iss.it/ITA/Items/GetPDF?uuid=a46408e5-13c8-4d3b-8bc4-b8af90b69560#page= 33*].

Singh, H., Husain, T., Agnihotri, P., Pande, P.C. and Khatoon, S. 2014. An ethnobotanical study of medicinal plants used in sacred groves of Kumaon Himalaya, Uttarakhand, India. *Journal of Ethnopharmacology*, **154**(1): 98-108.

Süntar, I. 2020. Importance of ethnopharmacological studies in drug discovery: Role of medicinal plants. *Phytochemistry Reviews*, **19**(5): 1199-1209.

Thakur, S., Kumar, V., Das, R., Sharma, V. and Mehta, D.K. 2024. Biomarkers of hepatic toxicity: An overview. *Current Therapeutic Research, Clinical and Experimental*, **100**: 100737. [*doi: 10.1016/j.curtheres.2024.100737*].

Tiwari, D., Sah, A.N., Bawari, S. and Bussmann, R.W. 2020. Ethnobotanical investigations on plants used in folk medicine by native people of Kumaun Himalayan region of India. *Ethnobotany Research and Applications*, **20**: 1-35.

Wang, Y., Njateng, G. S. S., Zhao, T., Yang, M., Wang, Y., Cao, J., *et al.*, 2021. Evaluation of acute and subacute toxicity of two different extracts from *Que Zui* tea in rats. *Efood*, **2**(2): 81-91.

Wu, Z., Ma, Y., Zhao, L., Cai, S. and Cheng, G. 2018. Acute and subchronic toxicities of the ethanol and hot-water extracts from Chinese sumac (*Rhus chinensis* Mill.) fruits by oral administration in rats. *Food and Chemical Toxicology*, **119**: 14-23.

Yang, L.E., Meng, Y., Peng, D.L., Nie, Z.L. and Sun, H. 2018. Molecular phylogeny of *Galium* L. of the tribe Rubieae (Rubiaceae) - Emphasis on Chinese species and recognition of a new genus *Pseudogalium*. *Molecular Phylogenetics and Evolution*, **126**: 221-232.

Yang, M., Wu, Z., Wang, Y., Kai, G., Njateng, G.S.S., Cai, S. *et al.*, 2019. Acute and subacute toxicity evaluation of ethanol extract from aerial parts of *Epigynum auritum* in mice. *Food and Chemical Toxicology*, **131**: [*110534.10.1016/j.fct.2019.05.042*].

Yasin, G., Ahmad, M. and Hussain, M. 2020. Pharmacological potential of plants from Himalayan region of Pakistan-assay for antioxidant indices. *Pharmacophore*, **11**(3): 82-88.