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Studies on Pharmacological and Toxicological Aspects of Trianthema Portulacastrum in Rats

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ABSTRACT
The study was taken up to evaluate the pharmacological and toxicological property of aerial parts of the plant T. portulacastrum. Proteins, alkaloids, saponins, flavonoids, phenolic compounds, tannins, phytosterols, and triterpenoids were all identified through phytochemical study. Carrageenan-induced paw oedema was used in the study to assess the plant extract's in vivo anti-inflammatory properties. The extract exhibits considerable anti-inflammatory action at doses of 400 and 600 mg/kg. Acetic acid-induced writhing was used as a peripheral pain model for testing in vivo analgesic effectiveness, and the extract at dosages of 400 and 600 mg/kg demonstrated notable analgesic activity.According to OECD guidelines 423 and 407, respectively, acute oral and repeated dosage 28-day oral toxicity tests of methanol extract were conducted in Wistar albino rats. doses of 500, 1000, and 1500 mg/kg were given daily for 28 days to rats with subacute oral toxicity and compared to control rats. No mortality or toxicity indications were seen in either of the toxicity studies. More than 5000 mg/kg was discovered to be the MTD value. These results are consistent with the observed histopathological features in the groups treated with extract. Haematological and biochemical parameters did not exhibit any appreciable changes. The aerial parts of the plant showed significant anti-inflammatory and analgesic property that can be utilized to explore the therapeutic efficacy.

Key words:T. portulacastrum, phytochemical analysis, Anti-inflammatory activity, Toxicity study, Wistar albino rats.

I. INTRODUCTION

Compounds found in natural products have served as either templates or specific agents for the treatment of several different types of diseases. It has been reported that approximately 50% of approved drugs since 1994 were based on natural products [1].Today, use of natural products is still prevalent in traditional and folkloric systems of medicine worldwide, particularly in developing countries where access to modern therapies may be challenging or expensive [2].

Specifically, India is considered as one of the largest producers of medicinal herbs, where 2500 species of plants known to have medicinal properties are found, and 150 of which are harvested for commercial use on a grand scale. India is also one of the countries that produces large amounts of herbal raw materials [3].

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Plant-based medicines have been used by mankind since time immemorial. The earliest record of medicinal use of plants in Hindu culture is found in "Rig-Veda", which dates to 4500-1600 BC and is one of the oldest repositories of human knowledge .Currently, almost 80% of people use herbal medicine for some part of basic healthcare. In addition, the WHO stated that of 122 pharmaceuticals originating from plants, nearly 80% are utilised in modern medicine, directly correlating with their traditional applications as plant medicines by indigenous societies.[4].

All known types of agro-climatic, ecologic, and edaphic conditions are met within India. India is rich in all the three levels of biodiversity such as species diversity, genetic diversity, and habitat diversity due to its unique biogeographic position

The acquired knowledge passed from generation to generation for centuries, with the use of medicinal plants is closely linked to the day-to-day life. There was a decline in the use of natural products at the expense of the drugs produced synthetically, especially after the second world war. Since 1990's, the use of herbal medicines has increased considerably, and even with the advancement of allopathic medicine, population's practical need continued to make use of medicinal plants due to the high cost and difficulties in access to allopathic medical care. There is also the fact that popular knowledge, in many cases, has already been scientifically proven, thus legitimizing the effectiveness of the use of plants in therapeutics [5].

The increasing demand for safer and cost-effective herbal medicines in the present world has led to the extraction and development of several drugs and chemotherapeutic agents from plants which are our traditional herbal remedies [6]. Plants are nowadays described as the sleeping giant of the pharmaceutical industry [7], which when fully exploited will provide novel compounds to fight many infectious diseases [8].

One of the countries with a wealth of traditional medical systems and a diverse ecosystem to support the herbal demands of traditional medical systems' treatments is India.The recognized Indian systems of medicine are Ayurveda, Siddha, and Unani, which use herbs and minerals in the formulations [9], but it is mandatory to prove traditional concepts scientifically in the laboratory. Researchers have been working to identify and validate plant-derived compounds for the treatment of numerous ailments during the past few years. It's interesting to note that over 25% of current medicines are thought to be derived either directly or indirectly from plants[10]. The global demand for herbal medicine is not only large, but also growing. Nowadays, collection of medicinal plants from forest is very difficult due to government's forest policy, rapid loss of diversity of plants, natural habitats, traditional community life, cultural diversity, and knowledge of medicinal plants. To solve this problem weeds may provide medicines with low cost, more potential, and without adverse side effects

Bishkhapra [Trianthema portulacastrum Linn.], belonging to the family Aizoaceae, is one of the common weeds, which has enormous traditional uses against diseases and some bioactive compounds have been isolated from this weed [11].

Many suspected cases of cattle suffered with the clinical signs of hemogalactia due to the consumption of the plant T. portulacastrum in various villages of Hassan district, Karnataka state, India. There are also a number of further probable toxicology occurrences in various regions of the state of Karnataka. Therefore, it was necessary to investigate how hazardous T. portulacastrum's aerial portions were. In addition to their toxicity, the leaves of the plant are said to have numerous medical uses in folklore. The pharmacological confirmation of the plant's reputed medicinal capabilities will make it easier to treat a variety of animal illnesses with it. Therefore, the following goals were pursued in the current investigation, which examined the pharmacological and toxicological effects of a methanol extract of T. portulacastrum's aerial parts in rats:To conduct the toxicity evaluation of methanol extract of aerial parts of the plant T. portulacastrum in rats.

1. To correlate the findings with gross observations and histopathological features.

II. MATERIALS AND METHODS

The experiment was carried out in the Lab Animal House, Veterinary College, Shivamogga. Present study was taken up to evaluate the phytochemical analysis, in vivo anti-inflammatory and analgesic activity, and to conduct the toxicity evaluation of the aerial parts of T. portulacastrum. The experimental protocol was approved by the Institutional Animal Ethics Committee, Veterinary college, Shivamogga, with the approval **No.VCS/IAEC/SA-60/2020-21** and dated: **31.08.2021.**

Collection of plant material and authentication

The fresh aerial parts of the plant were collected in the month of April and May 2021 from

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the villages of Shikaripura taluk, Shivamogga district, Karnataka State, India. The taxonomic identification of the plant was confirmed by Dr. K. G. Gopalakarishna Bhat, Professor and Head [Retd.], Department of Botany, Poornaprajna College, Udupi. The collected plant materials were washed under running tap water and allowed to drain before air drying under shade for two weeks. Thus, dried aerial parts of the plant were grounded mechanically using the household mixer-grinder and the obtained powder was sieved to get the fine powder which was kept in airtight containers for further use.

Preparation of the extract

The first and most important step in producing high-quality research results is the preparation of plant extract for experimental purposes. Before moving further with the desired biological testing, it entails the extraction and assessment of the quality and amount of bioactive elements. Menstruum is another name for the extraction solvent used to extract medicinal herbs. The type of plant, the portion of the plant to be extracted, the makeup of the bioactive chemicals, and the solvent's availability all influence the choice of solvent. [12].

The efficacy of detecting medicinal or toxic ingredients in plants is highly dependent on the method of extraction. None of the extraction methods can be said to be an ideal method as each extraction procedure is unique to the plants [13].

One thousand grams [1000 g] of the fine powder was weighed using an electronic weighing balance and soaked in 5 litres of methanol [99% SDFCL], at a ratio of 1:5 [powder: solvent] in closed glass flasks at room temperature. The mixture was agitated using an electric orbital shaker [REMI RS-12 plus] to enhance proper mixing of the solvent with the powder for the first 6 hour. Shaking and stirring of flasks done thrice a day for seven days. [14].

After seven days, the contents were first filtered through muslin cloth and Buchner's funnel later with Whatman No.1 filter paper [Himedia, 24 cm]. The filtrates were then separately concentrated in vacuum using Rotary Evaporator [DLAB RE100-Pro, China] at 37-40 °C till solvent got evaporated and extract settled down. These were concentrated to complete dryness in the incubator [SLM-INC-OS-250] at 50 °C. The extracts were stored in a refrigerator in air-tight containers from where aliquots were used for the phytochemical analysis and pharmacological formulation preparation [14].

Calculation of yield

The per cent yield [dry weight of extract] calculated after solvent extraction using the formula given below.

$$\% \text{ yield} = \frac{\text{Final weight of extract}}{\text{Initial weight of the powder}} \times 100$$

Toxicity studies of methanol extract of T. portulacastrum

Acute oral toxicity and Repeated dose 28-day oral toxicity studies of methanol extract of T. portulacastrum were conducted in rats as per the broader outlines of OECD 423 and OECD 407 respectively. [15,16]

III. DESIGN OF THE EXPERIMENT

Experimental animals :

In the present study, Wistar albino rats in the age group of 5 to 6 weeks with the body weight ranging from 170 ±20 g were procured from CPCSEA approved vender i.e. Adita biosys private limited, Tumkur [Reg No: 1868/PO/RcBt/S/16/CPCSEA. Date of registration: 23.02.2016 Valid up to D:08.04.2026]. The animals were maintained in standard conditions as per the CPCSEA guidelines in Small Animal House, Veterinary College, Shivamogga in a controlled temperature of 25 ±3 °C and relative humidity of 55 ±5% and 12 h light/dark cycle. The female rats were nulliparous and non-pregnant. The rats were given standard rat pellets and reverse osmosis [RO] water ad libitum. The toxicity study was conducted with prior permission from Institutional Animal Ethics Committee [IAEC].

Acute oral toxicity study

Acute oral toxicity study of methanol extract of T. portulacastrum aerial partswas conducted in female Wistar albino rats as per the Organization for Economic Co-operation and Development [OECD] guideline for testing of chemicals, Acute oral toxicity – toxic class method [16].

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Study procedure

Animal preparation

Adult healthy Wistar albino female rats aged 5 to 6 weeks weighing 170 ±20 g were acclimatized to the laboratory conditions for 7 days prior to test and before assigning the animals to treatment groups.

Animal groups and number of animals

Acute oral toxicity study was conducted in nulliparous and non-pregnant female Wistar albino rats. The animals were divided into four groups of three animals each were used for the study.

Selection and preparation of doses

According to OECD guidelines 423, selected the starting dose as 300 mg/kg [no mortality observed at 300 mg/kg] and the upper limit dose of 5000 mg/kg [limit test dose]. Hence the treatment group in this study received dosage of 300 mg/kg [Low dose], 2000 mg/kg [Medium dose] and 5000 mg/kg [high dose] of methanol extract of T. portulacastrum whereas the control group received the dosage of 10 ml/kg of distilled water as a vehicle. Previous pharmacological studies on methanolic extract of **T. portulacastrum** in mice suggested that up to dose of 3000 mg/kg body weight did not show any toxicity [17].

Administration of doses

The extract was dissolved in distilled water to make the solution of required quantity not exceeding 2 ml for the ease of administration. Weight of the animals was recorded, then doses of extract were administered to overnight fasted [water was not withheld] animals as a single dose by oral gavage. The grouping of animals [with the respective treatment] has been done as shown in Table 1.

Table 1: Experimental design for Acute oral toxicity study of METP

Group	Dose [mg/kg, p.o.]	No. of female rats
Group I	Distilled water [10 ml/kg]	3
Group II	300	3
Group III	2000	3
Group IV	5000	3

Observation of animals

After oral dosing of methanol extract of T. portulacastrum, food was withheld for further 3-4 h [16]. All the animals were observed individually at least once during first 30 min after dosing, periodically during the first 24 h [with special attention during the first 4 hours] and daily thereafter for a period of 14 days for the symptoms of toxicity and death. General clinical observations were made every day. All the animals were observed for changes in the level of activity, gait, posture, reactivity to handling or sensory stimuli, altered strength, health conditions, morbidity, and mortality.

Repeated dose 28-day oral toxicity study

Repeated dose 28-day oral toxicity study for the T. portulacastrum aerial parts extract was conducted in both male and female Wistar rats as per the broader outlines of Organization for Economic Co-operation and Development [15] guidelines for testing of chemicals, Repeated dose 28-day oral toxicity study in rodents OECD-407.

Study procedure

Animal preparation

Healthy young adult Wistar male and female rats aged around 7 to 8 weeks weighing 170 ±20 g were acclimatized to the laboratory conditions for 7 days prior to the study and before assigning the animals to different groups.

Selection and preparation of doses

Based on toxicological review, there were no published literature available on repeated 28-day sub-acute toxicity study. Only available information was on acute toxicity study where the extract was not toxic up to doses of 3000 mg/kg. Taking into consideration of this acute toxicity results, half of this dose [i.e. 1500 mg/kg] was taken as highest dose for sub-acute toxicity study. Other two doses selected were 1000 mg/kg and 500 mg/kg as medium and low dose of extract treatment respectively.

Animal groups and number of animals

The control and treatment groups were made up of male and female laboratory-trained rats. For the current investigation, 5 male and 5 female rats in each group were used. The methanol extract of T. portulacastrum was given to the animals once

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daily by oral gavage for a total of 28 days at various dose levels.The grouping of animals [with the respective treatment] has been done as shown in Table 2.

Table 2: Experimental design for Repeated dose 28-day oral toxicity study of METP

Group	Dose [mg/kg, p.o]	No. of male rats	No. of female rats
Group I	Distilled water [1 ml]	5	5
Group II	500	5	5
Group III	1000	5	5
Group IV	1500	5	5

Administration of doses

The animals were administered with the methanol extract of T. portulacastrum at different dose levels, daily for a period of 28 days by oral gavage as a single dose per day.

Observation of animals

General clinical observations were made once a day throughout the study period of 28 days considering the period of anticipated effects after dosing. All the animals were observed for health condition, morbidity, and mortality. Changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions, and autonomic activity were observed, and changes in gait, posture and response to handling as well as the presence of clonic or tonic movements and stereotypical behaviours were also recorded.

Body weight

Bodyweight of each animal in both acute and sub-acute oral toxicity studies was recorded weekly once till the completion of the study on day 14 and day 28 in acute and sub-acute oral toxicity study respectively.

Haematology

On day 14 in the acute toxicity study and day 28 in the sub-acute toxicity study blood was subjected for analysis of haematological parameters viz erythrocyte count [RBC], leucocyte count [WBC], haemoglobin concentration [Hb] and platelet count by using The Exigo® veterinary haematology analyser, Sweden. Using micro haematocrit capillary tubes, the terminal blood sample was collected by retro-orbital plexus puncture technique using diethyl ether as inhalant sedative.

Serum biochemistry

The serum biochemistry profile with respect to the following parameters was performed on day 14 in the acute toxicity study and day 28 in the sub-acute toxicity study using biochemical analyser [HY-SAC® Vet Version: A/6 Semi-auto Chemistry Analyzer, Hycel Handelsges Austria]. Using micro haematocrit capillary tubes, the terminal blood sample was collected by retro-orbital plexus puncture technique using diethyl ether as inhalant sedative. **.**Aspartate aminotransferase [AST] Alanine aminotransferase [ALT] Creatinine [CRT] Blood urea nitrogen [BUN] Calcium Phosphorous

Pathology

At the end of study period of 14 days and 28 days for acute and sub-acute oral toxicity study respectively, all the ailing rats in control and treated groups were humanely sacrificed using approved methods and subjected to detailed gross necropsy for the organs including examination of the external surface of the body, all orifices and cranial, thoracic and abdominal cavities and their contents for the changes.

Collection of organs for histopathological study

In all the study groups of acute and sub-acute oral toxicity study, the rats were weighed individually and sacrificed humanely by using CO_2 chamber. Necropsy was conducted on each carcass to observe any gross pathological changes. The organs viz., liver, kidney, spleen, duodenum, and heart were separated from the adhering tissues and preserved in 10% Neutral buffered formalin [NBF] for the period of 72 hour for tissue fixation. Further the tissues were processed for histopathology using automatic tissue processor followed by routine paraffin embedding technique. Sections of 5 microns thickness were cut and stained with Haematoxylin and Eosin using the standard protocol [18].

Statistical analysis

Data was expressed as mean ±SEM. Differences were considered significant at ***P<0.001, or **P < 0.01 or * P<0.05 when compared test groups v/s control group. For statistical analysis, wherever necessary either one-

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way or two-way Analysis of Variance [ANOVA] with Dunnet's multiple comparisions test was performed using Graph Pad Prism 9.3.1 Software

IV. RESULTS

Table3 :Mortality and morbidity pattern of rats in repeated dose 28-day oral toxicity study of methanolic extract of aerial parts of T. portulacastrum

Groups	Treatment and dose	No. of female rats	No. of male rats	Mortality observed	Mortality [%]
Group I	Distilled water	5	5	0	0
Group II	500 mg/kg	5	5	0	0
Group III	1000 mg/kg	5	5	0	0
Group IV	1500 mg/kg	5	5	0	0

Body weight

The effect of methanolic extract of T. portulacastrum aerial parts on body weight of male and female rats is depicted in Figure 1 and 2, respectively. The mean±SEM values of body weight of male and female rats in different groups have been shown in Table 4 and 5, respectively. All animals exhibited a gradual increase in body weight of both the control and treated groups. The percentage changes in body weight of the treated groups were not significantly different compared to the control rats except the male rats in Group III [1000 mg/kg] and Group IV [1500 mg/kg] have shown significant increase in body weight.

Table 4: Body weight of male rats in repeated dose 28-day oral toxicity study of methanolic extract of T. portulacastrum aerial parts

Days	Groups			
	Control	**500 mg/kg**	**1000 mg/kg**	**1500 mg/kg**
0	178±7.52	187±3.39	195±5.70	207±7.68
7	194±9.14	203±4.64	217±8.15	234±12.08*
14	209±9.67	222±8.00	244±9.14*	260±12.75**
21	233±9.43	259±10.65	267±10.20*	290±14.83***
28	255±5.92	278±10.20	292±11.58*	316±16.61***

Note: Data were analysed by two-way ANOVA followed by Dunnett's multiple comparisons test. Data were compared with the control group at different time intervals. Values are mean ±SEM, n=5, *p<0.05, **p<0.01, ***p<0.001.

Figure 1: Body weight of male rats in repeated dose 28-day oral toxicity study of methanolic extract of T. portulacastrum aerial parts

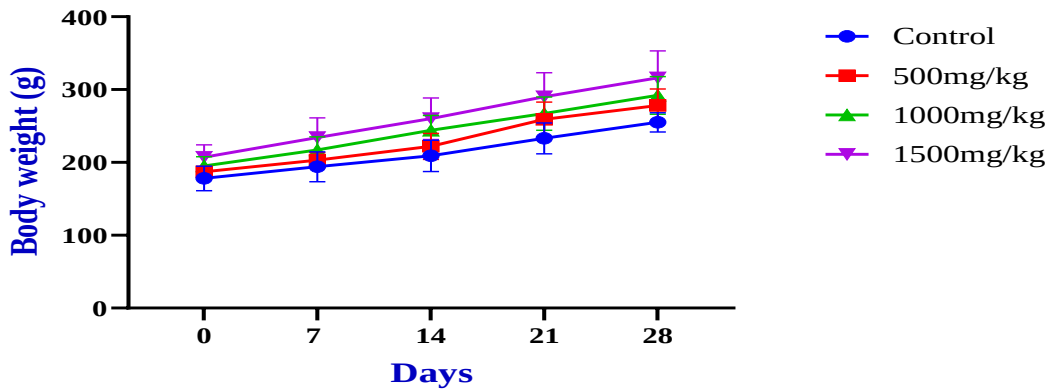

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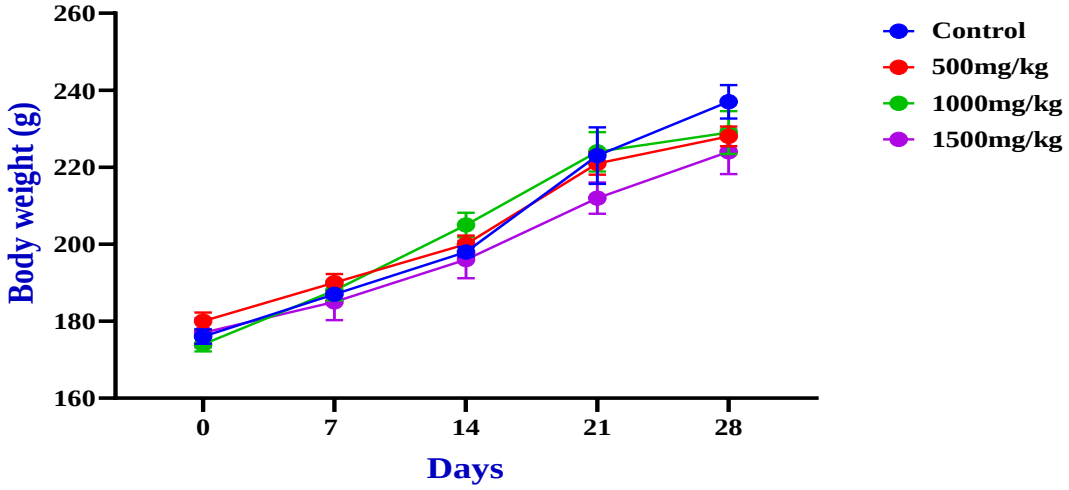
Table 5: Body weight of female rats in repeated dose 28-day oral toxicity study of methanolic extract of T. portulacastrum aerial parts

Days	Groups			
	Control	500 mg/kg	1000 mg/kg	1500 mg/kg
0	176±1.87	180±2.24	174±1.87	177±3.74
7	187±1.22	190±2.24	188±2.55	185±4.74
14	198±1.22	200±2.24	205±3.16	196±4.85
21	223±7.35	221±2.92	224±5.10	212±4.06
28	237±4.36	228±2.55	229±5.57	224±5.79

Note: Data were analysed by two-way ANOVA followed by Dunnett's multiple comparisons test. Data were compared with the control group at different time intervals. Values are mean ±SEM, n=5, *p<0.05.

Figure 2: Body weight of female rats in repeated dose 28-day oral toxicity study of methanolic extract of T. portulacastrum aerial parts



Haematology

Haematological parameters of male and female rats are presented in Table 6 and Table 7 respectively. In both male and female rats of subacute toxicity study, there was no significant [p<0.05] difference in haematological parameters [WBC, RBC and Hb] between control and treatment groups. But in female rats of Group III [1000 mg/kg]& Group IV [1500 mg/kg], there was significant decrease in the platelet count, conversely, there was significant increase in platelet count in male rats of Group III [1000 mg/kg] and Group IV [1500 mg/kg].

Table 6: Haematological parameters of male rats in repeated dose 28-day oral toxicity study ofmethanolic extract of T. portulacastrum aerial parts.

Parameter	Group and treatment			
	Control	500 mg/kg	1000 mg/kg	1500 mg/kg
WBC [10^3/mm^3]	9.70±1.49	10.54±0.87	14.20±3.03	10.80±0.38
RBC [10^6/mm^3]	7.83±0.30	8.19±0.27	6.88±0.43	8.33±0.12
Hb [g %]	13.98±0.49	14.18±0.32	13.16±0.98	14.68±0.31
Platelet [10^3/mm^3]	786.40±65.52	763.20±55.08	715.60±14.75	795.00±36.60

Note: Data were analysed by two-way ANOVA followed by Dunnett's multiple comparisons test and compared with the control group. Values are mean ±SEM, n=5, *p<0.05.

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Table 7: Haematological parameters of female rats in repeated dose 28-day oral toxicity study ofmethanolic extract of T. portulacastrum aerial parts.

Parameter	Group and treatment			
	Control	**500 mg/kg**	**1000 mg/kg**	**1500 mg/kg**
WBC [10^3/mm^3]	12.54±0.73	10.92±1.67	12.26±1.96	11.66±1.31
RBC [10^6/mm^3]	7.93±0.20	7.44±0.38	6.88±0.47	7.51±0.11
Hb [g %]	14.14±0.57	13.42±0.40	12.70±0.62	13.36±0.28
Platelets [10^3/mm^3]	730.80±32.55	704.60±37.60	694.00±36.59	717.00±24.47

Note: Data were analysed by two-way ANOVA followed by Dunnett's multiple comparisons test and compared with the control group. Values are mean ±SEM, n=5, *p<0.05.

Serum biochemistry

The serum biochemical parameters of male and female rats in repeated dose 28-day oral toxicity study are presented in Table 8 and Table 9 respectively. There was no significant [p<0.05] change observed in all biochemical parameters.

Table 8: Serum biochemical parameters of male rats in repeated dose 28-day oral toxicity study of methanolic extract of T. portulacastrum aerial parts.

Parameter	Groups			
	Control	**200 mg/kg**	**400 mg/kg**	**600 mg/kg**
ALT [IU/l]	33.22±1.53	35.03±1.34	35.85±1.83	35.28±1.77
AST [IU/l]	185.32±7.23	173.96±21.1	174.84±13.2	172.16±8.67
Creatinine [mg/dl]	1.62±0.13	1.65±0.06	1.45±0.08	1.47±0.07
BUN [mg/dl]	43.92±3.20	46.16±2.80	41.72±3.21	54.48±3.46
Calcium [mg/dl]	11.28±0.91	13.42±1.00	13.56±1.40	10.77±0.53
Phosphorous [mg/dl]	5.40±0.37	5.08±0.46	5.14±0.28	5.00±0.28

Note: Data were analysed by two-way ANOVA followed by Dunnett's multiple comparisons test and compared with the control group. Values are mean ±SEM, n=5, *p<0.05.

Table 9: Serum biochemical parameters of female rats in repeated dose 28-day oral toxicity study of methanolic extract of T. portulacastrum aerial parts.

Parameter	Groups			
	Control	**200 mg/kg**	**400 mg/kg**	**600 mg/kg**
ALT [IU/l]	36.94±2.22	34.98±1.72	39.94±3.62	33.18±1047
AST [IU/l]	182.20±8.87	169.40±21.41	165.60±13.80	173.60±5.39
Creatinine [mg/dl]	1.47±0.15	1.43±0.12	1.56±0.11	1.48±0.05
BUN [mg/dl]	45.08±4.80	44.66±2.81	43.40±3.08	48.64±4.54
Calcium [mg/dl]	12.84±0.39	11.00±0.78	14.48±1.30	11.48±0.75
Phosphorous [mg/dl]	4.56±0.26	4.98±0.38	5.28±0.43	5.04±0.49

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Note: Data were analysed by two-way ANOVA followed by Dunnett's multiple comparisons test and compared with the control group. Values are mean ±SEM, n=5, *p<0.05.

Pathology of rats in Acute Oral and Repeated Dose 28-day Oral toxicity studies of methanolic extract of T. portulacastrum aerial parts.

Acute toxicity study

Gross pathology

At necropsy, heart, liver, kidney, spleen, and duodenum of control group rats were normal with no observed abnormality.

Histopathology

Group I [Control group]

The histological appearance of the organs namely heart, liver, kidney, spleen and duodenum in the control group were found to be normal .

Groups treated with methanolic extract of T. portulacastrum aerial parts.

Microscopic analysis revealed that the treated animals' livers had normal cellular architecture, well-defined cytoplasm and nuclei, and no deformities in comparison to the control groups. Additionally, there were no visible symptoms of liver damage, necrosis, congestion, fatty infiltration, or hemorrhagic areas near the central vein or sinusoids. Hepatocytes are conspicuously present and tightly packed in branching cords. In the group with acute oral toxicity, there were no neutrophil, lymphocyte, or macrophage infiltrations in the liver's cross-section. For all groups that received extract treatment, there was no morphological change in the kidney histology.

The appearance of the cortex architecture with well-defined glomeruli, convoluted tubules with compact and uniformly arranged cuboidal renal epithelial cells were normal like the control groups. The glomeruli, distal, and proximal tubules in the kidney appeared normal in both male and female rats. In addition, there was no interstitial and intraglomerular congestion or tubular atrophies. All the nephron cells were apparently normal and with no degeneration, haemorrhages, necrosis, infiltration in acute oral toxicity.

In both the control and extract treated male and female rats, the heart shows normal cardio-myofibers and lungs show a normal alveolar structure with no treatment-related inflammatory response in acute oral toxicity group.

Similarly, in spleen, red pulp and white pulp with even distribution and normal appearance of lymphoid cells were evident. In addition, the normal structure and histology of duodenum observed in all the rats of acute oral toxicity study i.e. well-defined villi with columnar epithelial cells, goblet cells and duodenal crypts.

There were few very mild congestions and very mild degenerative changes seen in the lung, liver, and kidney of both the control and extract treated groups of both the sexes which were incidental, not consistent and spontaneous with no relation to methanol extraction treatment. Thus, the histopathological evaluations of the selected organs did not reveal any morphological abnormalities that could be attributed to the oral administration of methanol extract to the rats [Plate 7, 8 and 9].

Repeated Dose 28-day Oral toxicity study

Gross pathology

At necropsy, heart, liver, kidney, spleen, and duodenum of control group rats were normal with no observed abnormality.

Histopathology

Group I [control]

The control group's heart, liver, kidney, spleen, and duodenum were found to have normal histological appearances.

Groups treated with methanolic extract of T. portulacastrum aerial parts.

Microscopic analysis revealed that the treated animals' livers had normal cellular architecture, well-defined cytoplasm and nuclei, and no deformities in comparison to the control groups. Additionally, there were no visible symptoms of liver damage, necrosis, congestion, fatty infiltration, or hemorrhagic areas near the central vein or sinusoids. Hepatocytes are conspicuously present and tightly packed in branching cords.

In the group with acute oral toxicity, there were no neutrophil, lymphocyte, or macrophage infiltrations in the liver's cross-section.

Histologically in kidneys, there was no morphological change for all extract treated groups. The appearance of the cortex architecture with well-defined glomeruli, convoluted tubules with compact and uniformly arranged cuboidal renal epithelial cells were normal like the control groups. The glomeruli, distal, and proximal tubules in the kidney appeared normal in both male and female rats. In addition, there was no interstitial and intraglomerular congestion or tubular atrophies.

According to all appearances, there were no degeneration, haemorrhages, necrosis, or

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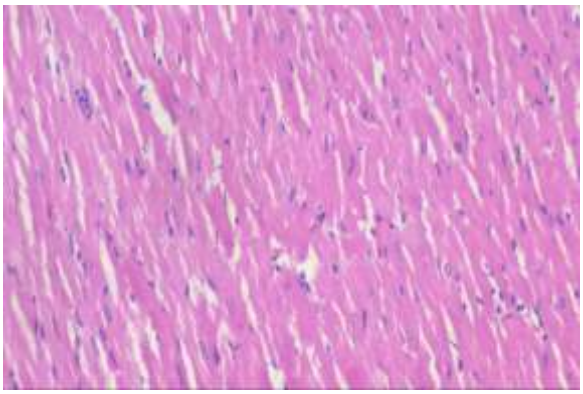
infiltration of acute oral toxicity in any of the nephron cells.

The heart and lungs in the acute oral toxicity group in both the control and extract-treated male and female rats display normal cardio-myofibers and a normal alveolar structure, respectively. Similar red and white pulp with uniform distribution and lymphoid cell morphology were visible in the spleen.

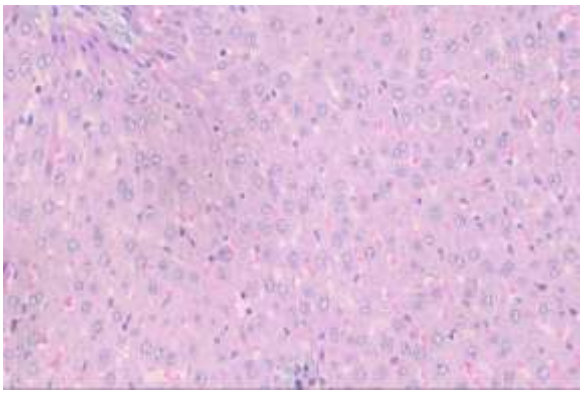
Additionally, the acute oral toxicity research rats' normal duodenal histology and structure were all observed.i.e. well-defined villi with columnar epithelial cells, goblet cells and duodenal crypts.

There were few very mild congestions and very mild degenerative changes seen in the lung, liver, and kidney of both the control and extract treated groups of both the sexes which were incidental, not consistent and spontaneous with no relation to methanol extraction treatment. Thus, the histopathological evaluations of the selected organs did not reveal any morphological abnormalities that could be attributed to the oral administration of methanol extract to the rats

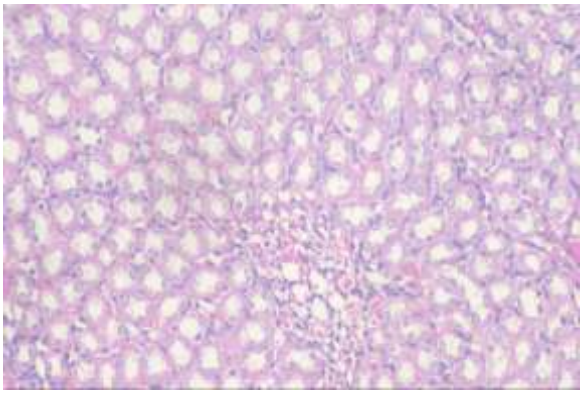
Histopathology: Acute oral toxicity - GroupII [300 mg/kg]



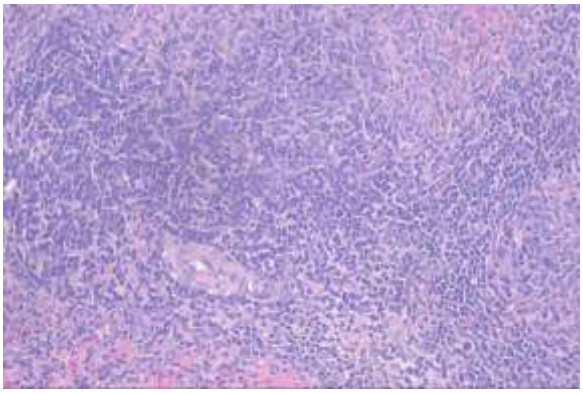
Heart [H&E, 20X]



Liver [H&E, 20X]



Kidney [H&E, 20X]



Spleen [H&E, 10X]

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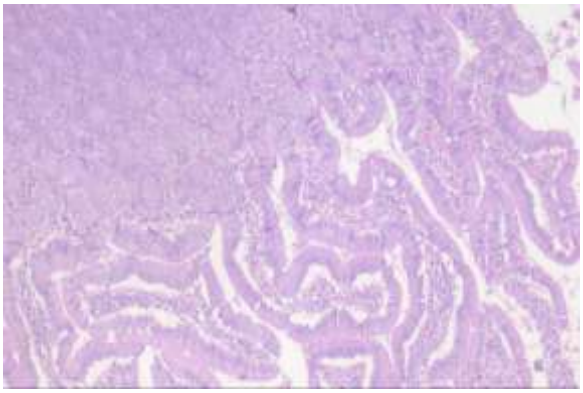
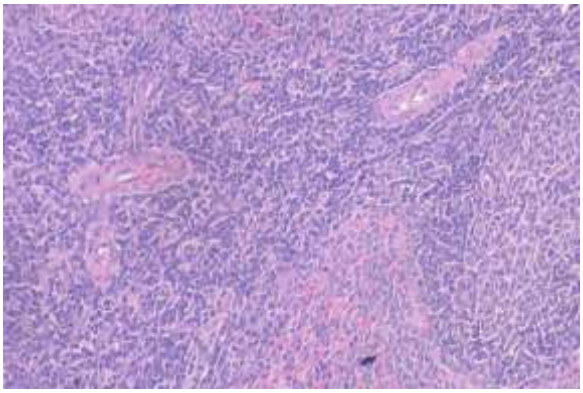


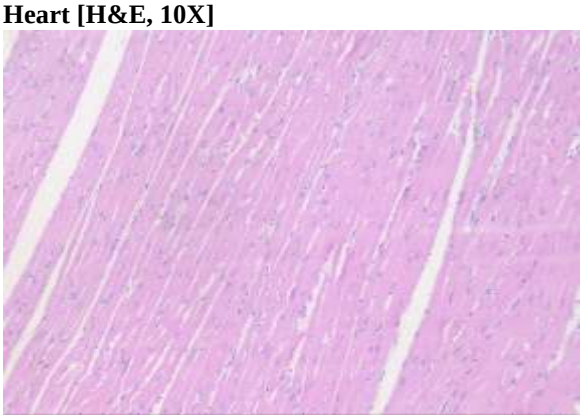
Duodenum [H&E, 10X]



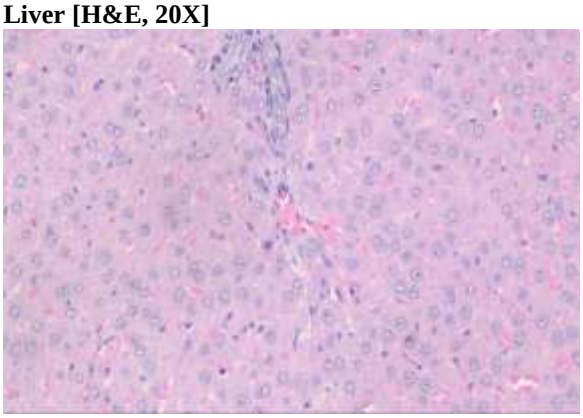
Spleen [H&E, 10X]

Histopathology: Acute oral toxicity - Group III [2000 mg/kg]

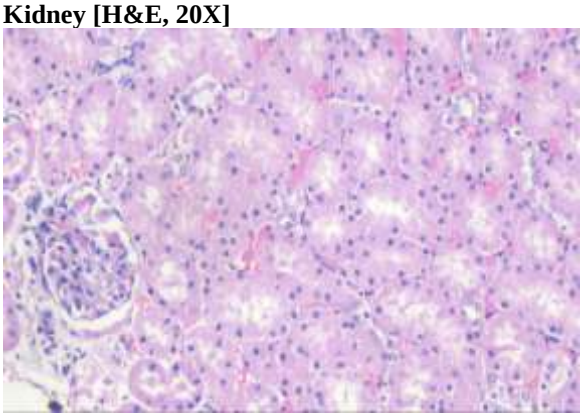
Heart [H&E, 10X]

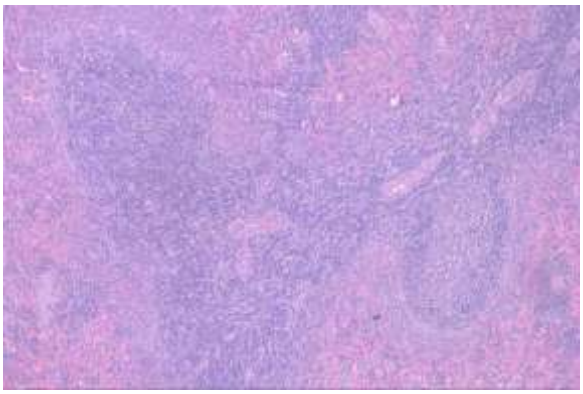


Liver [H&E, 20X]



Kidney [H&E, 20X]





Spleen [H&E, 10X]

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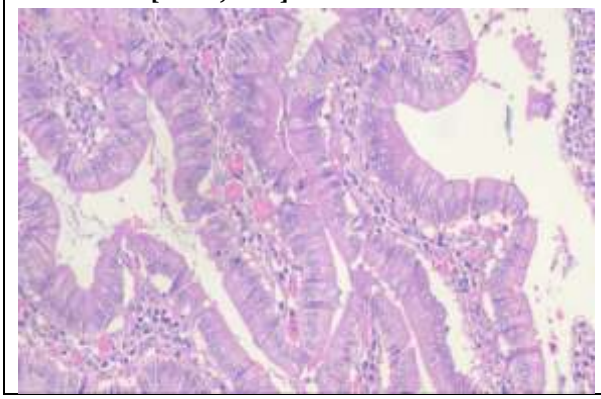
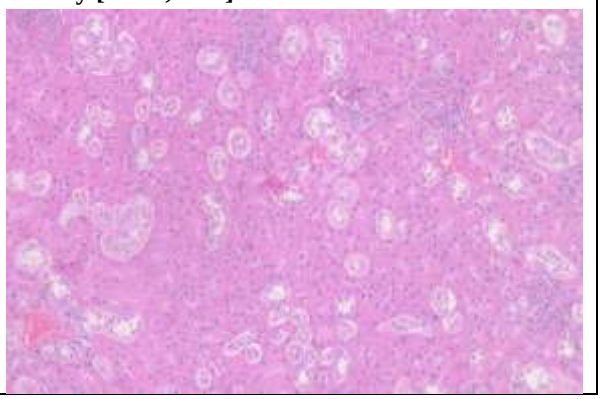
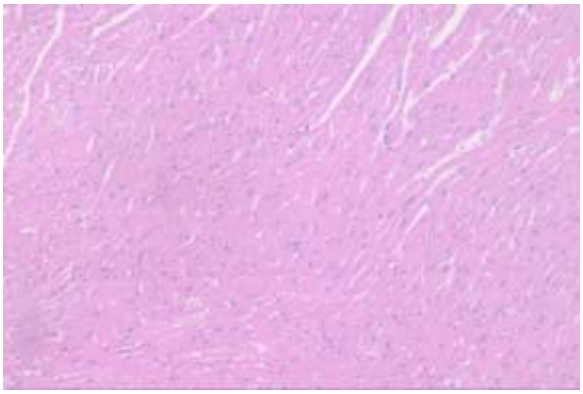
Duodenum [H&E, 10X]



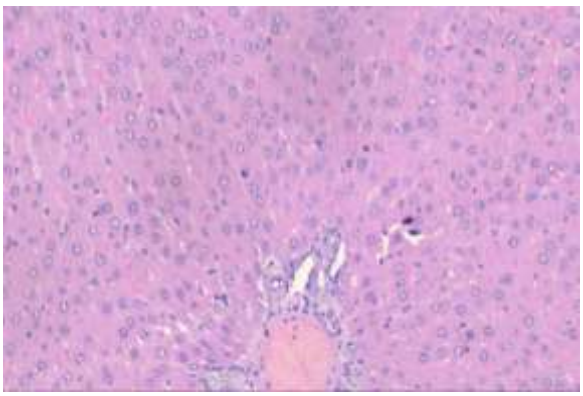
Kidney [H&E, 20X]



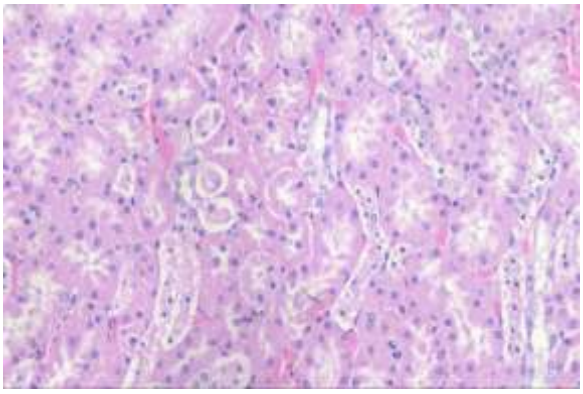
Histopathology: Acute oral toxicity - Group IV [5000 mg/kg]



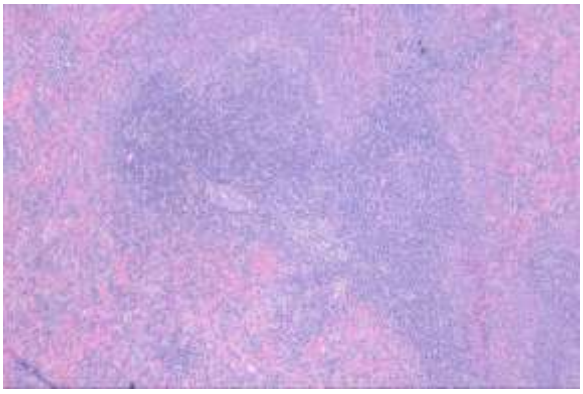
Heart [H&E, 10X]



Liver [H&E, 20X]



Kidney [H&E, 20X]



Spleen [H&E, 10X]

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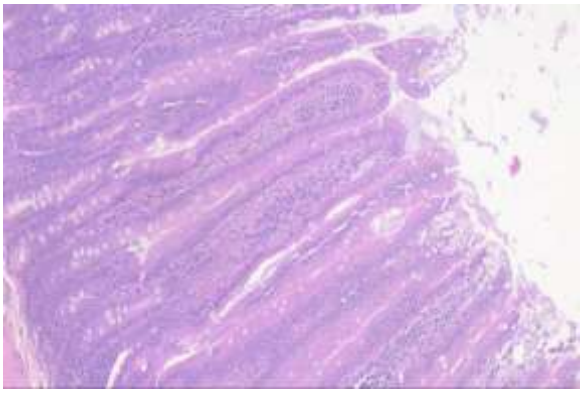
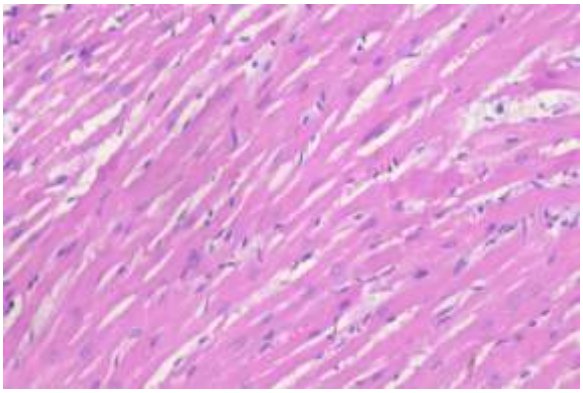
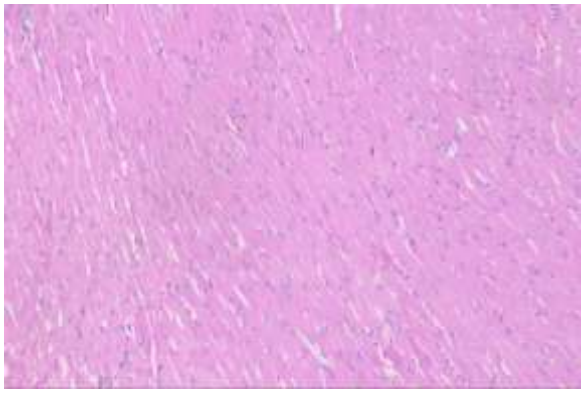


Duodenum [H&E, 10X]

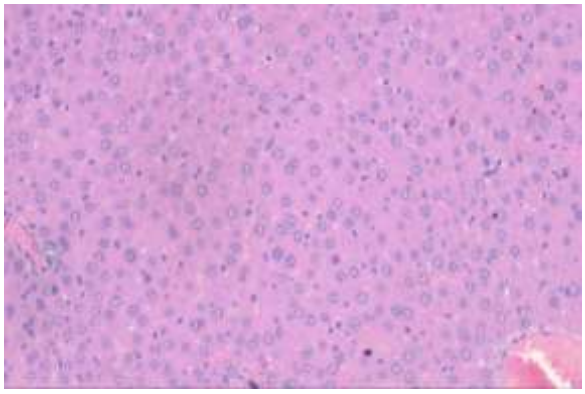


Heart [H&E, 20X]

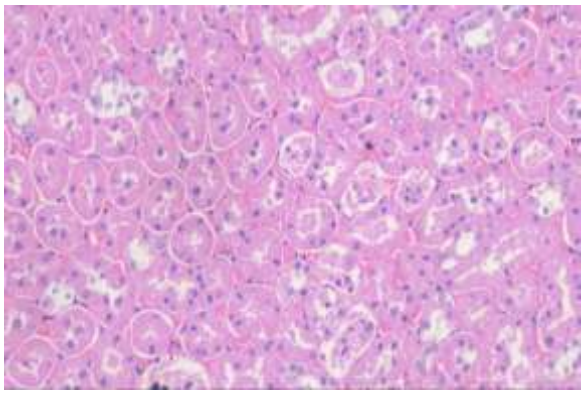
Histopathology: Sub-acute oral toxicity - Group I [Control]



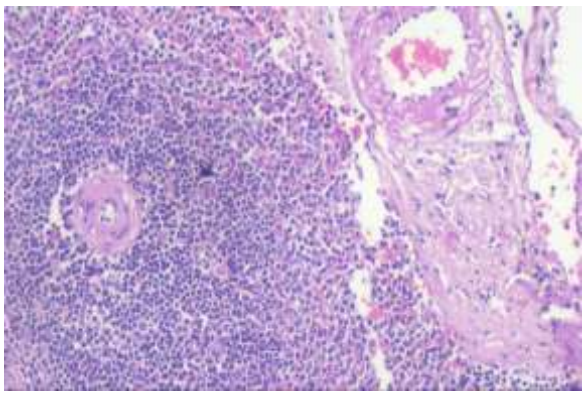
Heart [H&E, 10X]



Liver [H&E, 20X]



Kidney [H&E, 20X]



Spleen [H&E, 20X]

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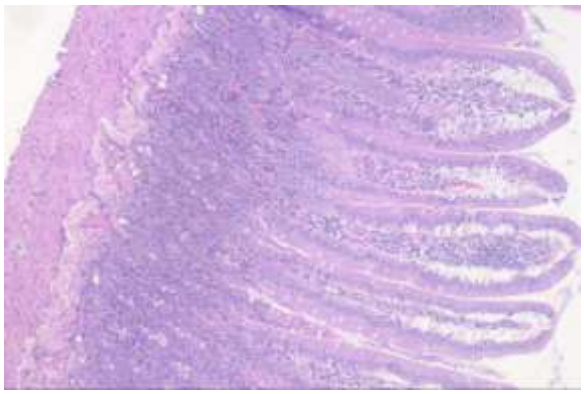
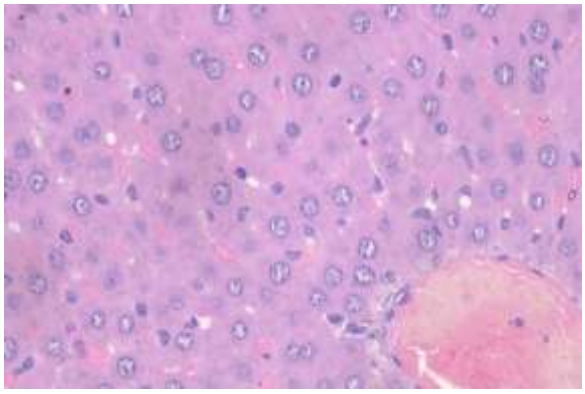
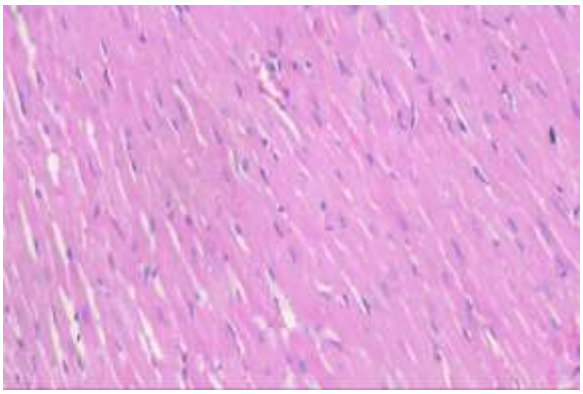


Duodenum [H&E, 10X]

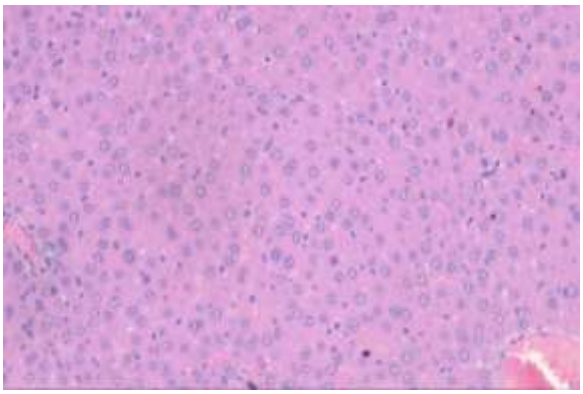


Liver [H&E, 20X]

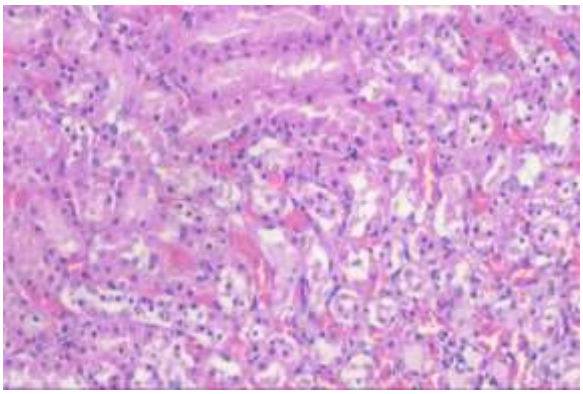
Histopathology: Sub-acute oral toxicity - Group II [500 mg/kg]



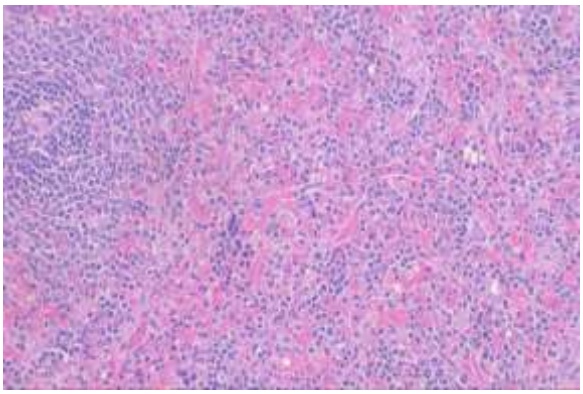
Heart [H&E, 20X]



Liver [H&E, 20X]



Kidney [H&E, 20X]



Spleen [H&E, 20X]

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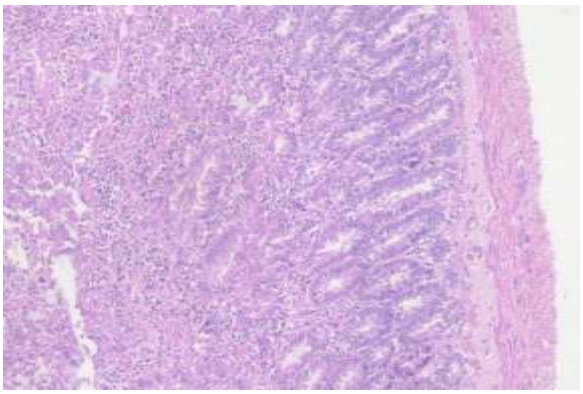
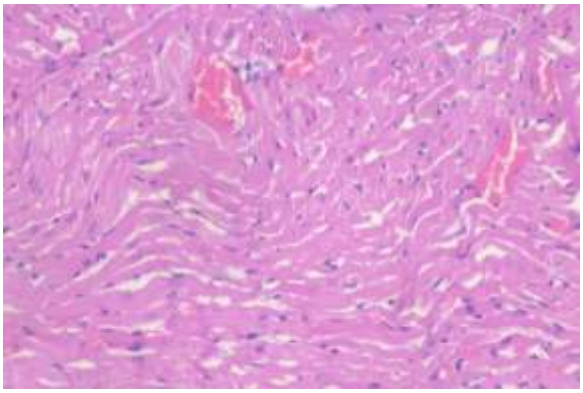
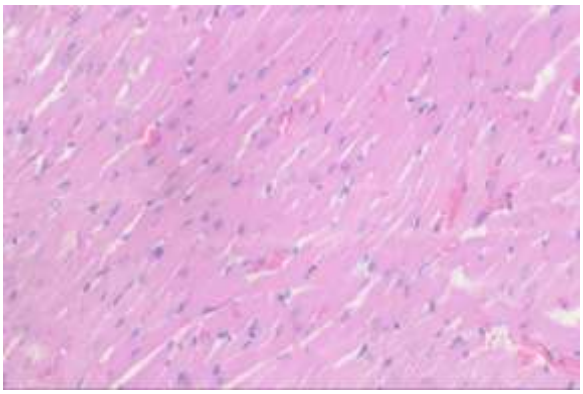


Duodenum [H&E, 20X]

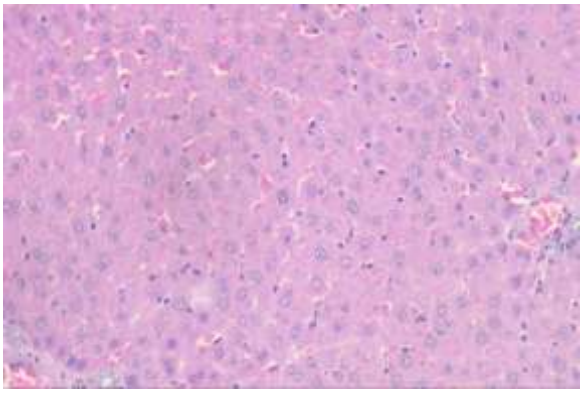


Heart [H&E, 10X]

Histopathology: Sub-acute oral toxicity - Group III [1000 mg/kg]

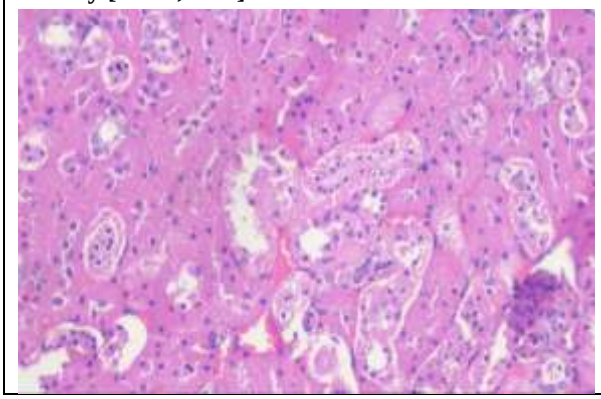


Heart [H&E, 10X]

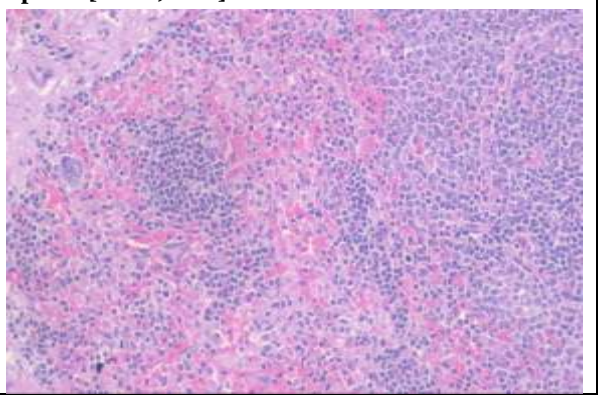


Liver [H&E, 20X]

Kidney [H&E, 20X]



Spleen [H&E, 10X]



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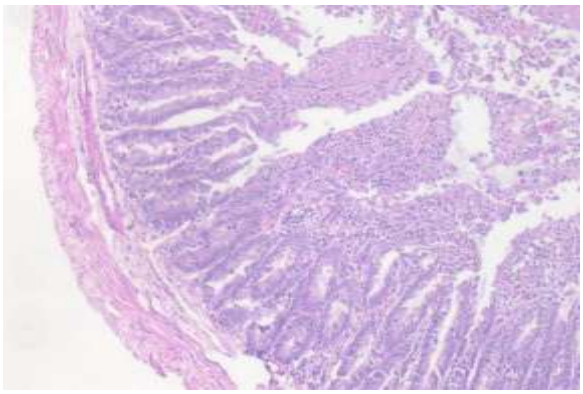
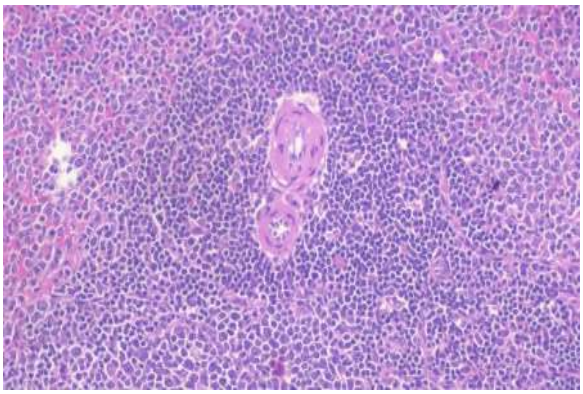


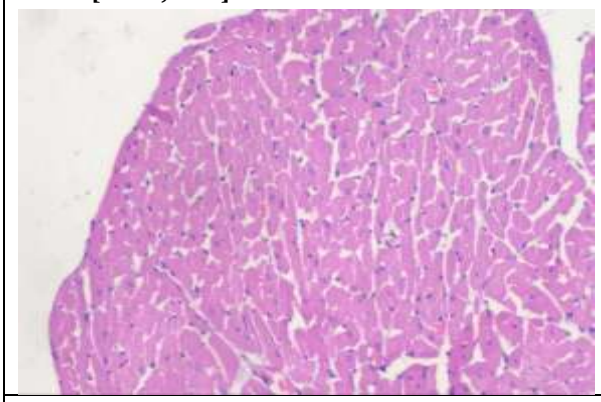
Duodenum [H&E, 10X]



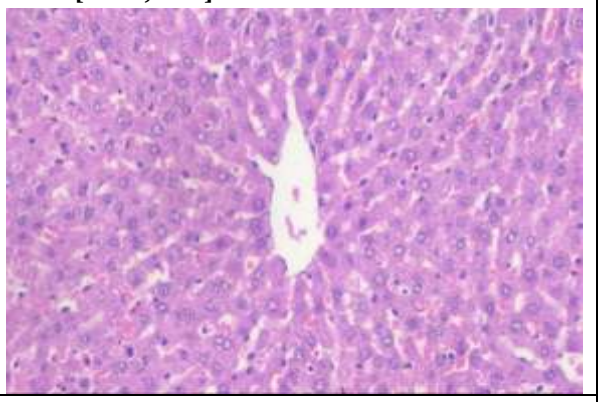
Spleen [H&E, 10X]

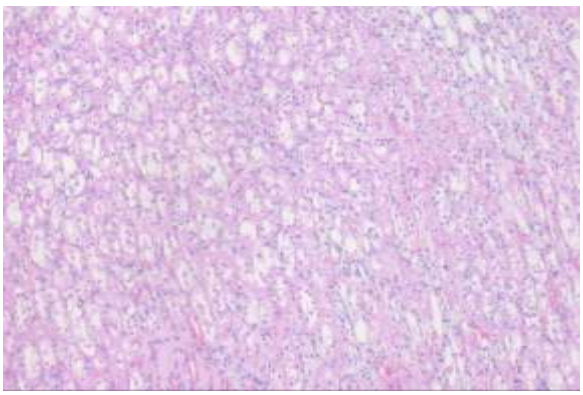
Histopathology: Sub-acute oral toxicity - Group IV [1500 mg/kg]

Heart [H&E, 10X]

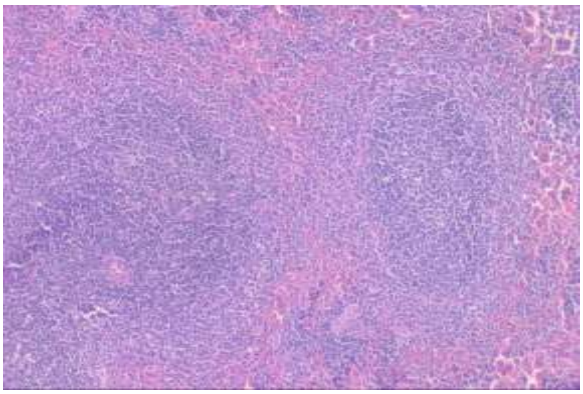


Liver [H&E, 20X]





Kidney [H&E, 20X]



Spleen [H&E, 10X]

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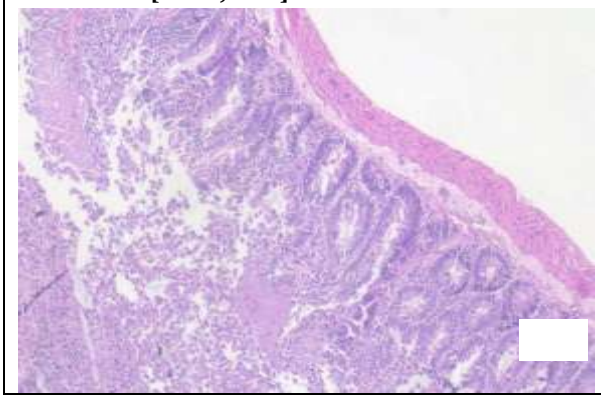
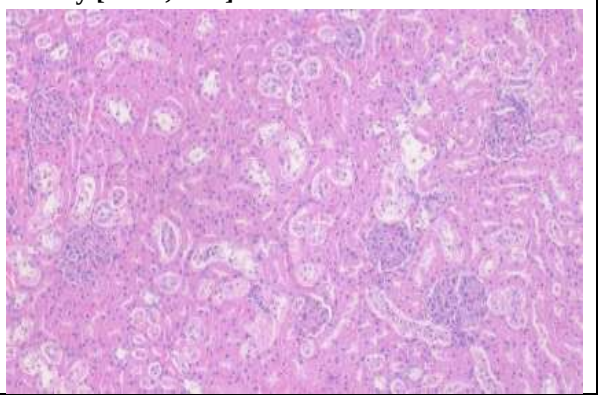
Duodenum [H&E, 10X]



Kidney [H&E, 20X]



V. DISCUSSION

Toxicity studies of methanolic extract of T. portulacastrum aerial parts

When there is no evidence to suggest that the test substance is likely to be hazardous, the investigator will typically use a test procedure with a starting dose of 300 mg/kg body weight [16]. From the experiment, it can be concluded that the experimental animals did not die at the initial dose of 300 mg/kg body weight. In accordance with the OECD standards 423, the next higher dose of 2000 mg/kg and 5000 mg/kg body weight was chosen.

One of the most crucial findings to show the toxicity effects on the treated groups' organs is the clinical symptom [19]. Because cumulative toxic effects do happen even at very low doses, knowledge on acute toxicity has little therapeutic use.Consequently, multiple dose studies are typically useful in evaluating the safety profile of phytomedicines. Therefore, sub-acute [Repeated dose 28-day oral toxicity] test has been used.

Body weight fluctuations are a sign of unfavourable side effects [20].Losing more than 20% of the animal's body weight is considered critical and has been outlined as one of the humane end points in numerous international recommendations [21,22].

To evaluate the harmful effects of methanol plant extract, a sub-acute oral toxicity test was performed. It was done so that information could be obtained about the potential health risks that could result from sub-acute exposure over time, the potential for cumulative effects, and an estimate of the dose at which no adverse effects are visible. Evaluation of safety margin between different dose level that produces the therapeutic effect and that which produces the adverse effects is necessary. Evaluation of safety is exactly to provide benefit to risk assessment. Animal experimental model is the only method that can assess this matter [23].

In this study, all rats in the vehicle control and extract treated groups were gaining weight; however, from 2nd week onwards there were significant change [increase] in the body weight gain in male rats of Group III [1000 mg/kg] and Group IV [1500 mg/kg] in sub-acute toxicity study.

Mortality and morbidity pattern

Both the toxicity studies [Acute Oral toxicity study and repeated dose 28-day oral toxicity study] of methanolic extract of T. portulacastrum aerial parts revealed no obvious signs of distress, and there were no noticeable symptoms of either toxicity or deaths. All the rats showed no significant changes in wellness parameters. Physical appearance features such as skin, fur, eyes, mucous membrane, salivation, behavioural pattern, etc of the animals in control and extract treated groups were found to be normal. Lethargy, tremors, diarrhoea, and coma did not occur in any of the animals and no signs of toxicity and mortality were evident at all the doses tested till the completion of study.

The mortality and morbidity patterns from the present study are in accordance with the study conducted by [17,24,25] with extract of T. portulacastrum at different doses in mice and rats, and they reported that there were no mortality and no changes in behavioural pattern and motor reflexes.

Body weight

In the present study, regarding food consumption, there was no significant change observed in all groups [vehicle and extract treated groups] in both acute and subacute oral toxicity tests and this reveals that extract did not adversely

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affect the basic metabolic processes of the experimental animals.

Haematological parameters

All the haematological parameters were normal in all the extract treated groups when compared with control group.

Since there was no statistically significant difference between the groups in the acute and sub-acute oral toxicity tests, the haematological parameters data in the current investigation suggested that the methanol extract did not alter the generation of blood cells.

Serum biochemical parameters

All the serum biochemical parameters were normal in all the extract treated groups when compared with control group.

The biochemical analysis of the acute and subacute oral toxicity trials in the current investigation did not reveal any statistically significant changes. Thus, the non-significant change in serum concentration of ALT and AST in control and extract treated groups at all doses used in this study proposed that methanol extract do not damage the hepatocytes or secretory functions of the liver which in turn indicated the non-adverse effects of the extract.

Histopathology

a) Toxicity studies revealed that, the methanol extract did not cause any mortality and no organ specific histopathological changes, and these may be correlated with results of haematological and biochemical parameters. The present investigation demonstrates, at least in part, the safety of methanol extract suggesting its promising potential for pharmaceutical uses.

b) The results of the present study allow the substance to be ranked and classified according to the Globally Harmonized System of Classification and Labelling of Chemicals. Thus, the methanol extract can be classified as category 5 with low acute toxicity hazard, which was the lowest toxicity class. Therefore, it can be concluded that methanol extract has a direct relevance for preserving animal health because it is tolerated up to 5000 mg/kg body weight when given as a single dose. We can also draw the conclusion that methanol extract is safe when taken daily for 28 days in doses of 500, 1000, and 1500 mg/kg body weight.

VI. CONCLUSION

The goal of the current study was to assess the pharmacological effects, anti-inflammatory, analgesic, and toxicological qualities of a methanol extract of plant T's aerial parts, including its acute oral toxicity and repeated dose 28-day oral toxicity.

Wistar albino rats were given portulacastrum. The methanol extract of T. portulacastrum aerial parts' physical characteristics showed that the extract has an acidic pH and a powder-like consistency. The initial phytochemical evaluation of the methanol extract of T. portulacastrum aerial parts found the presence of numerous phytoconstituents, including proteins, carbohydrates, alkaloids, saponins, flavonoids, phenolic compounds, tannins, phytosterols, and triterpenoids.

At dosages of 200, 400, and 600 mg/kg, the methanol extract of T. portulacastrum aerial parts was tested for its in vivo anti-inflammatory and analgesic effects.

The extract at the dose of 400 and 600 mg/kg showed significant anti-inflammatory activity against carrageenan induced paw oedema and the same doses showed significant analgesic activity against acetic acid induced writhings in Wistar rats.

Acute oral toxicity study and repeated dose 28-day oral toxicity study was conducted in Wistar rats. The maximum tolerable dose of methanol extract of T. portulacastrum aerial partsin female rats was found to be more than 5000 mg/kg. The general condition of the animals did not change, and all the animals remained in normal health condition throughout the experiment.

In both toxicity studies, the haematology and serum biochemical parameters of the rats treated with extract did not significantly differ from the control groups, which could be explained by the non-toxic nature of the extract.

Furthermore, the heart, liver, kidney, spleen, and duodenum histopathological changes in the extract-treated rats did not exhibit any dose-dependent and no organ-specific inflammatory or degenerative microscopic changes, suggesting that the extract will not affect the functioning of visceral organs.

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