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Original Article

Acute and Sub-Acute Oral Toxicity Evaluation of Avicularin

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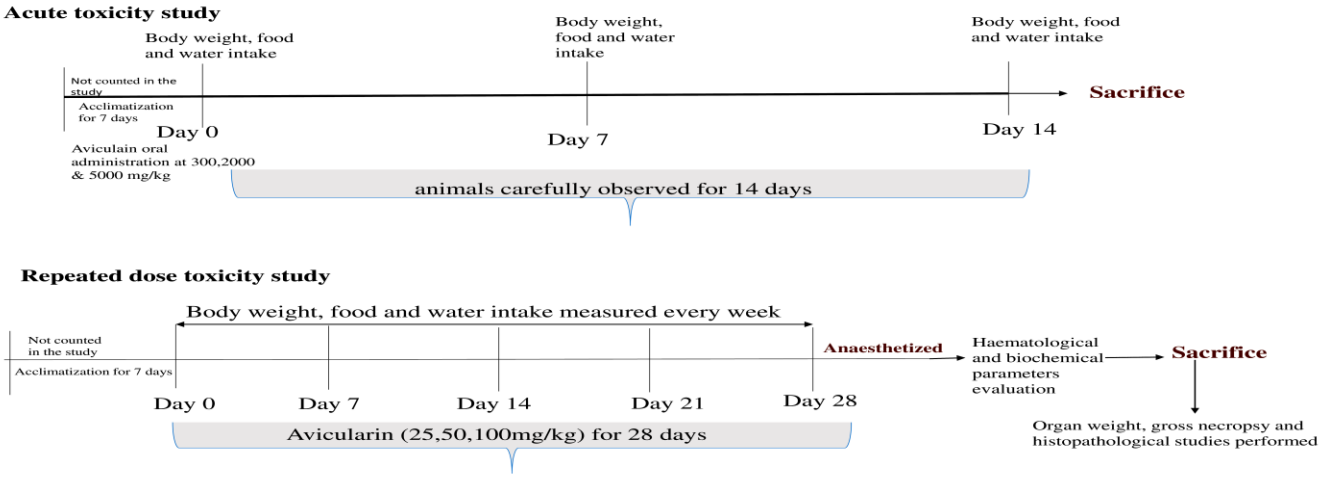
ABSTRACT

Avicularin is a bioactive flavonoid found mainly in blueberries, American cranberries, apples, and tea. Avicularin has been isolated from various plants, including *Polygonum aviculare, Rhododendron aureum,* and *Taxillus kaempferi*. Several preclinical studies have already demonstrated antioxidant, anti-inflammatory, antidepressant, and neuroprotective effects of avicularin. Moreover, avicularin is a promising phytoconstituent with broad therapeutic potential in various diseases. Therefore, a toxicity study is urgently needed so that the appropriate dose can be used in animal studies to demonstrate its potential effects. The main objective of this research article is to find out the maximum tolerated dose (MTD) and observe if there are any signs of toxicity in animals administered avicularin. The acute toxicity study was conducted according to the OECD guideline (TG 423). Single doses of 300, 2000, and 5000 mg/kg were administered, and the study was conducted for 14 days. In the acute toxicity study, the animals showed no mortality or changes in behavioural patterns. It was concluded that the maximum tolerated dose of avicularin is more than 5000 mg/kg. In a repeated dose toxicity study, we followed the OECD guideline (TG 407) and treated rats with 25, 50, and 100 mg/kg/day for 28 days. In the repeated toxicity study, the animals showed no differences in haematological and biochemical parameters. Histopathological examination of all organs revealed normal histology. Therefore, it is concluded from the results that repeated exposure to avicularin at 25, 50, and 100 mg/kg was considered safe in a 28-day toxicity study.

GRAPHICAL ABSTRACT



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Introduction

Flavonoids are the most commonly occurring phytochemicals in many plants, fruits, and vegetables [1, 2]. Avicularin is a quercetin-3-O-α-l-arabinofuranoside isolated from several plants, including *Polygonum aviculare*, *Rhododendron aureum*, and *Taxillus kaempferi* [3]. Avicularin is a bioactive flavonol that has a broad range of pharmacologic activities. Avicularin is considered as a potent antioxidant, hepatoprotective, anti-depressant, and anti-inflammatory agent [4]. According to Vo *et al.*, avicularin reduced lipopolysaccharide-induced inflammation by reducing the generation of nitric oxide, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) [5]. Avicularin has also been shown to improve human hepatocellular cancer [6] and rheumatoid arthritis via the MEK/NF-κB pathway [7], and decrease intracellular lipid accumulations [8].

Avicularin also exhibits good plasma concentration in a short period. Pharmacokinetic analysis of 5 mg/kg avicularin administered intravenously in six rats, revealed that avicularin has a faster clearance rate and short Tmax and half-life [9, 10]. Recently, avicularin also has been tested against SARS-COV-2 and showed good binding interaction with coronavirus. There are reports (computational studies), suggesting avicularin does not show any undesirable effect up to 5000 mg/kg [11], but no acute and repeated toxicity studies data available suggesting MTD of avicularin. Even though avicularin has numerous medicinal benefits, there are no available scientific reports or *in vivo* studies have been conducted on the safety profile of avicularin in animals. Moreover, it is vital to determine the avicularin toxicity in animals to learn more about its activities and potential effects. This study was carried out to determine the maximum tolerated dose (MTD) of avicularin and to look for any signs of toxicity in animals.

Materials and methods

Procurement of chemicals and kits

The avicularin was procured from Tokyo Chemical Industry Co., Ltd. Japan and supplied by TCI Chemical Pvt Ltd. Mumbai. Anti-coagulant (heparin) was procured from a local pharmacy shop and other biochemical diagnostic kits were purchased from Transasia Biomedicals Ltd., India.

Experimental animals

Both male and female *Sprague Dawley* rats aged 6-8 weeks (180-220 g) were procured from the National Institute of Biosciences in Pune, India. At the start of the experiment, all the animals were acclimatized for 7 days. The animal experimental and housing conditions such as 22 ± 2 ºC temperature, 75 ± 5% relative humidity, and 12 hr of light/dark cycle were maintained according to the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The protocol was approved by the Institutional Animal Ethics Committee (approval number- CPCSEA/IAEC/P-32(R)/2021).

Dose preparation and drug administration

Avicularin was dissolved in 0.5% CMC and administered to all the animals by oral gavage. All the animals were divided into 4 groups, group 1 served as a normal control group. Groups 2, 3, and 4 received 300, 2000, and 5000 mg/kg of avicularin, respectively. The body weight of the individual animal was considered while calculating the dose and that particular dose was administered to the animals.

Acute toxicity study

Acute study doses of avicularin were selected based on previous research papers and pilot studies. As per OECD guideline (TG 423), 300, 2000, and 5000 mg/kg doses were selected for the acute toxicity study [12, 13]. The female animals were split into three groups with three females in each group. Group 1 belonged to the normal control group. Animals fasted overnight, and then a single dose of avicularin was administered to groups 2, 3, and 4 at 300, 2000, and 5000 mg/kg.

The animals were carefully observed for changes in physical appearance such as changes in fur, skin, eye colour, pupil size, and behaviour for 14

days [14]. All animals were further observed for toxic signs like salivation, diarrhea, tremors, coma and lethargy, and change in body posture, rearing, grooming, and motor activity [15]. Bodyweight, food, and water intake were measured on 0, 7, and 14 days.

Sub-acute oral toxicity study

The subacute toxicity study of avicularin was conducted as per OECD guideline (TG 407) in male and female rats. Each group contains 10 animals (5 males and 5 females). Male and female animals were housed in separate cages. 0.5% CMC was provided to Group 1 (normal control group) for 28 days and groups 2, 3, and 4 were receive 25, 50, and 100 mg/kg/day of avicularin, respectively. The highest dose (100 mg/kg) in sub-acute toxicity study was selected based on acute toxicity of avicularin. Furthermore, bodyweight, food, and water intake were measured every week till the 28th day. Graphical abstract depicts a thorough methodology for a repeated toxicity study.

Haematological and biochemical analysis

Pentobarbital sodium was given to the rats via intraperitoneal injection for anaesthesia and blood was collected from the retro-orbital sinus in a tube containing 30 µl of EDTA (disodium ethylenediamine tetraacetic acid). Hematological parameters such as white blood cell (WBC) count, red blood cell (RBC) count, hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), mean corpuscular platelet count, red blood cell distribution width (RDW), and procalcitonin (PCT) were analysed using (Nihon kohden hematology analyzer). For biochemical estimation, serum was extracted from collected blood. The blood was allowed to clot for 30 minutes at 25 °C and thereafter centrifuged at 1500×g for 15 min at 4 °C to collect the serum and stored at -20 °C till further evaluation. Various biochemical parameters such as glucose, total protein, bilirubin, albumin, creatinine, total cholesterol (TP), HDL-Cholesterol, LDL-Cholesterol, triglyceride, alanine transaminase (ALT), blood urea nitrogen (BUN), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were estimated using (Erba Chem 7, Erba Mannheim, Germany). Electrolyte levels including sodium, potassium, and chloride levels were further measured.

Organ weight, gross necropsy, and histopathological study

At the end of the study, all the animals were sacrificed with the help of carbon dioxide asphyxiation. All vital organs such as the brain, heart, lungs, kidney, intestine, liver, testes, and ovaries have been removed, washed with the help of saline solution, placed on Petri plates, and weighed by using a weighing balance, and each organ weight was recorded. Thereafter, all the organs were collected in 10% formalin solution and were processed routinely and embedded in paraffin. The sections of 3-5 µm thickness were cut and stained with hematoxylin-eosin stain [16]. Histopathological studies of all vital organs were performed [17].

Statistical analysis

All of the data is presented as a mean ± SEM. The statistics were analysed using Graph Pad Prism (version 8) software. For the significance level study, a two-way ANOVA was used, accompanied by Bonferroni multiple comparison test.

Results and Discussion

Acute toxicity study

Body weight, food, and water intake

Bodyweight, food, and water intake were measured every week. In comparison to a normal control group, rats given 300, 2000, and 5000 mg/kg showed no noticeable changes in body weight, food, or water intake (Figure 1).

Behavioural parameters

All the animals showed no change in their physical appearance or behaviour. Similarly, all animals did not show abnormal salivation, diarrhea, lethargy, gripping force, reflex action, skin colour, fur, or pupil size.

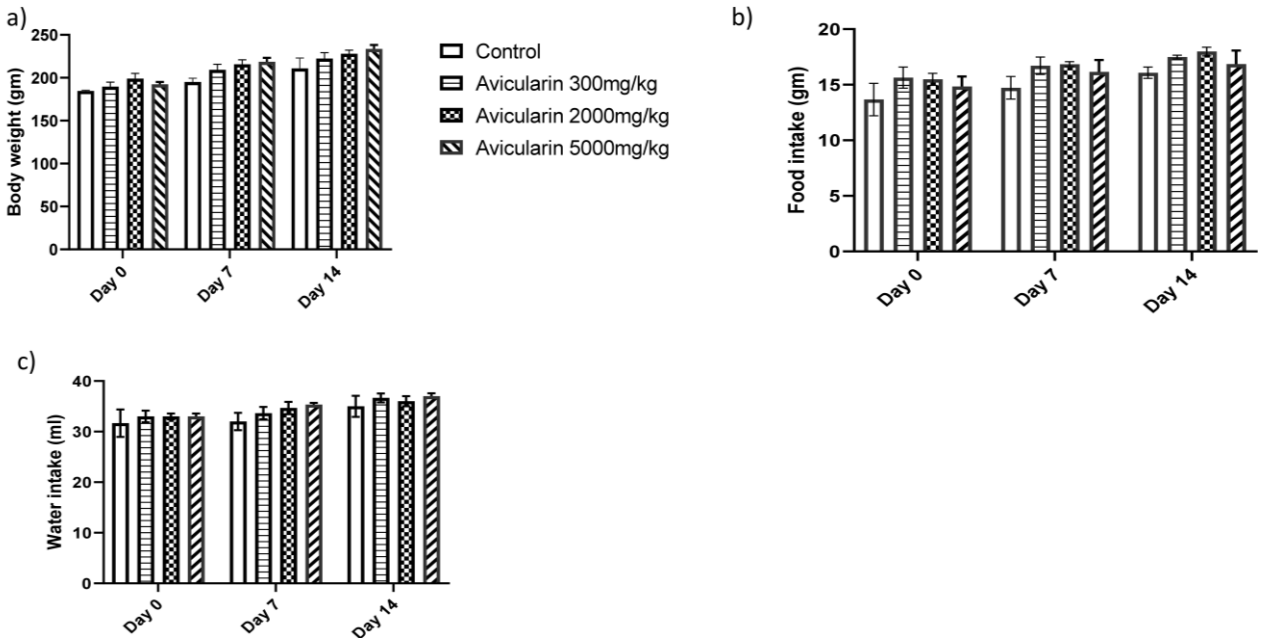

Figure 1: Effect of avicularin on (a) body weight, (b) food intake, and (c) water intake in acute toxicity study. Each point represents mean ± SEM (n= 3)

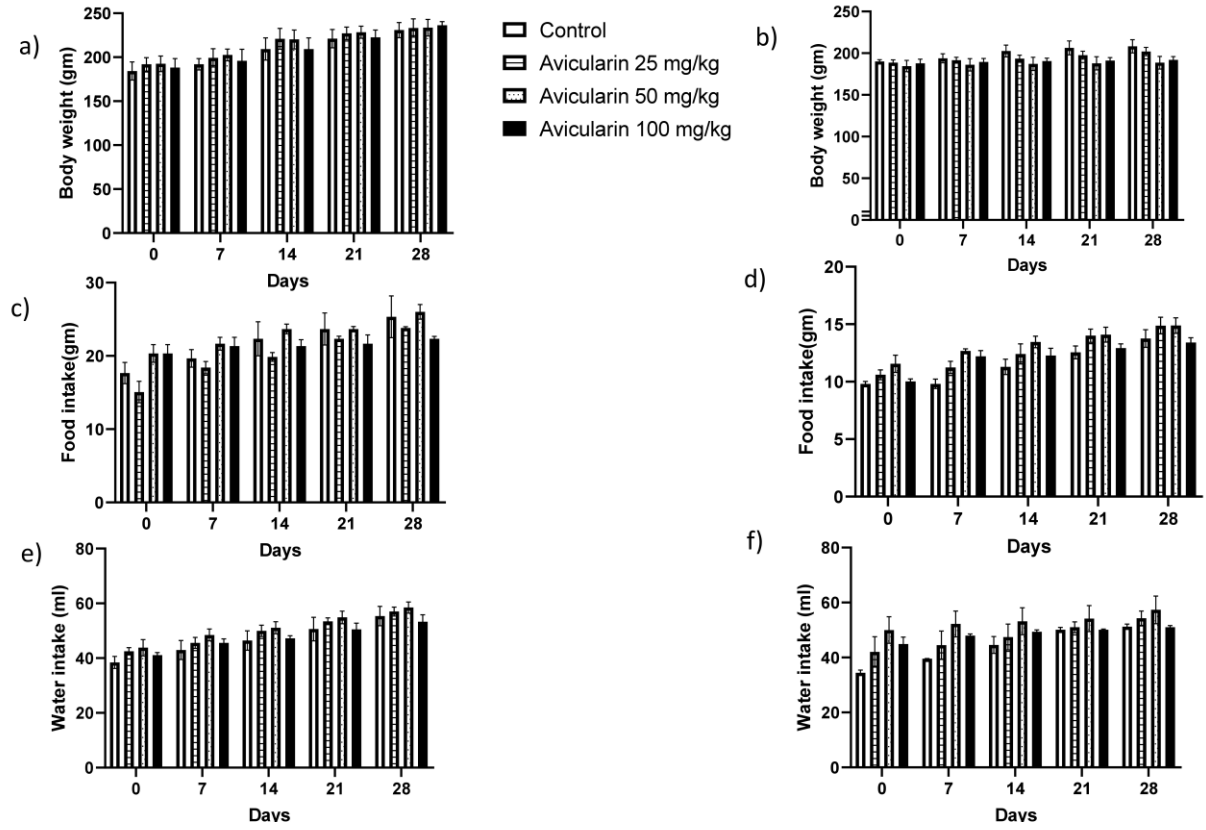

Figure 2: Effect of avicularin on body weight, food, and water intake in repeated toxicity study. Figure a, c, and e represent body weight, food, and water intake in male rats. Figures (b, d, and f) represent body weight, food, and water intake in female rats. All values are expressed as mean ± SEM (n=5

Body weight, food, and water consumption

Bodyweight, food, and water intake were measured weekly for both male and female rats. There were no significant changes in body weight, food consumption, or water intake was observed after 28 days of avicularin

administration (Figure 2). As a result, avicularin appears to have no impact on body weight, food intake, or water consumption, as compared with the normal control group.

Haematological and biochemical parameters

A range of haematological and biochemical parameters was also measured after 28 days in all animals. Avicularin does not affect haematological parameters (Tables 1-4). Avicularin-treated animals did not show any significant difference in haematological parameters when compared to the normal control group.

Table 1: Effect of avicularin on haematological parameters (male rats) in repeated toxicity study

Male animals				
Haematological Parameters	Control	Avicularin (25 mg/kg)	Avicularin (50 mg/kg)	Avicularin (100 mg/kg)
WBC 10^9/L	23.64 ± 2.18	22.1 ± 2.14	20.56 ± 2.57	19.26 ± 1.24
RBC 10^{12}/L	9.70 ± 0.17	7.98 ± 0.10	7.14 ± 0.41	10.99 ± 0.78
HGB g/L	148.8 ± 2.12	151.4 ± 2.69	149.6 ± 2.94	155.8 ± 3.90
HCT %	31.26 ± 1.23	43.98 ± 0.46	33.10 ± 1.96	40.86 ± 1.44
MCV fL	43.16 ± 1.56	52.18 ± 1.64	48.5 ± 1.54	53.46 ± 1.43
MCH Pg	20.12 ± 0.43	21.74 ± 0.42	20.64 ± 0.20	19.38 ± 0.42
MCHC g/L	334.2 ± 1.13	358.2 ± 2.15	314.8 ± 1.33	325.4 ± 1.25
PLT count 10^3/lL	711.2 ± 26.64	667.6 ± 12.31	689.8 ± 16.66	698.6 ± 25.82
RDW %CV	14.26 ± 0.90	13.54 ± 0.23	16.84 ± 0.34	15.22 ± 0.42
PCT %	0.29 ± 0.02	0.31 ± 0.02	0.29 ± 0.03	0.26 ± 0.04

(WBC: White Blood Cells, RBC: Red Blood Cells, HG: Haemoglobin, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, PLT count: Platelet count, RDW: Red Blood Cell Distribution Width, and PCT: Plateletcrit).

Table 2: Effect of avicularin on hematologic parameters (Female rats) in repeated dose toxicity

Female animals				
Haematological Parameters	Control	Avicularin (25 mg/kg)	Avicularin (50 mg/kg)	Avicularin (100 mg/kg)
WBC 10^9/L	18.64 ± 2.18	18.11 ± 1.14	16.46 ± 2.57	16.16 ± 1.24
RBC 10^{12}/L	7.13 ± 0.14	7.10 ± 0.20	6.26 ± 0.41	8.92 ± 0.78
HGB g/L	128.8 ± 3.42	137.4 ± 1.69	124.7 ± 1.94	136.4 ± 2.40
HCT %	32.46 ± 1.14	36.41 ± 1.86	35.21 ± 2.36	34.46 ± 2.44
MCV fL	40.26 ± 2.16	41.28 ± 1.43	38.15 ± 2.44	43.16 ± 2.11
MCH Pg	17.12 ± 1.23	20.92 ± 1.32	17.19 ± 0.17	15.18 ± 1.21
MCHC g/L	316.2 ± 2.16	313.1 ± 1.24	308.2 ± 2.33	311.4 ± 1.25
PLT count 10^3/lL	532.2 ± 18.24	569.4 ± 12.72	475.2 ± 16.26	545.9 ± 14.21
RDW %CV	12.41 ± 0.80	12.41 ± 0.32	15.18 ± 1.24	12.31 ± 1.25
PCT %	0.16 ± 0.01	0.19 ± 0.02	0.21 ± 0.03	0.16 ± 0.03

Table 3: Effect of avicularin on biochemical parameters (male rats) in repeated dose toxicity

Male animals				
Biochemical Parameters	Control	Avicularin (25 mg/kg)	Avicularin (50 mg/kg)	Avicularin (100 mg/kg)
Blood glucose (mg/dl)	109.21 ± 2.13	110.27 ± 1.53	116.24 ± 2.18	118.26 ± 3.28
Total protein, g/dL	8.16 ± 0.26	7.68 ± 0.31	8.12 ± 0.38	7.61 ± 0.21
Albumin, g/dL	4.34 ± 0.18	4.025 ± 0.83	3.132 ± 0.41	3.41 ± 0.58
Creatinine, mg/dL	0.6125 ± 0.01	0.6279 ± 0.03	0.7839 ± 0.03	0.6413 ± 0.03
BUN, mg/dL	17.19 ± 0.51	16.47 ± 0.95	18.81 ± 0.18	20.23 ± 0.49
ALT, IU/L	57.18 ± 1.67	59.61 ± 3.42	53.56 ± 1.63	58.45 ± 2.29

AST, IU/L	144.2 ± 2.19	120.4 ± 1.32	135.2 ± 3.52	118.1 ± 4.61
ALP, IU/L	22.42 ± 2.19	26.19 ± 1.60	29.43 ± 0.82	21.34 ± 0.94
Bilirubin (mg/dl)	0.29 ± 0.01	0.34 ± 0.02	0.38 ± 0.03	0.43 ± 0.02
Total cholesterol (mg/dl)	58.21 ± 2.45	64.41 ± 2.88	55.43 ± 3.89	62.30 ± 2.16
HDL-Cholesterol (mg/dl)	43.54 ± 3.08	48.29 ± 1.53	32.10 ± 2.08	49.63 ± 1.47
LDL- Cholesterol (mg/dl)	11.57 ± 1.02	10.89 ± 1.25	12.25 ± 0.48	10.58 ± 0.85
Triglyceride (mg/dl)	68.28 ± 7.68	57.09 ± 4.87	72.85 ± 10.29	65.46 ± 6.19
Sodium, Mmol/L	137.4 ± 2.13	103.6 ± 2.04	120.3 ± 2.10	124.5 ± 2.10
Potassium, Mmol/L	3.46 ± 0.16	3.15 ± 0.69	3.11 ± 0.63	2.63 ± 0.47
Chloride, Mmol/L	83.5 ± 0.63	92.1 ± 1.98	96.2 ± 2.18	89.9 ± 0.52

Table 4: Effect of avicularin on biochemical parameters (Female rats) in repeated dose toxicity

Female animals				
Biochemical Parameters	Control	Avicularin (25 mg/kg)	Avicularin (50 mg/kg)	Avicularin (100 mg/kg)
Blood glucose (mg/dl)	122.10 ± 5.57	113 ± 5.02	108.70 ± 4.90	126.90 ± 6.58
Total protein, g/dL	8.27 ± 0.19	7.71 ± 0.54	7.43 ± 0.44	8.73 ± 0.57
Albumin, g/dL	5.64 ± 0.18	4.086 ± 0.17	3.471 ± 0.32	4.27 ± 0.22
Creatinine, mg/dL	0.31±0.24	0.32 ±0.17	0.28 ± 0.22	0.25±0.19
BUN, mg/dL	0.68 ± 0.05	0.65 ± 0.03	0.67 ± 0.13	0.6 -2 ± 0.02
ALT, IU/L	21.54 ± 0.28	18.59 ± 1.25	20.37 ± 1.16	24.76 ± 0.83
AST, IU/L	47.24 ± 2.23	49.97 ± 4.29	56.21 ± 2.88	45.78 ± 3.46
ALP, IU/L	134.5 ± 1.14	134.6 ± 2.20	125.6 ± 4.10	114.0 ± 3.45
Bilirubin (mg/dl)	28.46 ± 1.17	22.02 ± 2.10	28.36 ± 1.72	26.44 ± 0.74
Total cholesterol (mg/dl)	42.14 ± 3.07	46.29 ± 1.23	51.10 ± 1.08	49.63 ± 1.37
HDL-Cholesterol (mg/dl)	9.57 ± 1.03	11.49 ± 1.27	11.23 ± 0.43	12.28 ± 0.87
LDL- Cholesterol (mg/dl)	76.32 ± 5.02	88.21 ± 8.23	87.49 ± 3.47	68.25 ± 6.12
Triglyceride (mg/dl)	64.17 ± 3.39	67.26 ± 4.13	59.34 ± 4.07	57.17 ± 1.46
Sodium, mmol/L	143.5 ± 1.93	123.5 ± 1.64	129.3 ± 1.20	135.0 ± 1.40
Potassium, mmol/L	2.80 ± 0.15	4.75 ± 0.12	2.22 ± 1.18	4.80 ± 0.10
Chloride, mmol/L	103.1 ± 1.29	110.9 ± 1.13	104.2 ± 3.08	103.7 ± 0.50

(BUN: Blood Urea Nitrogen, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, and ALP: Alkaline Phosphatase).

Histopathology study was performed at the end of the 28th day. No significant changes were found in the histopathological studies compared with the normal control group. All organs such as brain, heart, kidney, intestine, lungs, liver, ovaries, and testes showed normal histology after treatment with avicularin (100 mg/kg) for 28 days (Figures 3 and 4).

The purpose of this study is to investigate the toxicological profile of avicularin via the intragastric route. The study focused on measuring the safety and effect of acute and repeated doses of avicularin in animals. Earlier results specified the anti-depressant, anti-inflammatory, and antioxidant activity of avicularin. Some researchers also demonstrated the beneficial role of avicularin in multiple studies [18, 10]. In our previous research study, we have demonstrated the neuroprotective activity of avicularin against Aβ induced Alzheimer's Disease [19]. Furthermore, computation studies revealed that safety of avicularin, hence acute toxicity study was conducted at 300, 2000, and 5000 mg/kg. Despite the good therapeutic efficacy of avicularin, there is no systematic toxicity study has been conducted to date. Hence, a toxicity study is urgently needed so that the appropriate dose can

be used in animal studies to demonstrate its potential effect.

In addition, by considering its potential actions of avicularin and investigating the possible action of avicularin we have conducted a toxicity study of avicularin. In the acute toxicity study, rats treated with 300, 2000, and 5000 mg/kg did not display any change in body weight, food and water intake, behavioural parameters, external physical appearance, and mortality. This condition indicates that avicularin did not produce any mortality, or toxic signs and is safe up to 5000 mg/kg. A repeated-dose toxicity study was conducted after the acute toxicity study. In the acute toxicity study, the highest dose (5000 mg/kg) did not show any mortality or toxic signs, thus, 1/50th dose of 5000, i.e. 100 mg/kg selected as a maximum dose for repeated toxicity study [20]. To demonstrate dosage-related response and no-observed-adverse-effect level (NOAEL), a descending sequence of dose levels (50 and 25 mg/kg) of avicularin was selected for repeated toxicity study. Hence, repeated toxicity study was carried out on 25, 50, and 100 mg/kg doses [21]. The body weight, food and water consumption, behavioural parameters, and physical appearance of rats treated with 25, 50, and 100 mg/kg avicularin showed no significant changes throughout the study. Locomotor activity and respiratory activity were also found to be normal until the 28th day. Measurement of the haematological endpoint acts as a clinical indicator to study the presence or instigation of any disease. The haematological study primarily determines the initial effect of the drug on various blood parameters [20-24]. All the haematological parameters were found to be normal in the repeated dose toxicity study. Other than haematological parameters, biochemical parameters were also estimated. Concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are primarily evaluated to determine the hepatic function [25]. Abnormalities in ALT, AST, ALP, and bilirubin levels indicate liver damage or abnormality in liver function [26]. In the repeated-dose toxicity study, rats did not exhibit altered levels of ALT, AST, ALP, and bilirubin. Moreover, it is estimated that blood urea nitrogen and creatinine in the blood mainly control kidney function. High levels of BUN and creatinine suggest glomerular lesions in the kidney [27, 28]. Rats treated with avicularin showed no abnormal deviation in BUN and creatinine levels. Furthermore, total protein, cholesterol, and glucose levels were also found to be normal and did not exhibit any abnormal alterations. Sodium, potassium, and chloride concentrations were normal. All the rats showed a normal level of glucose, total protein (TP), bilirubin, albumin, urea nitrogen (BUN), creatinine, ALT, total cholesterol, AST, and ALP levels when compared with the normal control group (Table 5).

Table 5: Effect of avicularin on organ weight (male and female rats) in repeated dose toxicity

Relative organ weight (%)	Male animals			
	Control	Avicularin (25 mg/kg)	Avicularin (50 mg/kg)	Avicularin (100 mg/kg)
Brain	0.57± 0.04	0.55± 0.0333	0.54± 0.05	0.61 ± 0.07
Heart	0.53 ± 0.03	0.55 ± 0.02	0.48 ± 0.02	0.54 ± 0.02
Lung	0.63±0.156	0.66±0.0147	0.67±0.132	0.78±0.163
Liver	4.54 ± 0.16	4.42 ± 0.64	4.16 ± 0.35	4.26 ± 0.22
Kidney	0.39 ± 0.01	0.36 ± 0.03	0.33 ± 0.01	0.36 ± 0.02
Testes	0.53 ± 0.01	0.51 ± 0.02	0.51 ± 0.05	0.519 ± 0.04
Relative organ weight (%)	Female animals			
Brain	0.63 ± 0.03	0.69 ± 0.04	0.76 ± 0.04	0.79 ± 0.05
Heart	0.35 ± 0.02	0.41 ± 0.02	0.38 ± 0.04	0.36± 0.05
Lung	0.86 ± 0.129	0.82 ± 0.247	0.78 ± 0.124	0.79 ± 0.213
Liver	3.81 ± 0.19	3.76 ± 0.52	3.95 ± 0.31	4.09 ± 0.27
Kidney	0.37 ± 0.03	0.29 ± 0.05	0.32 ± 0.04	0.35 ± 0.02
Ovary	0.021 ± 0.001	0.027 ± 0.001	0.028 ±0.002	0.023 ± 0.003

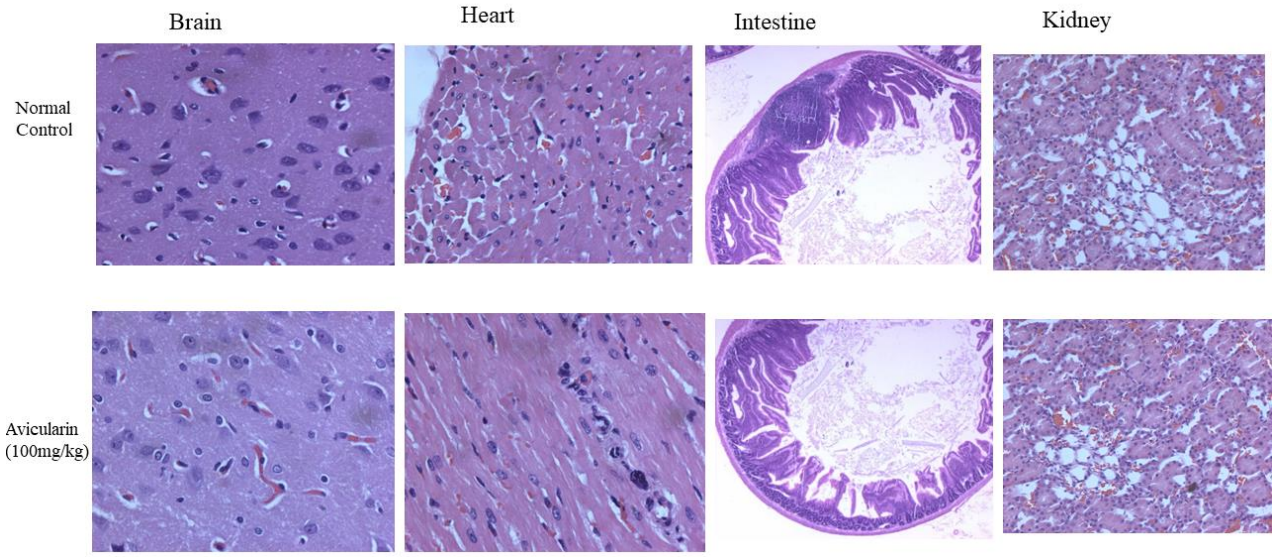

Figure 3: Histopathology of organs in repeated dose toxicity study (400X): The normal control group revealed normal histology brain, heart, lungs, kidney, liver, intestine, ovaries, and testes (Photograph courtesy of Dr. Bagal. Copyright 2021") this image is free domain

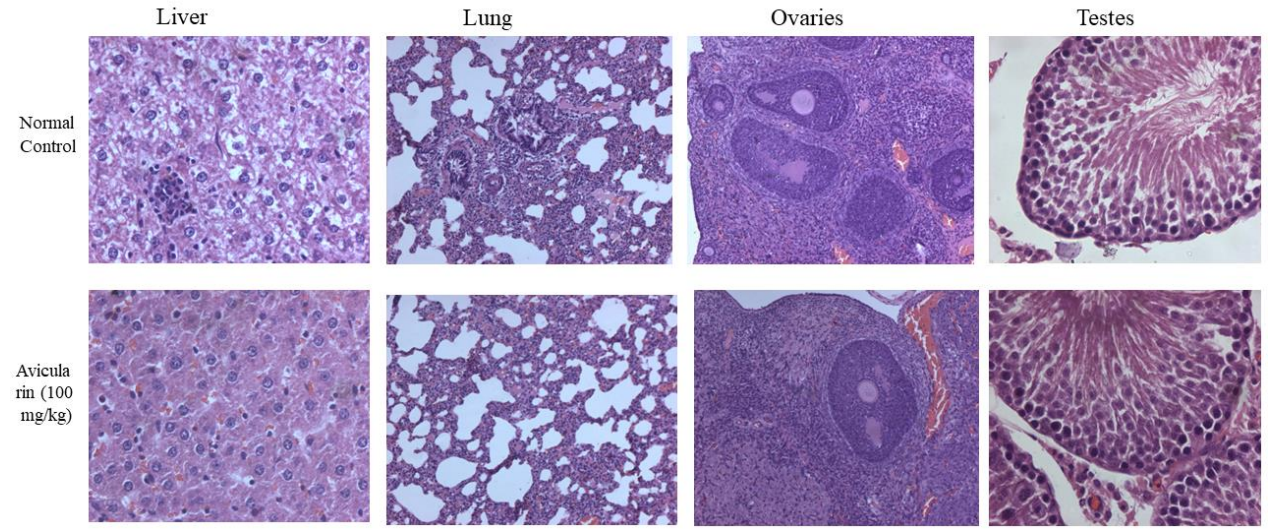

Figure 4: Histopathology of organs in repeated dose toxicity study (400X): Avicularin 100 mg/kg treated group revealed normal histology brain, heart, lungs, kidney, liver, intestine, ovaries, and testes. (Photograph courtesy of Dr. Bagal. Copyright 2021") this image is free domain

At the end of the study, individual organs were weighed and observed for any external morphological changes, rats treated with 25, 50, and 100 mg/kg showed no difference in organ weight, and all organs were found to be normal during gross necropsy. Histological studies of all vital organs revealed normal histology such as the brain shows normal morphology of neurons and glial cells in the cerebral cortex, heart histopathology study revealed normal morphology of myocytes of the myocardium of the heart, normal morphology of mucosal layer was found in the intestine, kidneys revealed normal morphology of tubule and glomerulus, normal morphology of hepatocytes in the liver was observed, normal morphology of alveoli in the lung, ovaries revealed normal morphology of follicular cells, and testes revealed normal morphology of spermatogonial cells. From these histopathology study results, avicularin did not cause any toxicity in organs in a repeated-dose toxicity study. Haematological, biochemical, and histological studies also support the safety of avicularin up to 100 mg/kg. To assess the clinical

applicability of avicularin, it's very important to evaluate the maximum tolerated dose level of avicularin. According to past research studies, MTD is an essential endpoint for assessing drug safety and selecting a range of therapeutic doses [29]. As a result, the maximum tolerate dose of avicularin was found to be more than 5000 mg/kg via the oral route in the acute toxicity study.

Conclusion

Animals administered via the intragastric route of avicularin at 5000 mg/kg dose did not produce any mortality or toxic signs. Avicularin in repeated dose toxicity study (25, 50, and 100 mg/kg) did not show any changes in behavioural pattern, or physical appearance, moreover, haematological, biochemical, gross necropsy, and histology of all organs were found to be normal. From the results, we can conclude that avicularin is safe up to 5000 mg/kg, and repeated dose of avicularin at 25, 50, and 100 mg/kg does not produce any undesirable side effects.

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Authors' Contributions

Mrs. Nikita Patil Samant has done all the lab work, literature search, and research activities. The accuracy of the research work was checked by Dr. Girdhari Lal Gupta. Both authors drafted and finalized the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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