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Research Article

Acute and Sub-Acute Toxicity Studies of Ethyl Acetate Extract of Roots and Rhizomes of Rheum Webbianum Royle in Wistar Albino Rats

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Abstract. Rheum webbianum Royle, commonly referred to as Himalayan rhubarb, has been extensively utilized in traditional medicine systems such as Ayurveda and Unani. Despite its traditional and pharmacological significance, comprehensive toxicity studies are necessary to ensure its safety. This study investigates the acute and the sub-acute oral toxicity of the ethyl acetate extract of Rheum webbianum Royle in Wistar rat models. The acute toxicity assessment was performed using female rats, administered a single oral dose of 500 mg/kg and 2500 mg/kg body weight, and observed over 14 days for any toxicological manifestations. Similarly, the sub-acute toxicity study involved the administration of daily oral doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg to both male and female rats for 28 days. Throughout the experimental period, all animals were rigorously monitored for any alterations in behavior, morphology, or physiological functions, as well as for any mortality occurrences. The findings from the treated groups were systematically compared with those of the control group to evaluate potential toxic effects. Body weight was recorded weekly, and on days 15 and 29, all animals were sacrificed for the assessment of organ weights, hematological and biochemical parameters, as well as gross and microscopic pathology. No mortality or notable behavioral alterations were observed in the rats at the administered doses, during the acute and sub-acute oral toxicity evaluations. Consequently, it was established that the median lethal dose (LD50) of the plant extract, exceeds 5000 mg/kg. The biochemical parameters were found to be within normal ranges, and histopathological analysis revealed no evidence of toxicity. This indicates that the extract concentrations used in the study do not exert toxic effects on organs such as the heart, liver, kidneys, and brain. Thus, we can conclude that the root and rhizome extract of Rheum webbianum Royle is non-toxic at high doses over short periods of use. However, further research may be needed for extended use of the extract.

Keywords: *Rheum webbianum Royle*, traditional medicine, toxicity, histopathology.

INTRODUCTION

Traditional medicine and healing practices, which have been passed down through generations, are increasingly recognized as valuable treatment options throughout the world. (Chung, 2021) (Yuan, 2016) This rapid adoption is driven by factors such as accessibility, cultural acceptance, and the gaps in modern healthcare systems. (Gureje, 2015) The 2023 WHO Traditional Medicine Global Summit, highlighted the growing popularity of traditional medicine and its promising role in achieving comprehensive healthcare. These remedies frequently use natural substances, such as herbs, plants, minerals, and animal products, and are deeply rooted in the cultural beliefs of a community. (Singh J. R., 2019)The Himalayan region

in India, in particular, is recognized for its exceptional biodiversity and is home to a vast variety of medicinal plants. (Elufioye, 2024) (Chaachouay, 2024) Local communities have historically harnessed these resources to address various health issues, from respiratory ailments to digestive concerns. (Che, 2024) India's rich biodiversity has led to extensive research on its medicinal plants, e.g., *Aconitum heterophyllum* (Talreja, 2023), *Curcuma longa* (Aggarwal, 2007), *Withania somnifera* (Singh N. B., 2011), *Piper betel* (Biswas, 2022), *Zingiber officinale* (Prasad, 2015). These plants are vital in traditional medicine and are gaining interest for modern pharmacology. (Liberale, 2021) (Balkrishna, 2024) Despite these promising applications, there is a growing concern about the scientific validation of the safety, efficacy, side effects, and toxicological properties of these herbal remedies. (Ekor, 2014) As the interest in medicinal plants continues to rise, it becomes increasingly important to conduct thorough investigations into their toxicological profiles to ensure their safety and effectiveness for the consumers. (Raju, 2024) Research in this area can protect public health and enhance the acceptance of the traditional healing methods in modern medicine.

Rheum webbianum Royle, commonly referred to as Indian rhubarb or Himalayan rhubarb, is a perennial herbaceous species indigenous to the Himalayan region. (Ballabh, 2008) (Tayade, 2012) It is classified within the family Polygonaceae and exhibits a preference for high-altitude ecosystems, typically thriving at elevations between 3,000 and 4,800 meters above sea level. (Tabin S. G., 2022)The plant has long been valued in traditional medicine for its diverse therapeutic properties, including astringent, antiseptic, antidiabetic (Zargar, 2011), antiviral, antimicrobial, antitumor (Khaja, 2024), antioxidant (Ma, 2024), cytotoxic, diuretic, liver stimulant (Shakya, 2020), nephroprotective, purgative, stomachic, anti-cancer, and tonic effects (Mohtashami, 2021). It is commonly used to treat conditions like boils, wounds, bone fractures, back pain, and joint or rheumatic pain. (Yadav, 2023) The leaves of this plant are also consumed as vegetables. The medicinal benefits of *Rheum webbianum* is primarily attributed to its rich content of bioactive compounds (Bhat, 2021), including anthraquinone derivatives such as emodin, rhein, aloe-emodin, and chrysophanol (Tabin S. G., 2019), stilbenoids (resveratrol and piceatannol), anthocyanins, flavonoids (Kuljarusnont, 2024), and polyphenols. (Pandith, 2014)

The roots and rhizomes of *R. webbianum* are the most pharmacologically significant parts of the plant and are well-documented for their myriad medicinal properties. (Yang, 2024) Traditionally, it has been used in both Ayurvedic and Tibetan medicinal practices. However, there has yet to be a thorough study on the safety and potential toxicity of the plant. Thus, we aim to conduct a toxicological assessment of *R. webbianum* in Wistar rats to support its beneficial pharmacological effects.

METHODOLOGY

Collection, identification, and preparation of plant material

The plant was collected from Pehjan Shepherds Grazing Valley, Budgam, Jammu and Kashmir, India (33°58' N, 74°27' E) at an altitude of 3500 m in September 2023. The plant material was identified and authenticated by Dr. Akhtar H. Malik, Taxonomist at the Centre for Biodiversity and Taxonomy (CBT) in the Department of

Botany, University of Kashmir under voucher specimen No. 9034-KASH. The roots and rhizomes of the plant were carefully washed and then dried in the shade at room temperature. Once dried, the roots and rhizomes were ground into a fine powder and macerated with ethyl acetate. The extract was then concentrated to dryness using a rotary evaporator and freeze-dried in a lyophiliser. The solid mass was stored at 4°C for future use. The percentage yield for ethyl acetate extract was about 2.6%.

Phytochemical screening

Preliminary qualitative analysis was performed to determine the presence of various classes of phytochemicals in the different extracts of the plant. Standardized reagents and established methodologies were utilized to identify key phytoconstituents, including alkaloids, glycosides, steroids, triterpenoids, flavonoids, phenolic compounds, carbohydrates, and saponins. (William Charles Evans, 2009)

Animal Selection and Care

Healthy male and female Wistar rats (8 to 12 weeks), weighing between 100 and 200 grams, were obtained from the Indian Institute of Integrative Medicine (IIIM), Jammu. The animals were separated by sex and housed in standard plastic cages under controlled environmental conditions, with a temperature maintained at 28 ± 2 °C and relative humidity ranging from 45% to 55%. A natural 12-hour light/dark cycle was implemented to mimic their day night cycle. During the acclimatization period, the rats were individually labeled for identification and randomly assigned to either control or experimental groups. They had unrestricted access to standard laboratory chow and clean drinking water, provided ad libitum.

All experimental procedures adhered to the ethical standards set forth by the Animal Ethics Committee and were formally approved by both the University Ethics Committee and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).The use of animals in our study adhered to the 3R principle, ensuring that a minimum possible number of animals were used to achieve reliable results. (Hubrecht, 2019) For the acute toxicity assessment, three groups with a total of 15 female rats were utilized. In contrast, the sub-acute toxicity study comprised four groups, each consisting of six animals (three males and three females). In total, 39 Wistar rats were employed to evaluate the toxicity of the *R. webbianum* extract. (FVSc/VCC-9/19/286-87)

Acute oral toxicity

Acute toxicity testing was conducted in compliance with the OECD Guideline 423, incorporating minor procedural modifications. (OECD-423, 2002) The study utilized non-pregnant, nulliparous female rats, which were randomly divided into 3 groups, consisting of 5 rats each. Following an overnight fasting period, the extract of *R. webbianum* was administered via oral gavage in single doses of 500 mg/kg and 2500 mg/kg. On the other hand, the control group received an equivalent volume of distilled water for comparison. The animals were continuously monitored for 30 minutes after dose administration, followed by hourly checks for four hours and then assessments over the next 48 hours, for signs of toxicity, mortality, and behavioral

changes. Key observations encompassed variations in body weight, salivation, the occurrence of tremors and convulsions, the presence of diarrhea, and alterations in the condition of the skin, fur, eyes, and mucous membranes. Food and water intake were systematically observed. Additional monitoring for delayed signs of toxicity continued daily for 12 days. On day 14 of the trial, blood samples were collected through cardiac puncture while the animals were under anesthesia, after which they were euthanized. Body weight was recorded at the start and conclusion of the trial. Finally, the LD50 value was calculated.

Sub-acute toxicity

A sub-acute toxicity study was conducted in accordance with the 28-day repeated dose toxicity protocol as outlined in OECD Guideline 407. (OECD_407, 2008) Adult Wistar rats of both sexes were randomly assigned to four groups, each comprising six animals (three males and three females). The control group (Group I) received water, while Groups II, III, and IV were administered *Rheum webbianum* extract at doses 200, 400, and 800 mg/kg body weight, respectively, via oral gavage once daily for 28 days. The animals were closely monitored weekly for changes in body weight, behavior, physiological parameters, and any occurrence of mortality. At the conclusion of the study, all animals were euthanized following standard ethical protocols to facilitate the collection of organ and blood samples for further analysis. Organs were carefully excised, weighed, and preserved in buffered formalin for gross examination. The weight of isolated organs was calculated using the below mentioned formula:

$$\text{Relative organ weight (\%)} = \frac{organ\ weight}{body\ weight} \times 100$$

Blood Sampling, Serum Isolation, and Organ Preparation

During the study, all the animals were anesthetized with isoflurane to enable blood collection via cardiac puncture. The collected blood samples were incubated at 37 °C to facilitate coagulation, followed by centrifugation at 3000 rpm for 10 minutes at 4 °C to separate the serum. The isolated serum was subsequently utilized for biochemical analyses, including assessments of liver and kidney function as well as measurements of electrolyte levels. At the conclusion of the trial, the animals were sacrificed, and vital organs such as the liver, kidneys, brain, spleen, pancreas, heart, and lungs were excised, washed, and weighed. These organs were then preserved in 10% formalin for subsequent histopathological evaluation, allowing for a detailed examination of tissue structure and potential pathological changes.

Hematological analysis

Blood samples collected from the animals were subjected to comprehensive hematological analysis, which included the evaluation of white blood cell (WBC) count, red blood cell (RBC) count, hematocrit levels, hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count.

Biochemical analysis

Biochemical parameters like total protein, triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), urea and creatinine, were measured using commercially available diagnostic kits.

Histopathological studies

Sections of the organs were carefully placed in tissue cassettes to ensure their preservation. These cassettes underwent a rigorous twelve-hour cycle in a tissue processor, which effectively replaced the water in the tissues. The tissues were embedded in molten paraffin wax and sectioned into precise 4 µm slices using a rotary microtome. The selected sections were mounted onto slides and incubated in an oven at 58 °C overnight to ensure proper adherence and fixation. Subsequently, the slides were stained using the Harris hematoxylin and eosin (H&E) staining technique, effectively enhancing the visualization of cellular structures. Ultimately, the slides were carefully examined under a light microscope to identify any potential deformity (inflammation, necrosis, etc).

Statistical analysis

Statistical analysis was conducted using appropriate statistical tools like graph, descriptive statistics, ANOVA to evaluate group differences. The analyses were performed using GraphPad Prism software (version 9.0.0). Results are presented as the mean ± standard error of the mean (SEM), and differences were considered statistically significant at a p-value < 0.05.

RESULTS

Phytochemical analysis

The results of the qualitative phytochemical analysis are summarized in Table 1. The extracts derived from the roots and rhizomes of *Rheum webbianum Royle* revealed significant presence of flavonoids, phenols, glycosides, steroids, and triterpenoids, indicating their phytochemical richness. Conversely, alkaloids and saponins were largely absent across all the tested extracts. Flavonoids and carbohydrates were specifically detected in the ethyl acetate and methanol extracts only.

Table 1 Phytochemical analysis of different extracts of *R. webbianum Royle*

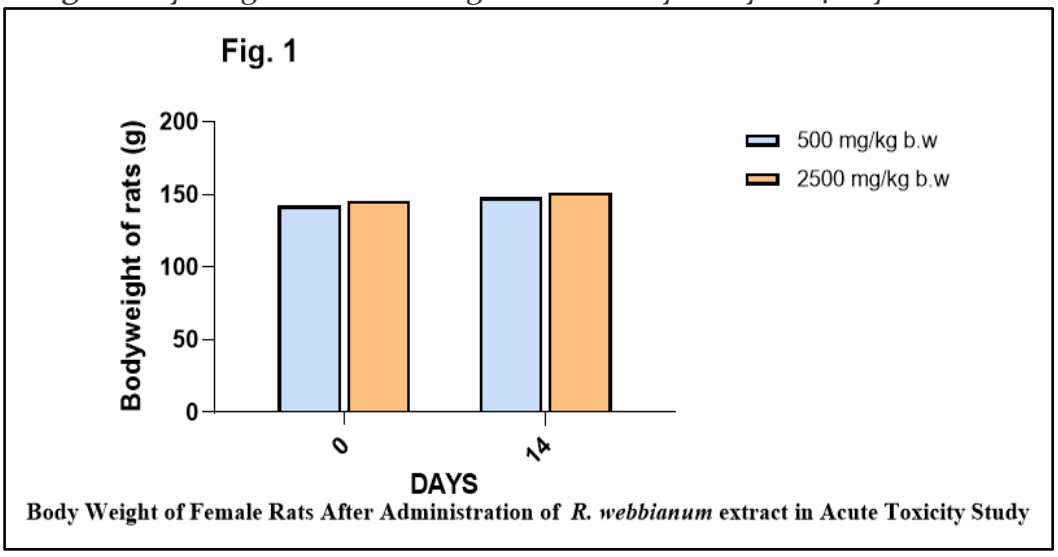
Phytoconstituents	Tests	Hexane extract	Ethyl acetate extract	Methanolic extract
Alkaloids	Dragendorff's test	-	-	-
	Wagner's test	-	-	-
	Mayer's test	-	-	-
	Hager's test	-	-	-
Glycosides	Bontrager's test	+	+	+
	Legals test	+	+	+
	Keller–Killiani test	+	+	+

Steroids and triterpenoids	Libermann-Burchard's test	+	+	+
	Salkowski's test	+	+	+
Flavonoids	Shinoda's test	-	+	+
	Alkaline reagent test	-	+	+
	Zinc HCl test	-	+	+
Phenols	Ferric chloride test	+	+	+
	Lead acetate test	+	+	+
Carbohydrates	Fehling's test	-	+	+
	Benedict's test	-	+	+
Saponins	Foaming test	-	-	-

Acute toxicity

During the acute toxicity study, mortality was not observed in any of the test animals, indicating that the plant extract is likely to have low toxicity. Both dosages tested at 500 mg/kg and 2500 mg/kg of body weight, were safe, indicating that the lethal dose of the extract may be greater than the administered doses. Furthermore, no signs of illness or toxicity were observed in the animals, reinforcing the conclusion of its safety at these dosages. Thus, it is estimated that the LD50 for the ethyl acetate extract of *R. webbianum* is above 5000 mg/kg. Also, there was no significant difference in overall weight gain between the treated groups. (Fig. 1)

Fig. 1 Body weight of rats during acute toxicity study of 14 days



Sub-acute toxicity

No mortality or toxicity signs were observed in any of the groups treated with doses of 200, 400, and 800 mg/kg over a period of four weeks. Interestingly, there was no significant difference in body weight (Fig. 2) and relative organ weight between the control group and the treated groups over the course of the study. (Table 2)

Fig. 2 Weekly body weight of rats during Sub-acute toxicity study of 28 days

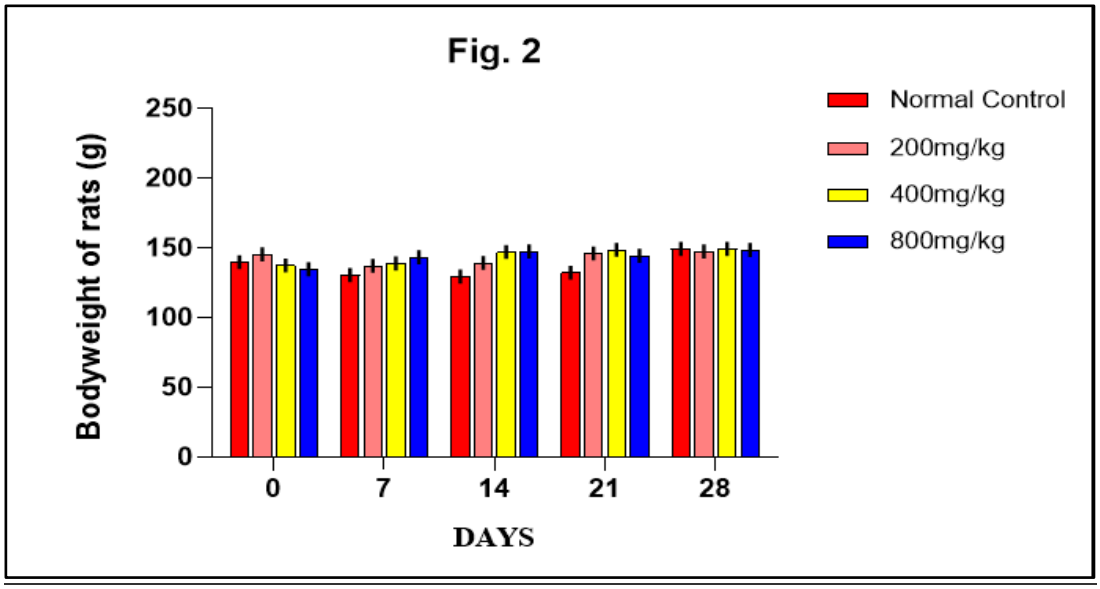


Table 2 Relative organ weight of male and female rats after 28 days of Sub-acute toxicity study

Organs	Gender	Normal control	*R. webbianum* extract dosage in mg/kg body weight		
			200	400	800
Liver	Female	4.48 ± 0.03	4.55 ± 0.15	4.43 ± 0.07	4.72 ± 0.16
	Male	3.60 ± 0.084	3.61 ± 0.138	3.51 ± 0.132	3.73 ± 0.129
Kidney	Female	0.91 ± 0.06	0.86 ± 0.04	0.90 ± 0.02	0.90 ± 0.05
	Male	0.63 ± 0.02	0.67 ± 0.01	0.77 ± 0.017	0.68 ± 0.03
Heart	Female	0.63 ± 0.02	0.61 ± 0.03	0.59 ± 0.04	0.62 ± 0.02
	Male	0.41 ± 0.019	0.43 ± 0.015	0.38 ± 0.010	0.415 ± 0.012

Brain	**Female**	0.96 ± 0.01	0.85 ± 0.08	0.94 ± 0.11	0.96 ± 0.04
	Male	0.76 ± 0.07	0.87 ± 0.07	0.75 ± 0.13	0.85 ± 0.00
Ovary	**Female**	0.20 ± 0.002	0.25 ± 0.001	0.22 ± 0.002	0.29 ± 0.001
Testis	**Male**	0.44 ± 0.022	0.45 ± 0.015	0.45 ± 0.020	0.47 ± 0.017
Lungs	**Female**	1.61 ± 0.29	2.09 ± 0.30	1.78 ± 0.24	1.82 ± 0.15
	Male	1.91 ± 0.27	1.81 ± 0.08	1.73 ± 0.19	1.71 ± 0.24

Hematological inferences

The hematological parameters in both the male and female rats treated with different doses of *R. webbianum* extract resulted in mild differences only, indicating minimal adverse effects on the vascular systems. The results are presented in Table 3.

Biochemical inferences

The serum biochemical analysis conducted during the sub-chronic toxicity study showed that the levels of key parameters such as total cholesterol (TC), triglycerides (TG), creatinine, and urea demonstrated remarkable stability in both male and female rats when compared to the control group. (Table 4)

Histopathological analysis

After 28 days of the subchronic study, tissues from vital organs such as the heart, liver, kidneys, and brain were examined under a microscope for histopathological assessment. The microscopic examination revealed no significant pathological lesions in these organs for both the control and the treatment groups. (Fig. 3 a-f)

Table 3 Hematological profile of male and female rats after 28 days of Sub-acute toxicity study

Hematological Parameters	**Gender**	**Normal control**	***R. webbianum* extract dosage in mg/kg body weight**		
			200	**400**	**800**
WBC ($10^3/\mu L$)	**Female**	5.43 ± 0.45	5.38 ± 0.32	6.45 ± 0.68	6.85 ± 0.12
	Male	6.06 ± 0.58	6.20 ± 0.64	6.84 ± 0.25	6.63± 0.38
Neutrophils	**Female**	19.32 ±	17.40±	24.80 ± 2.02	29.53± 3.59

(%)		4.62	3.40		
	Male	29.41 ± 1.78	24.47± 3.39	20.69 ± 4.27	31.03 ± 2.90
Lymphocytes (%)	**Female**	62.38 ± 2.55	79.00 ± 3.63	75.27± 2.11	75.62 ± 3.32
	Male	58.52 ± 2.90	83.24± 5.85	79.01± 3.92	62.03 ± 1.75
Monocytes (%)	**Female**	5.83 ± 0.31	2.15 ± 0.28	3.42 ± 0.18	2.31 ± 0.18
	Male	7.05 ± 0.78	6.40 ± 0.87	5.20 ± 1.32	6.20 ± 0.73
Eosinophils (%)	**Female**	3.52 ± 0.65	1.63 ± 0.45	1.38 ± 0.75	2.44 ± 0.52
	Male	1.90 ± 0.82	1.15 ± 0.65	1.70 ± 0.50	1.80 ± 0.78
Erythrocytes (10^6/µL)	**Female**	7.45 ± 0.76	8.65 ± 0.30	7.85 ± 0.61	8.90 ± 0.29
	Male	8.03 ± 0.13	7.48 ± 0.12	8.15 ± 0.19	7.85 ± 0.07
Hb (g/dL)	**Female**	13.21± 1.32	14.95 ± 0.39	14.12± 0.78	14.75± 0.55
	Male	15.31 ± 0.22	14.66 ± 0.15	15. 51± 0.37	15.27 ± 0.24
MCV (fL)	**Female**	52.48 ± 1.15	51.45 ± 0.18	53.15± 0.94	55.32 ± 0.63
	Male	59.12 ± 0.26	56.82 ± 0.43	57.81 ± 0.39	57.54 ± 0.33
MCH (pg)	**Female**	18.15 ± 0.12	17.05 ± 0.02	16.80± 0.25	17.65 ± 0.10
	Male	18.55 ± 0.13	17.56 ± 0.22	17.88 ± 0.23	17.91 ± 0.27
PLT (10^3/µL)	**Female**	1021 ± 210.4	710.5± 78.90	735.5 ± 51.02	689.3± 72.10
	Male	969.03± 49.6	989 ± 23.9	906.42 ± 20.2	839.06± 30.45
Basophils (%)	**Female**	0.0041± 0.0029	0.00 ± 0.001	0.00 ± 0.00	0.01 ± 0.00

	Male	0.0 ± 0.0	0.60 ± 0.24	0.010 ± 0.00	0.00 ± 0.00

Table 4 Biochemical profile of male and female rats after 28 days of Sub-acute toxicity study

Biochemical Parameters	**Gender**	**Normal control**	***R. webbianum* extract dosage in mg/kg body weight**		
			200	**400**	**800**
Creatinine (mg/dL)	**Female**	0.38 ± 0.04	0.30 ± 0.03	0.34 ± 0.08	0.39 ± 0.05
	Male	0.36 ± 0.04	0.32 ± 0.03	0.38 ± 0.08	0.49 ± 0.05
Urea (mg/dL)	**Female**	13.20 ± 1.40	11.15 ± 2.60	14.30 ± 0.47	13.60 ± 4.41
	Male	12.80 ± 1.32	10.90 ± 2.48	13.75 ± 0.52	14.10 ± 4.30
LDL (mg/dL)	**Female**	42.85 ± 2.60	39.20 ± 2.90	45.45 ± 3.22	38.57 ± 3.58
	Male	41.75 ± 2.45	40.30 ± 2.85	44.60 ± 3.10	39.50 ± 3.40
HDL (mg/dL)	**Female**	46.6 ± 2.38	57.6 ± 6.92	45.4 ± 1.22	57.8 ± 4.39
	Male	47.42 ± 5.42	55.64 ± 4.17	58.70 ± 2.42	59.00 ± 5 .22
Triglycerides (mg/dL)	**Female**	213.50 ± 4.80	250.00 ± 7.00	242.30 ± 7.22	229.30 ± 3.18
	Male	220.50 ± 5.00	245.00 ± 6.50	235.30 ± 7.10	230.00 ± 4.00
Total Protein (g/dL)	**Female**	6.80 ± 0.15	6.90 ± 0.30	6.38 ± 0.09	6.93 ± 0.56
	Male	6.75 ± 0.12	6.87 ± 0.25	6.45 ± 0.08	6.95 ± 0.50

Fig. 3 Histopathological examination of rat organs following Sub-acute treatment with *R. webbianum* extract (H&E:10X, 20X and 40X). (a) Heart; (b) Liver; (c) Kidney; (d) Lung; (e) Brain; (f) Intestine

Fig-3 a: Photomicrograph of **heart** of a normal control	**Fig-3 a1:** Photomicrograph of **heart** revealing normal histological structures of myocardial fibers
Fig-3 b: Photomicrograph of **liver** of a normal control	**Fig-3 b1:** Photomicrograph of **liver** revealing normal architectural details of hepatocytes within the liver
Fig-3 c: Photomicrograph of **kidney** of a normal control	**Fig-3 c1:** Photomicrograph of **kidney** revealing normal morphological structure of glomerulus within the kidney
Fig-3 d: Photomicrograph showing typical histological structure of **lung**s of a normal control	**Fig-3 d1:** Photomicrograph showing typical histological structure of **lung**s
Fig-3 e: Photomicrograph of **Brain** of a normal control	**Fig-3 e1:** Photomicrograph of **Brain** with distinct clusters of neurons that regulate various bodily functions
Fig-3 e: Photomicrograph of **Intestines** of a normal control	**Fig-3 f1:** Photomicrograph of **Intestine** suggesting normal architectural details of tunica muscularis (Green arrow) & intestinal glands (yellow arrow)

DISCUSSION

Although herbal medicines are more commonly utilized in developing countries, there is a widespread perception that these natural remedies are inherently safe. However, toxicological evaluations have demonstrated that not all herbal products are free from adverse effects. (Korth, 2014) (Valdivia-Correa, 2016) The current study evaluated the potential acute and subchronic toxicological effects of the ethyl acetate extract of *R. webbianum* in Wistar rats. The findings indicated that the extract exhibits a favorable safety profile, as no mortality or adverse behavioral changes were observed even at a dose of 2500 mg/kg. The LD50 value was estimated to be greater than 5000 mg/kg, indicating it as a promising candidate for potential therapeutic use.

In toxicity studies, the overall health and metabolic status of the test animals is reflected by their body weight. Maintenance of normal body weight levels in the treated groups suggest that the extract did not induce any systemic toxicity or disrupt metabolic functions. (Ramsingh, 2010)Throughout this study, no significant deviations in body weight or behavioral patterns were observed in the treated animals compared to the control group. Similarly, the organ weight changes are often a sensitive indicator of organ-specific toxicity. During the sub-acute study of 28 days, the relative organ weight of the organs like liver, kidneys, brain, heart, and lungs, did not show any significant variation.

Hematological analysis revealed no significant alterations in red blood cell counts, white blood cell counts, hemoglobin, or platelet parameters, indicating that the extract did not interfere with normal hematopoiesis or immune function. Similarly, biochemical parameters remained within normal physiological ranges across all treatment groups. These findings suggest the absence of hepatotoxicity or nephrotoxicity, which are common concerns in toxicity evaluations. (Xiaofen Xu, 2020) Histopathological examination provided additional confirmation of the extract's safety profile. All vital organs appeared structurally normal, with no observable treatment-related tissue damage.

The findings of this study demonstrate that the extract exhibits low acute and sub-acute toxicity profiles, making it a promising candidate for further pharmacological investigations. However, several limitations must be acknowledged. The relatively short duration of exposure limits the ability to draw definitive conclusions regarding long-term toxicity, reproductive effects, or genotoxic potential.

CONCLUSION

In summary, the results of this study demonstrate that the ethyl acetate extract of *R. webbianum* is relatively safe under both acute and sub-acute toxicity conditions. The absence of mortality, adverse effects on body weight, hematological and biochemical parameters, organ weights, and histopathology, suggests that the extract is well-tolerated in experimental models. These findings align with the historical use of *R. webbianum* in traditional medicine and indicate its potential as a safe natural product for therapeutic development. Further studies are essential to explore its long-term safety and pharmacological potential in higher models, paving the way for its integration into clinical applications.

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Credit authorship contribution statement:

Aneequa Rafiquee: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Wajid Mohammad Sheikh, Shazia Javed, Shafia Bashir:** Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Mubashir Hussain Masoodi, Mohammad Younis Dar:** Supervision Project administration, Conceptualization, Review & editing, Formal analysis. Data curation. **Majid Shafi, Showkeen Muzamil Bashir:** Review & editing, Data curation.

Bilal Ahmad Bhat: Statistical Analysis

Declaration of competing 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.

Data availability: Data will be made available on request.

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