

Acute Toxicity Evaluation of Methanolic Peel Extract of Radish (*Raphanus sativus* L.) in Swiss Albino Mice

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Keywords: *Raphanus sativus*, Toxicity profiling, Diabetes, Biochemical analysis, Hematological analysis, Histopathology

Received on 3 Oct 2025

Accepted on 5 Nov 2025

Published on 10 Nov 2025

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Abstract

Raphanus sativus L., (radish) has been utilized to treat various diseases, and possess significant antidiabetic properties. The present work aimed to conduct the acute toxicity studies in Swiss albino mice to evaluate the short-term detrimental effects of the methanolic peel extract of *R. sativus*. For this purpose, Swiss albino mice were provided with the methanolic extract of *R. sativus* peel, that was administered orally in a single dose of 2000 mg/kg of body weight and the mice kept under observation for the eight consecutive days. At the end of trial, mice were dissected and their blood was collected for different biochemical (ALT, AST, ALP, Uric acid, Urea, Creatinine, LDL, And HDL) and hematological parameters (RBCs, MCV, WBCs, Hematocrit, Platelets, and Hemoglobin) and some major organs (liver, kidneys, spleen, lungs, and heart) were surgically collected for histopathological

examination. It was concluded that oral administration of the methanolic peel extract of *R. sativus* had no detrimental effects on biochemistry and hematology of the mice. Moreover, the histopathological examination revealed non-toxic effects on major organs of mice, suggesting that methanolic peel extract of *R. sativus* is safe and can be used as an alternative therapy for various ailments.

Introduction

Human civilization has relied on flora throughout history. For thousands of years, people have used plants for medicine. Using herbal plants to cure and prevent illness is crucial (Shakya, 2016). This interaction between plants and man has evolved and most plants came to be utilized as medicines. Phytomedicine or herbal medicine is the medical and therapeutic use of plants in curing and improving human health (Yadav *et al.*, 2016). Radish (*Raphanus sativus*) is a flowering plant that belongs to the mustard family and it was domesticated in Asia initially (Nishio and Kitashiba, 2017). Multi-

nutritional medicinal properties are exhibited by different portions of radish fruit such as roots, seeds, and leaves. The radish seeds have been applied to treat asthma and other chest complaints. Radish oil has been known to possess numerous varying health benefits as is the case with the radishes that are usually eaten in salads (Waheed *et al.*, 2019). Studies indicate that the antidiabetic properties of radish may stem from its ability to enhance antioxidant defenses and mitigate oxidative stress (Banihani *et al.*, 2017).

The main aim of *in vitro* and *in vivo* toxicity profiling of the methanolic *R. sativus* peel extract is to determine its safety margin and therapeutic window before the pharmacological assessment (Saha *et al.*, 2025). Profiling of this type is essential to pharmacological valorization of *R. sativus* peel as a good source of bioactive flavonoid and terpenes by defining dose-dependent biological responses and toxicity (Geng *et al.*, 2025). Median lethal dose (LD50) and safe dosage ranges can be determined by acute toxicity testing in Swiss albino mice and mechanistic data on the cellular-level cytotoxicity can be obtained by *in vitro* testing, such as the MTT cell viability assay (Riaz *et al.*, 2023). This is a combinatorial strategy that will also be used to determine the efficacy of subsequent studies such as antioxidant, hepatoprotective, or anti-inflammatory efficacy at non-toxic concentrations, thus verifying the traditional application of peel and avoiding the negative effects (Sarwari *et al.*, 2019). *R. sativus* is a common traditional medicinal plant in Morocco which is used to treat different kidney ailments like nephropathy (Zrouri *et al.*, 2021). *R. sativus* oil in diabetic male rats was also investigated in terms of its therapeutic effect. The effect of this natural material on the pancreas, liver, and kidneys of diabetic rats was studied by histopathology, biochemical parameters, and immunohistochemistry (Abed *et al.*, 2015). Consistent with the biochemical studies performed, the histopathological findings provide further evidence that *R. sativus* juices have hepatoprotective and nephroprotective effects (Islam *et al.*, 2014). This study therefore is aimed at providing information on the effects of the methanolic leaf extract of *R. sativus* on biochemistry, hematology and histopathology of Swiss albino mice.

2. Materials and methods

2.1. Collection of plant material

Fresh vegetables (*Raphanus sativus*) were purchased from the local market and a peeler was used for the removal of peels.

2.2. Preparation of methanolic extract of *Raphanus sativus* peel

Fresh peels of the *R. sativus* were thoroughly cleaned with tap water. Then shade dried under room temperature. After that, an electric grinder was used to grind them to make a coarse powder and was sieved to separate large particles and kept in a locked plastic bag for further use. A 50 g peel powder was dissolved in 75% methanol at 1 g: 10mL, then let run in a conical flask on an orbital shaker at 24 °C with the rotational speed of 250 rpm for 72 hours. The mixture was filtered using Whatman filter paper No. 1 and exposed to heat at 40°C using an incubator to eliminate water. The dry extract was refrigerated at 4 °C for later use (Nauroze *et al.*, 2023).

2.3. Toxicity profiling in mice model

2.3.1. Maintenance of animals

The animals used in this study were Swiss albino mice, both male and female, weighing 20-30 g and aged between 5 and 6 weeks. They were obtained from the University of Veterinary and Animal Sciences, Lahore, Pakistan. Animal handling and care were all done according to the requirements of the University of Okara Ethical Research Committee (Punjab, Pakistan). The mice were kept in the control conditions (23-25°C, 12 h/ 12 h light/ dark cycle) and were allowed to freely access to the normal pellet food

and water. A week was allowed to them to adapt to the conditions of laboratories before the trial began

2.3.2. Ethical approval

The Ethical Research Committee, University of Okara, Punjab, Pakistan gives ethical clearance under the letter No. UO/ERC/2024/Z13, dated: 22-12-2023, before this experimental animal study could be conducted.

2.3.3. Measurement of body weight

Prior to the commencement of the experiment, the body weights of all mice were measured separately using electric weighing balance. The weight of all the control and treatment groups was measured daily at the exposure period. The doses of mice were all done based on average weight of mice.

2.4. Acute toxicity study

Swiss albino mice were then separated into two groups consisting of 5 mice each. The treatment group of mice was administered with a 2000 mg/kg body weight of methanolic peel extract of *Raphanus sativus* through oral gavage. The control group in the experiment that fed the mice on regular food and water. The first 24 hours were observed keenly on the abnormal behavior and general signs of toxicity in the animals after being administered the dosage. The observations were then done appropriately over a period of 8 days. The mice were required to starve one night at the culmination of the experiment and then subjected to dissection. Mice were put under general anesthesia with the use of chloroform and blood was taken out through cardiac puncture. Blood was gathered in two distinct blood tubes one of them was used to carry out serum and the other in the hematological studies (Kanwal *et al.*, 2022).

2.4.1. Biochemical analysis

After incubating the samples of blood at room temperature between 5 and 10 minutes, the plasma and serum were centrifuged at 3000 rpm in the regular evacuated biochemical tubes to separate the plasma and serum of the blood. Some of the biochemical parameters that were assessed using commercially available diagnostic kits included Serum samples of mice; Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Urea, Uric acid, Creatinine, low-density lipoprotein (LDL), and High-density lipoprotein (HDL) (Nauroze *et al.*, 2023).

2.4.2. Hematological analysis

In the case of hematological analysis, the blood samples of the mice were stored in EDTA-filled tubes. Hemolytic samples were assessed using an automatic hematology analyzer (Coulter counter S-plus VI) as the total number of white blood cells (WBCs), platelets (PLT), red blood cells (RBCs), mean corpuscular volume (MCV), hematocrit, and hemoglobin (Hb) (Nauroze *et al.*, 2023).

2.4.3. Histopathological examination

The liver, kidneys, spleen, lungs, and heart were removed by surgery and treated with phosphate buffer saline during dissection to clear off debris. Subsequently, the weight of each organ was measured using an analytical balance and represented in grams. These organs were then incubated in a new solution of 10 per cent formalin (pH 7.4) in 24 hrs. This was followed by dehydration of the spleen, kidneys and liver in increasing levels (70, 90 and 100 percent alcohol) followed by the alcohol being eliminated using xylene and then fixed in paraffin wax at 58-60 °C. Blocks were prepared and sections of 5 mm were cut with the help of a microtome. All tissue sections were stained by hematoxylin and eosin (H&E). The stained sections were examined in a

photomicroscope using different magnifications (Kanwal *et al.*, 2022).

2.5. Statistical analysis

The findings were in a tabular form of means with a standard error of the mean. The data were assessed by Graph pad Prism (version 5), t-test. The values were taken as statistically significant with P value below 0.05.

3. Results

All mice lived for a duration of eight days after getting a single oral administration (2000mg/kg body weight) of the methanolic peel extract. Both control and treated groups were monitored carefully. There was no significant change in different behavioral patterns (Table 1). Body weight of both control and treated group was recorded daily. Table (2) shows that the control and treatment groups had no significant difference in their body weight. Mice organs (kidneys, liver, spleen, heart, and lungs) were surgically removed after dissection. These organs did not alter in size, shape, color, or composition. No statistically significant difference existed between the organs weight of control and treatment groups (Table 3). The mice's blood biochemical profiles showed no significant variations in AST, ALT, ALP, Creatinine, Uric acid, Urea, HDL, and LDL (Figure 1). There was no significant effect of methanolic peel extract of *R. sativus* on the level of RBCs, WBCs, PLT, MCV, Hb, and Hematocrit in mice's blood (Figure 2). The histopathological examination of the liver, heart, spleen, kidneys, and lungs of dissected mice was carried out to ascertain the toxicity of plant extract. The histopathology of vital organs revealed no damage, indicating that these organs were unharmed by the methanolic *R. sativus* peel extract (Figure 3).

Table 1. The Behavioral pattern of extract-treated mice observed during acute toxicity study.

Sr. No.	Parameters	Swiss albino mice	
		Control group	Treated group
1.	Convulsions	Not Found	Not Found
2.	Salivation	Not Found	Not Found
3.	Lethargy	Not Found	Not Found
4.	Lacrimation	Not Found	Not Found
5.	Drowsiness	Not Found	Not Found
6.	Nasal bleeding	Not Found	Not Found
7.	Food consumption	Normal	Normal
8.	Water consumption	Normal	Normal
9.	Body weight	Normal	Normal
10.	Mortality	Not Found	Not Found

Table 2. Body weight of control and treated group of mice.

Days	Body weight (g) of Swiss albino mice	
	Control group	Treated group
Before Dose	24.6 ± 1.5	24.1 ± 1.3
After Dose		
Day 1	23.5 ± 1.5	23.8 ± 1.2
Day 2	24.0 ± 1.8	24.3 ± 1.5
Day 3	24.4 ± 1.5	24.2 ± 1.2
Day 4	24.2 ± 1.5	24.2 ± 1.3
Day 5	24.9 ± 1.1	24.0 ± 1.1
Day 6	24.5 ± 1.0	23.0 ± 0.9
Day 7	24.9 ± 1.0	23.4 ± 1.2

Values are articulated as Mean ± SEM. Values are the mean of five replicates.

Table 3. Organ weight of control and treated group of mice.

Sr. No.	Organs	Control group mice	Treated group mice
1.	Kidney	0.3 ± 0.0	0.3 ± 0.0
2.	Liver	1.5 ± 0.2	1.5 ± 0.2
3.	Spleen	0.2 ± 0.1	0.4 ± 0.2
4.	Heart	0.1 ± 0.0	0.2 ± 0.1
5.	Lungs	0.2 ± 0.0	0.2 ± 0.0

Values are articulated as Mean ± SEM. Values are the mean of five replicates.

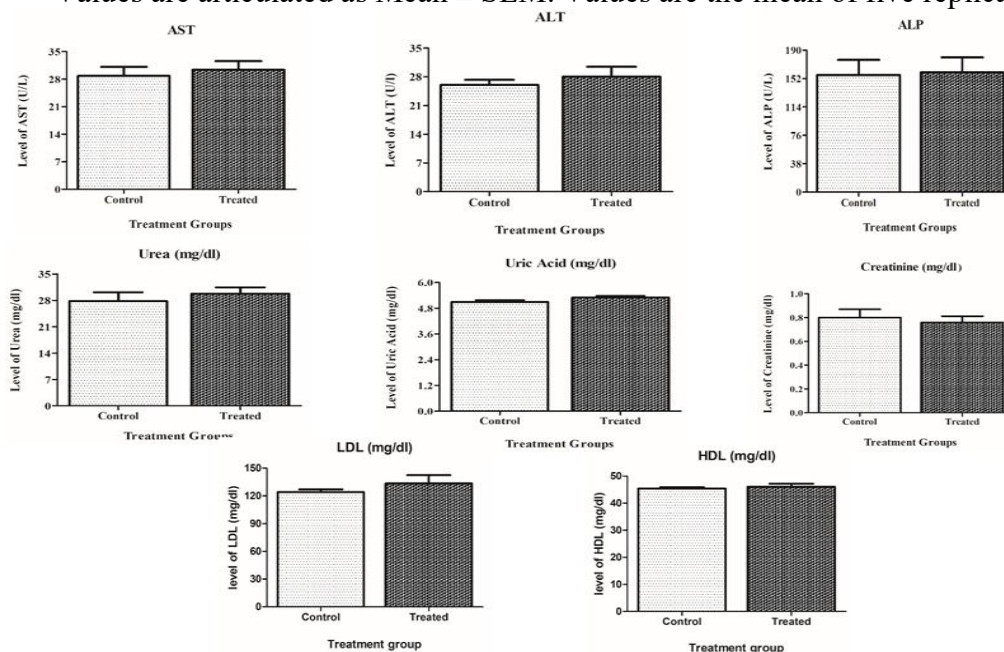


Figure 1. Effect of methanolic peel extract of *Raphanus sativus* on the level of AST, ALT, ALP, Urea, Uric acid, Creatinine, LDL, and HDL of Swiss albino mice in acute toxicity study.

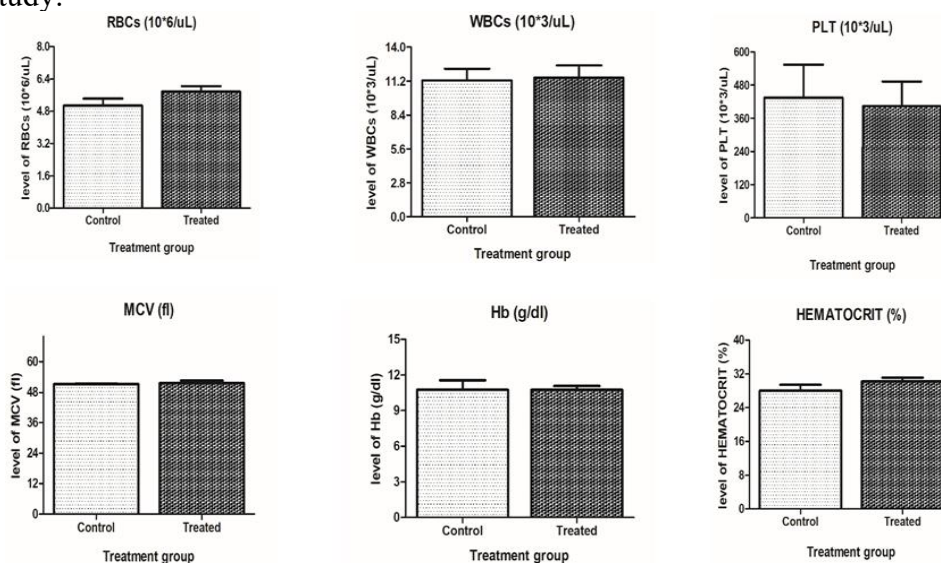


Figure 2. Effect of methanolic peel extract of *Raphanus sativus* on the level of RBCs, WBCs, PLT, MCV, Hb, and Hematocrit of Swiss albino mice in acute toxicity study.

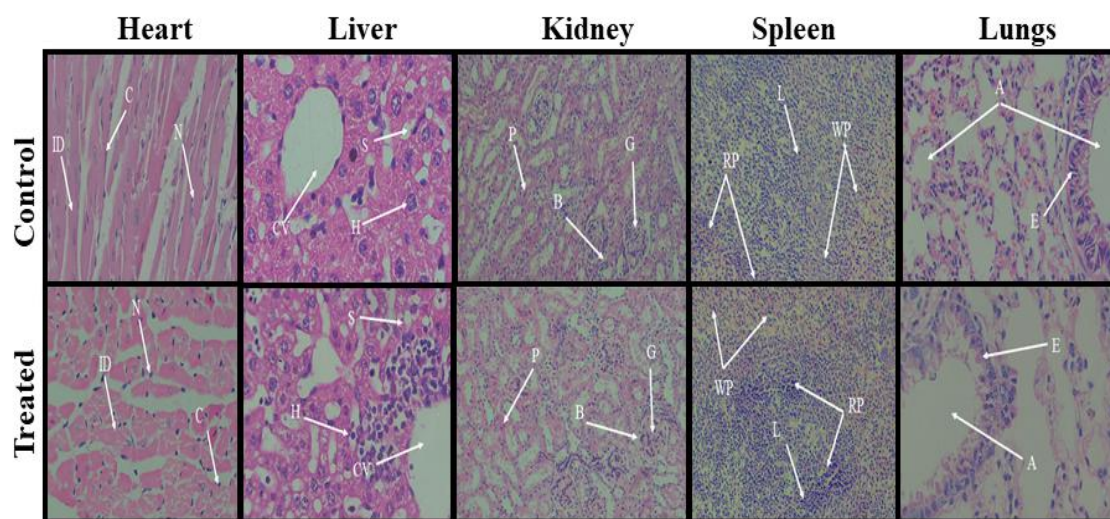


Figure 3: Histopathological comparison of vital organs in control versus *Raphanus sativus* peel-extract treated mice. Hematoxylin Eosin-stained sections were examined at 10 x and 40 x. Abbreviations: ‘C’ for Capillary, ‘N’ for Nuclei, ‘ID’ for Intercalated disc ‘H’ for Hepatocytes, ‘S’ for Sinusoidal cells, ‘CV’ for Central vein ‘G’ for Glomerulus, ‘B’ for Bowman’s space, ‘P’ for Podocytes ‘WP’ for White pulp, ‘RP’ for Red pulp, ‘L’ for Lymphocytes ‘E’ for Epithelium, ‘A’ for Alveoli.

4. Discussion

The previous study conducted by Ur Rehman *et al.*, (2021) suggested that medicinal plants (MPs) and their plant extracts (PEs) are applicable in the treatment of many diseases. These illnesses are present in both plant and human pathology. People generally think of herbs and plants as good things that can be used for healing and restoring health. Our works also explains the importance of medicinal plants. We also suggested that plants have commercial as well as medicinal importance.

Generally referred to as "radish," *Raphanus sativus* is a root vegetable of Brassicaceae family. Among other healthful components, they abound in potassium, vitamins C and B, and others. We also noted that radishes contain many vitamins, phytochemicals, and antioxidants that help treat and eliminate many ailments (Ahmad *et al.*, 2012).

Tomson *et al.*, (2024) suggested that the peel of the radish plant, *R. sativus*, is standardized by a solid-liquid extraction method in the context of methanolic extraction. Peels that have been crushed, cleaned, and dried in the shade to keep the thermolabile parts of the peels. When the product is evaporated in methanol, phytochemicals can be taken out of it. We also make methanolic extract under standardized conditions and analyze the toxicity profiling in Swiss Albino mice.

Usually known as radish, *R. sativus* has tremendous use in medicine. Among the bioactive molecules in the plant, many anthocyanins, glucosinolates, and flavonoids with anti-inflammatory, antibacterial, and antioxidant action exist. As earlier scientists saw that radish has many biological activities, we also noticed that in our plant radish has antioxidant, anti-inflammatory, antibacterial, antifungal, and many other biological activities (Ahmad *et al.*, 2012).

One of the main signs of diabetes is having high blood sugar levels all the time, which is a metabolic disorder. If we don't treat diabetes, it can harm a number of organ systems over time. People with diabetes are far more likely to suffer heart disease, strokes, heart attacks, and angina. This is a big reason to be worried. Banihani, (2017) observed that radishes have a lot of beneficial substances in them, such as anthocyanins, flavonoids, and glucosinolates. These chemicals help lower blood sugar levels by making insulin work better and lowering oxidative stress. Radishes are a good source of these things. We also noted that *R. sativus* plays an important role in controlling diabetes because it

contains many antioxidants.

In agreement to this study, previous research conducted by Zrouri *et al.*, (2021) concluded that biochemical analysis after 28 days of administering *R. sativus* methanolic peel extract orally at two dosages of 100 and 200 mg/kg body weight did not show sub-acute toxicity and physiological impairment, compared to treatment.

The effects of radish and leek (leaves and roots) juices on blood parameters were examined in a study that included both healthy and dimethoate-treated groups. Significant decreases ($P < 0.05$) were noted in HCT, Hb concentration, RBCS count, MCH, MCV, and MCHC in the dimethoate group (Moram *et al.*, 2015). The potential benefits of *Raphanus sativus*, or radish, in promoting blood health are revealed by hematological analysis. We investigated the same hematological parameters as used in the previous study and found no definite variations in their levels.

Islam *et al.*, (2014) used biochemical parameters and histopathology to evaluate the effects of this natural material on the major organs of mice. Our works also based on histopathological examination of major organs (Kidney, spleen, liver, lungs, and heart) which shows that all the vital organs remain unaffected during the toxicity analysis.

5. Conclusion

Our study investigated that the methanolic peel extract of *Raphanus sativus* has a positive safety profile in the studied conditions. There were no significant changes in either hematological indices or serum biochemical indices measured in acute administration of the extract in Swiss albino mice in relation to control group indicating no hepatotoxicity, nephrotoxicity, or hematopoietic disruption. Moreover, the histopathological analysis showed that there were no morphological aberrations of the major organs as a result of the treatment that supports the biocompatibility of the extract. The present findings support traditional usage of *R. sativus* peel and place it as a promising source of development of novel nutraceuticals or functional food ingredients. Further researches are justified to investigate its therapeutic potential, especially in the context of metabolic and oxidative stress-related pathology.

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