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Toxicological Evaluation of Khellin in Rats: Acute and Sub-acute Toxicity Studies

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Abstract

Khellin (Khe), a furanochromone isolated from *Ammi visnaga* fruits, has been conventionally employed for its vasodilatory, anti-spasmodic, cardioactive, anti-asthmatic, and neuroprotective properties. Despite its broad pharmacological relevance, the comprehensive toxicological evaluation of Khe remains limited. This study intended to thoroughly evaluate acute and sub-acute oral toxicity of Khe in Wistar rats using OECD guidelines. Acute oral toxicity was assessed in female rats in accordance with OECD guideline 423 using a single dosage of 50 mg/kg. Sub-acute toxicity was evaluated in male and female Wistar rats given Khe orally at dosages of 5, 10, and 40 mg/kg/day for 28 days, per OECD guideline 407 (n = 6 per group). Clinical manifestations, death rates, body mass, and consumption of food and water were observed throughout the study. Hematological measures (red and white blood cell counts, haemoglobin, platelet indices, packed cell volume, erythrocyte indices, and differential leukocyte count) and serum biochemical markers associated with lipid profile, hepatic, and renal function were assessed at end of study. Gross necropsy, relative organ weights, and histopathological examination of major organs, including liver, kidney, heart, brain, spleen, colon, testes and ovaries were performed. The acute toxicity study at 50 mg/kg showed no clinical symptoms associated with mortality. In sub-acute research, repeated oral administration of Khe treatment doses produced no significant modifications in body weight, food and water intake, relative organ weights, haematological indices, or serum biochemical parameters compared with control animals. Histopathological evaluation of major vital organs revealed normal architecture with no evidence of treatment-related toxicity.

Major Findings: The results showed that Khe is well tolerated in Wistar rats after initial oral dose of 50 mg/kg and repeated oral administration up to 40 mg/kg/day for 28 days. This study establishes preliminary safety profile of Khe and supports its further preclinical development as a pharmacologically active natural compound.

Keywords: Acute Toxicity, Histopathology, Khellin, OECD Guidelines, Sub-acute Toxicity, Wistar Rats

1. Introduction

Complementary medicines, especially natural remedies made from medicinal plants, have gained popularity worldwide in recent decades as a means of treating and preventing a wide range of illnesses[1]. Medicinal plants remain a primary source of bioactive phytoconstituents and continue to contribute significantly to modern drug discovery and development[2]. Despite their widespread traditional use, many plant-derived compounds lack systematic toxicological evaluation, which is essential for ensuring safety and facilitating their translational and regulatory acceptance.

Acute and sub-acute toxicity studies are fundamental components of preclinical safety assessment, providing critical information regarding the potential hazards, target organ toxicity, and safe dose ranges of chemical substances and phytoconstituents. Acute toxicity studies involve single-dose exposure and are designed to identify early toxic manifestations and approximate lethal dose ranges, following internationally accepted guidelines such as OECD 423 [3]. In contrast, sub-acute toxicity studies involve repeated-dose administration over a defined period and are conducted to assess cumulative toxic effects, organ-specific toxicity and dose response relationship, in accordance with OECD 407 guidelines[4].

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These findings collectively contribute to risk assessment, hazard identification, and the safe development and therapeutic application of bioactive substances[5].

Ammi visnaga L. (Family: *Apiaceae/Umbelliferae*), commonly known as *khella*, is an aromatic flowering plant widely distributed across North Africa, Europe, the Eastern Mediterranean region, Atlantic Islands, Southwestern Asia and North America[6]. Traditionally, the fruits of *A. visnaga* have been employed for the management of mild angina pectoris, bronchial asthma, renal calculi, intestinal colic, dysmenorrhea and as a diuretic. Phytochemical investigations of *A. Visnaga* have been shown to contain diverse secondary metabolites, including furanochromones, coumarins, flavonoids, and essential oils[7].

Among these constituents, Khellin (Khe) and visnagin are the principal furanochromone derivatives present in the seeds of *A. visnaga*[8]. Khe, characterised by a γ-pyrone-containing furanochromone structure, has been extensively investigated for its potent vasodilatory and antispasmodic properties. These pharmacological effects led to its historical use in the management of angina pectoris and inspired the development of synthetic antiarrhythmic agents[9]. Beyond its cardiovascular applications, Khe has shown a variety of biological actions such as smooth muscle relaxant and anti-asthmatic properties, anti-inflammatory activity, melanin synthesis inhibition, nephrolithiasis prevention, cerebroprotective and anti-parkinsonian effects, as well as anticancer and chemopreventive properties[10-14]. Despite its extensive pharmacological profile and longstanding traditional use, the toxicological safety of Khe has not been comprehensively established through standardised experimental protocols. Medicinal plants remain important sources of bioactive secondary metabolites with therapeutic potential. Despite the extensive pharmacological profile of Khe, comprehensive toxicological evaluation using standardised OECD guidelines remains limited. Previous studies mainly focused on pharmacological efficacy and lacked repeated-dose toxicity assessment, detailed histopathology, and standardised safety evaluation. Therefore, the present study was designed to evaluate the acute and sub-acute toxicity profile of Khe in Wistar rats using OECD-compliant protocols.

To address this gap, in compliance with OECD guidelines, the current study sought to assess the acute and sub-acute oral toxicity of Khe in Wistar rats. Clinical symptoms, changes in body weight, and food and water intake were all methodically evaluated, along with other parameters, such as haematological, biochemical, relative organ weights, and histopathological alterations in major vital organs, to generate robust preclinical safety data for Khe.

2. Materials and Methods

2.1 Drug Procurement

Khe was procured from Tokyo Chemical Industry (India) Private Limited, India, with certified purity suitable for experimental use. Serum biochemical diagnostic kits were obtained from Accurex Biomedical Private Limited, India. Dimethyl sulfoxide and Polyethylene glycol (1:3) used as a solvent was purchased from Merck Specialities Private Limited, Mumbai, India. All other chemicals and reagents employed in the study were of analytical grade and obtained from standard viable sources.

2.2 Experimental Animals

Female and male Wistar rats (200-250 g) were obtained from the animal house of the National Institute of Bio Sciences in Pune. The animals were housed in polypropylene cages under conventional circumstances (temperature 25 ± 2 °C, relative humidity 65%, and light/dark cycle 12/12 hours). Fresh paddy husk is used as the bedding material. The animals were given a standard laboratory animal feed and *ad libitum* water in clean polypropylene bottles with stainless steel sipper tubes. The animals were acclimatised to laboratory conditions before the test. Experiments were conceived and carried out in compliance with ethical guidelines authorised by the Committee for the Control and Supervision of Experiments on Animals (CCSEA) and the Institutional Animal Ethical Committee (PCP/IAEC/2024/4-11).

2.3 Acute Toxicity Study

The acute toxicity test was performed in accordance with OECD guideline 423 for chemical testing (OECD, 2001). Before beginning the test, all animals were fasted overnight. Healthy young Wistar rats, nulliparous, non-pregnant females weighing between 200 and 250 g, were given a single oral dosage of 50 mg/kg of Khe. The animals were examined individually for the first 30 minutes for any signs of behavioural changes or physiological appearance, and then at regular intervals throughout the

first 24 hours, with special emphasis on the first 4 hours. All animals will be monitored for 14 days for symptoms of toxicity, morbidity, and mortality, such as changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, and central nervous systems, as well as behavioural patterns.

2.4 Sub-acute Toxicity Study

The sub-acute toxicity of Khe was assessed in accordance with OECD Guideline 407. Wistar rats were separated into eight groups, each with six male and female rats. The two-vehicle control (VC) male and female groups received distilled water and other six group of male and female received Khe low, medium and high dose of 5 mg/kg,10 mg/kg and 40 mg/kg respectively (VC-F: Vehicle Control Female, VC-M: Vehicle Control Male, Khe F-5: Khe Female Low Dose, Khe F-10: Khe Female Medium Dose, Khe F-40: Khe Female High Dose, Khe M-5: Khe Male Low Dose, Khe M-10: Khe Male Medium Dose, Khe M-40: Khe Male High Dose) were given Khe orally every day for 28 days. During the treatment period, the animals' body weight (BW), water intake, and food consumption were recorded before and after the trial. At the end of the trial (on the 29th day), the animals were anaesthetised with sodium thiopentone (50 mg/kg, i.p.) and blood was taken from the retro-orbital sinus for biochemical and hematologic analysis. Red blood cells (RBC cells/µL), haemoglobin (Hb g/dL), white blood cells (WBC cells/µL), mean corpuscular volume (MCV fL), lymphocyte percentage (LYM%), mid-sized cell percentage (MID%), neutrophil (NEUT%), hematocrit (HCT), and platelet (cells/µL) count. A blood samples was centrifuged at 3500 rpm for 10 minutes, and the separated serum was analysed for biochemical parameters, *viz*. aspartate aminotransferase (AST U/L), alanine aminotransferase (ALT U/L), Alkaline Phosphatase (ALPU/L), albumin (Ab g/dL), globulin (Gb g/dL), total protein (TP g/dL), total bilirubin (TB mg/dL), cholesterol (CH mg/dL), triglycerides (TG mg/dL), high density lipoprotein (HDL mg/dL), low density lipoprotein (LDL mg/dL), blood urea nitrogen (BUN mg/dL), creatinine (mg/dL), urea (mg/dL), and uric acid (mg/dL). The rats were euthanised via cervical dislocation, and the organs, liver, kidney, heart, brain, spleen, colon, testes and ovaries were removed, weighed, processed, and histopathologically examined. For histopathological evaluation, tissue samples were fixed in 10% neutral buffered formalin for 24–48 hours, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Tissue sections of 4–5 µm thickness were prepared using a rotary microtome and stained with hematoxylin and eosin (H and E). Histological examinations were performed under a light microscope at 10× and 40× magnifications by a blinded histopathologist to avoid observational bias.

2.5 Statistical Analysis

Data was compared between groups using one-way ANOVA, followed by the Tukey multiple comparison test. The repeated measures two-way ANOVA was used to examine the changes in water intake, food consumption, and body weight during the study. Results are shown as Mean ± Standard Error of the Mean (SEM). A P-value of less than 0.05 indicates statistical significance. All data were analysed by GraphPad Prism (Version 5).

3. Results

3.1 Acute Toxicity of Khe

Khe therapy in rats resulted in no mortality at a fixed dose, 50 mg/kg/body weight. Furthermore, no obvious evidence of acute toxicity was detected. For 14 days, no salivation, aggressiveness, increasing fur, or writhing were seen. Hence, according to OECD 423, the LD_{50} dose of Khe was 50mg/kg, indicating low to moderate acute toxicity at the tested dose.

3.2 Sub-acute Toxicity of Khe

3.2.1 Effect of Khe on Body Weight in Sub-acute Toxicity Study in Rats

Oral administration of Khe at 5, 10, and 40 mg/kg/day for 28 days did not kill any of the rats, and no dose showed symptoms of toxicity at any point during the experiment. There was no significant difference in BW between the VC and Khe (5, 10, and 40 mg/kg dose) treated rats (Figure 1).

The data were presented as Mean ± SEM (n = 6), and one-way ANOVA was used for analysis, followed by Tukey's multiple comparison test. VC-F: Female Vehicle Control Female, VC-M: Vehicle Control Male, Khe F-5: Khe Female Low Dose, Khe F-10: Khe Female Medium Dose, Khe F-40: Khe Female High Dose, Khe M-5: Khe Male Low Dose, Khe M-10: Khe Male Medium Dose, Khe M-40: Khe Male High Dose. There was no significant change in body weight in the Khe-treated and VC group ($P < 0.05$).

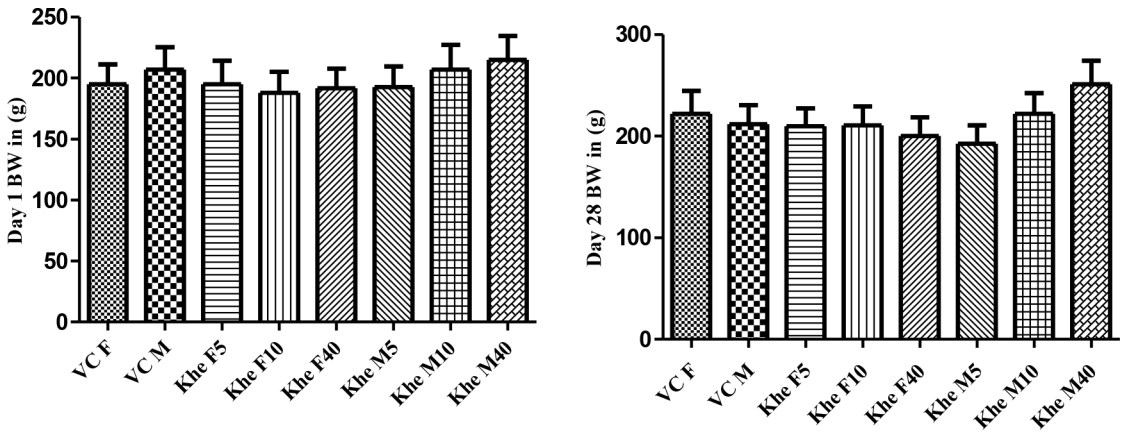


Figure 1. Effect of Khe (5, 10 and 40 mg/kg) on body weight (day 1 and day 28) in sub-acute toxicity in rats.

Table 1. Effects of Khe on water and food intake in sub-acute toxicity in rats

Parameters	Animal (Sex)	Day	Study Group			
			VC	Khe (5 mg/kg)	Khe (10 mg/kg)	Khe (40 mg/kg)
Water Intake (ml/24 h)	Female	Day 1	22.72±2.46	24.17±2.25	22.33±2.56	23.67±2.51
		Day 28	23.30±2.05	21.25±2.27	22.18±2.55	24.08±2.31
	Male	Day 1	24.00±2.03	24.67±2.09	25.42±2.95	26.05±2.08
		Day 28	23.08±2.12	25.62±2.26	24.58±2.28	24.43±2.15
Food Intake (g/24 h)	Female	Day 1	12.88±1.3	13.77±1.41	16.43±1.53	16.65±1.03
		Day 28	13.38±1.30	11.98±1.37	16.27±1.18	17.23±1.35
	Male	Day 1	13.07±1.63	15.75±1.38	16.00±1.16	17.00±1.04
		Day 28	15.33±1.68	15.50±1.09	17.83±1.20	16.10±1.68

The data were presented as Mean ± SEM (n = 6), and one-way ANOVA was used for analysis, followed by Tukey's multiple comparison test. There was no significant change in water intake and food intake in the Khe-treated and VC group ($P < 0.05$). VC-F: Vehicle Control Female, VC-M: Vehicle Control Male, Khe F-5: Khe Female Low Dose, Khe F-10: Khe Female Medium Dose, Khe F-40: Khe Female High Dose, Khe M-5: Khe Male Low Dose, Khe M-10: Khe Male Medium Dose, Khe M-40: Khe Male High Dose.

3.2.2 Effect of Khe on Water and Food Intake in a Sub-Acute Toxicity Study in Rats

Changes in water intake and food consumption on Day 1 and Day 28 were monitored and represented in Table 1. No changes were observed in food and water intake after the treatment of rats with Khe (5, 10 and 40 mg/kg) for 28 days in sub-acute toxicity tests. Animals treated with Khe (5, 10, and 40 mg/kg) did not significantly vary from VC animals in terms of their food and water intake on Days 1 and 28 (Table 1).

3.2.3 Effect of Khe on Organ Weights in a Sub-acute Toxicity Study in Rats

On the last day of the treatment period (28 days), the weight of the heart, liver, kidney, spleen, brain, and colon was measured. No significant change was observed in the organ weights (heart, liver, kidney, spleen, brain, and colon) of rats treated with Khe (5, 10 and 40 mg/kg) for 28 days compared to VC rats (Figure 2).

The data were presented as Mean ± SEM (n = 6), and one-way ANOVA was used for analysis, followed by Tukey's multiple comparison test. VC-F: Female Vehicle Control, VC-M: Male Vehicle Control, Khe F-5: Khe Female Low Dose, Khe F-10: Khe Female Medium Dose, Khe F-40: Khe Female High Dose, Khe M-5: Khe Male Low Dose, Khe M-10: Khe Male Medium Dose, Khe M-40: Khe Male High Dose, there was no significant change on Organs Weight (Heart, Liver, Kidney, Spleen, Brain, and Colon) in Sub-acute toxicity in rats in the Khe treated and VC group ($P < 0.05$).

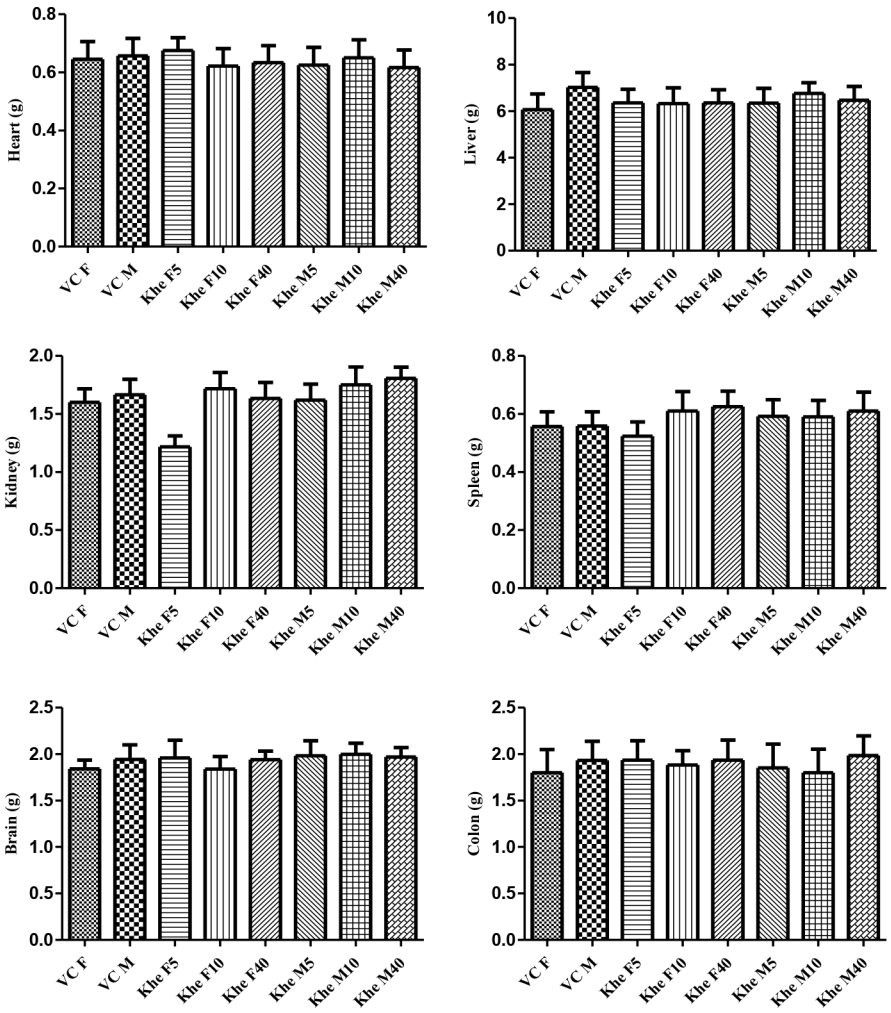


Figure 2. Effect of Khe (5, 10 and 40 mg/kg) on organs' weight (heart, liver, kidney, spleen, brain, and colon) in sub-acute toxicity in rats.

3.2.4 *Effect of Khe on Haematological Parameters in Sub-acute Toxicity Study in Rats*

Figure 3 showed the results of haematological examination (RBC, Hb, WBC, LYM, MID, NEUT, HCT, MCV and PLT) of Khe in sub-acute toxicity in rats. There were no significant differences in the RBC, Hb, WBC, LYM, MID, NEUT, HCT, MCV and PLT levels in male and female rats treated with Khe (5, 10 and 40 mg/kg) compared to VC rats (Figure 3).

The data were presented as Mean ± SEM (n = 6), and one-way ANOVA was used for analysis, followed by Tukey's multiple comparison test. VC-F: Female Vehicle Control, VC-M: Male Vehicle Control, Khe F-5: Khe Female Low Dose, Khe F-10: Khe Female Medium Dose, Khe F-40: Khe Female High Dose, Khe M-5: Khe Male Low Dose, Khe M-10: Khe Male Medium Dose, Khe M-40: Khe Male High Dose. There was no significant change in haematological parameters RBC, Hb, WBC, LYM%, MID%, NEUT%, HCT, MCV and PLT in sub-acute toxicity in Khe treated and VC group ($P < 0.05$).

3.2.5 *Effect of Khe on Serum Biochemical Parameters in a Sub-acute Toxicity Study in Rats*

Effect of Khe (5, 10 and 40 mg/kg) on various biochemical parameters, such as liver, renal and lipid levels, is represented in Figures 4, 5, 6 and 7. There was no significant changes observed in the serum liver (AST, ALT, ALP, Ab, Gb, TP and TB), Kidney (BUN, Creatinine, Urea, Uric acid) and Lipid parameters (CH, TG, HDL and LDL) levels in rats treated with Khe (5, 10 and 40 mg/kg) compared to VC rats (Figures 4-7).

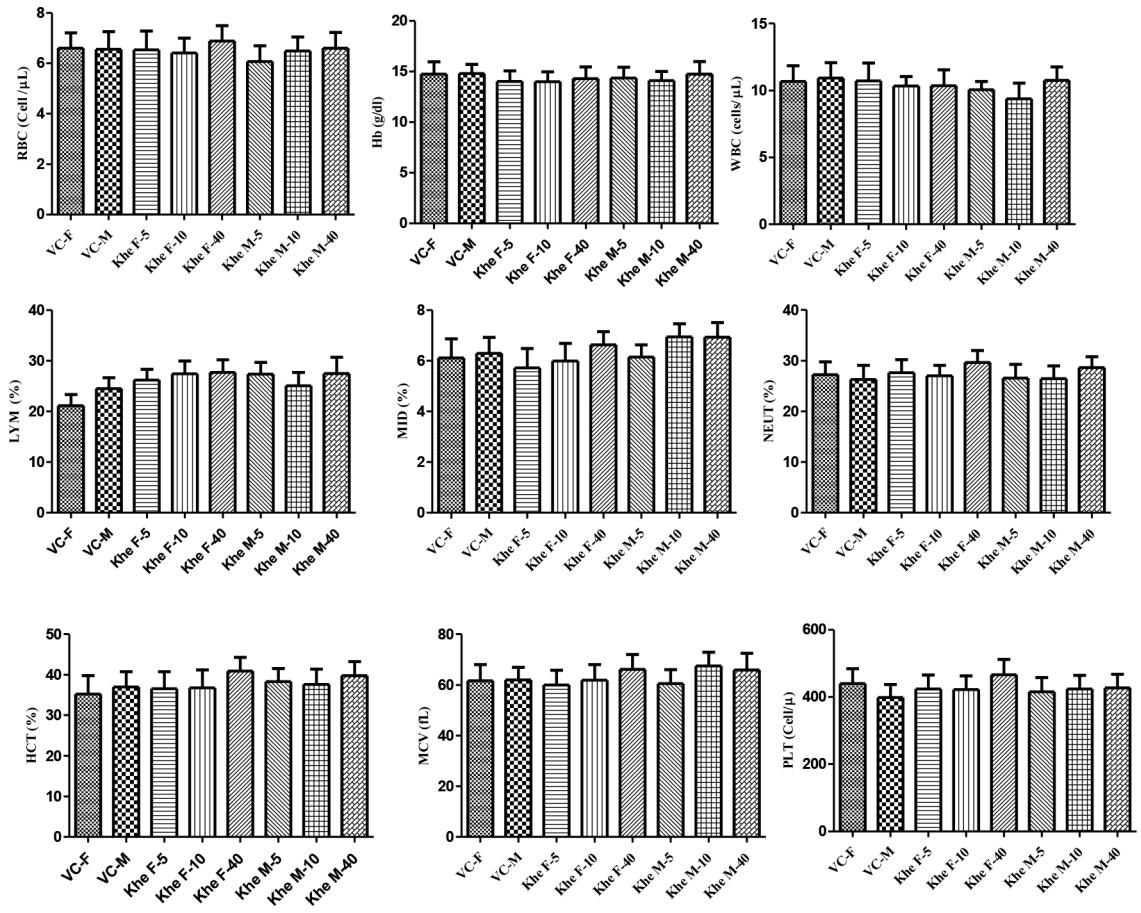


Figure 3. Effect of Khe (5, 10 and 40 mg/kg) on haematological parameters RBC, Hb, WBC, LYM%, MID%, NEUT%, HCT, MCV and PLT in sub-acute toxicity in rats.

The data were presented as Mean ± SEM (n = 6), and one-way ANOVA was used for analysis, followed by Tukey's multiple comparison test. VC-F: Female Vehicle Control, VC-M: Male Vehicle Control, Khe F-5: Khe Female Low Dose, Khe F-10: Khe Female Medium Dose, Khe F-40: Khe Female High Dose, Khe M-5: Khe Male Low Dose, Khe M-10: Khe Male Medium Dose, Khe M-40: Khe Male High Dose. There was no significant change in liver parameters (AST, ALT and ALP) in sub-acute toxicity in Khe treated and VC group ($P < 0.05$).

The data were presented as Mean ± SEM (n = 6), and one-way ANOVA was used for analysis, followed by Tukey's multiple comparison test. VC-F: Female Vehicle Control, VC-M: Male Vehicle Control, Khe F-5: Khe Female Low Dose, Khe F-10: Khe Female Medium Dose, Khe F-40: Khe Female High Dose, Khe M-5: Khe Male Low Dose, Khe M-10: Khe Male Medium Dose, Khe M-40: Khe Male High Dose. There was no significant change in liver parameters (Ab, Gb, TP and TB) in Sub-acute toxicity in Khe-treated and VC group ($P < 0.05$).

The data were presented as Mean ± SEM (n = 6), and one-way ANOVA was used for analysis, followed by Tukey's multiple comparison test. VC-F: Female Vehicle Control, VC-M: Male Vehicle Control, Khe F-5: Khe Female Low Dose, Khe F-10: Khe Female Medium Dose, Khe F-40: Khe Female High Dose, Khe M-5: Khe Male Low Dose, Khe M-10: Khe Male Medium Dose, Khe M-40: Khe Male High Dose. There was no significant change in kidney parameters (BUN, Creatinine, Urea and Uric acid) in Sub-acute toxicity in Khe treated and VC group ($P < 0.05$).

The data were presented as Mean ± SEM (n = 6), and one-way ANOVA was used for analysis, followed by Tukey's multiple comparison test. VC-F: Female Vehicle Control, VC-M: Male Vehicle Control, Khe F-5: Khe Female Low Dose, Khe F-10: Khe Female Medium Dose,

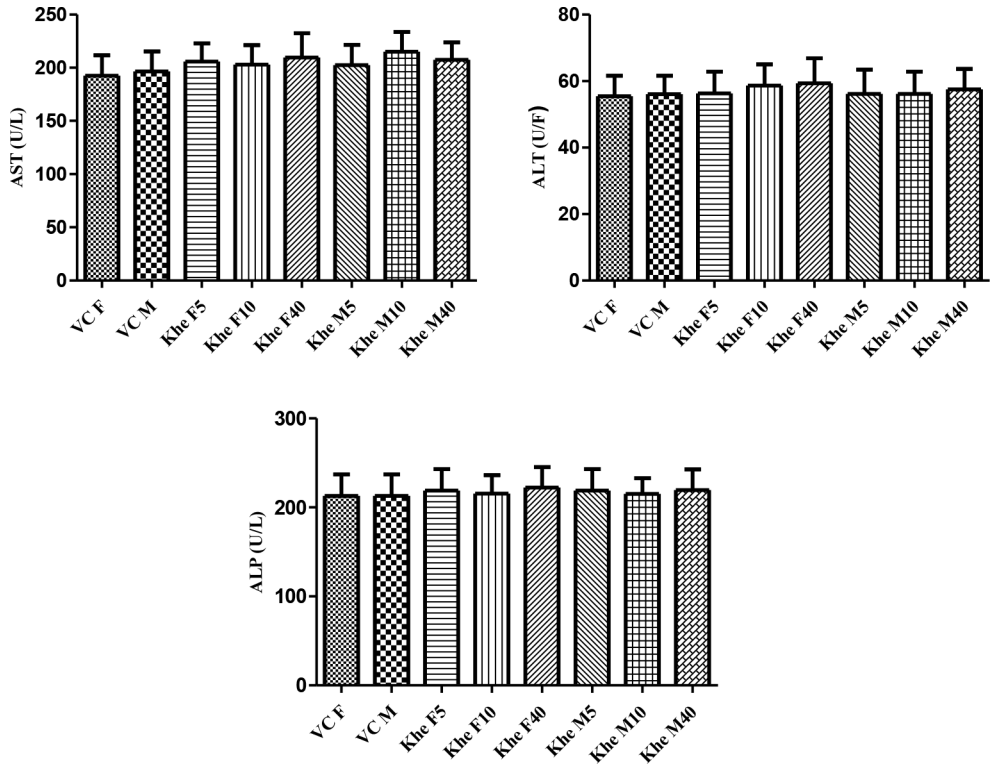


Figure 4. Effect of Khe (5, 10 and 40 mg/kg) on liver parameters (AST, ALT and ALP) in sub-acute toxicity in rats.

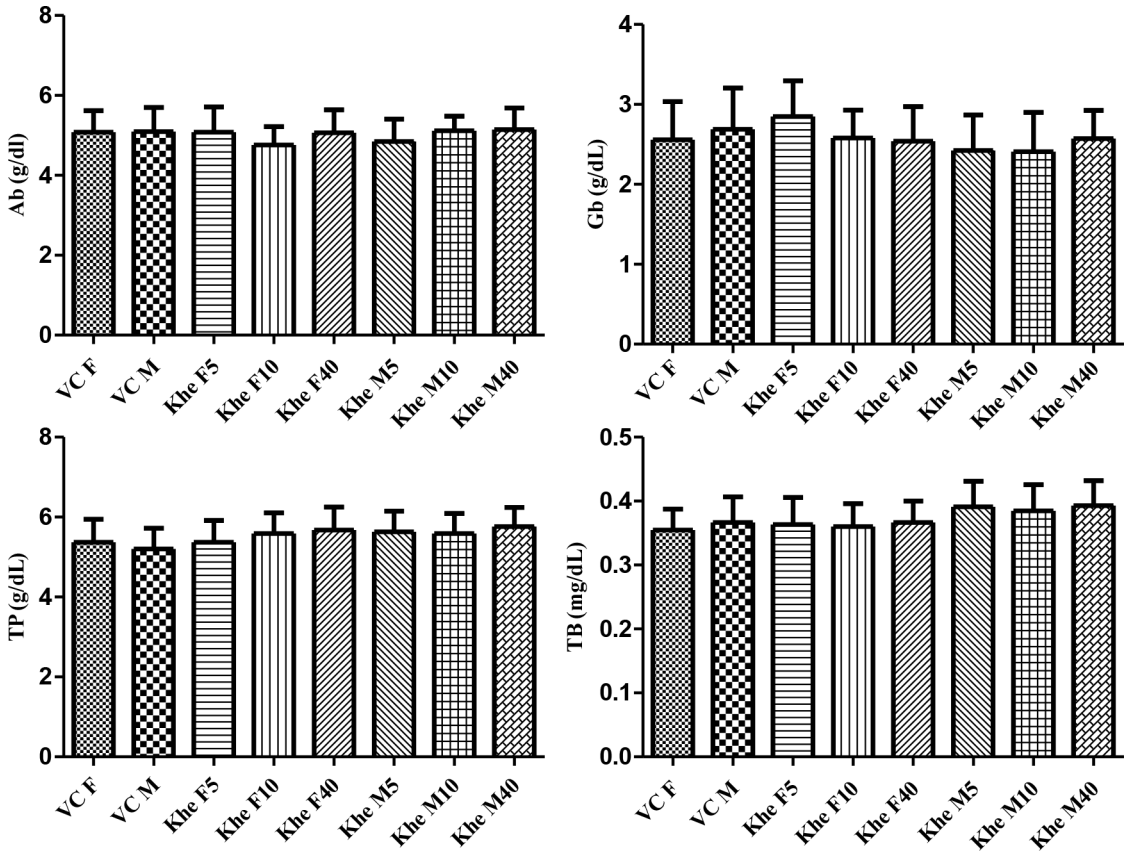


Figure 5. Effect of Khe (5, 10 and 40 mg/kg) on liver parameters (Ab, Gb, TP and TB) in sub-acute toxicity in rats.

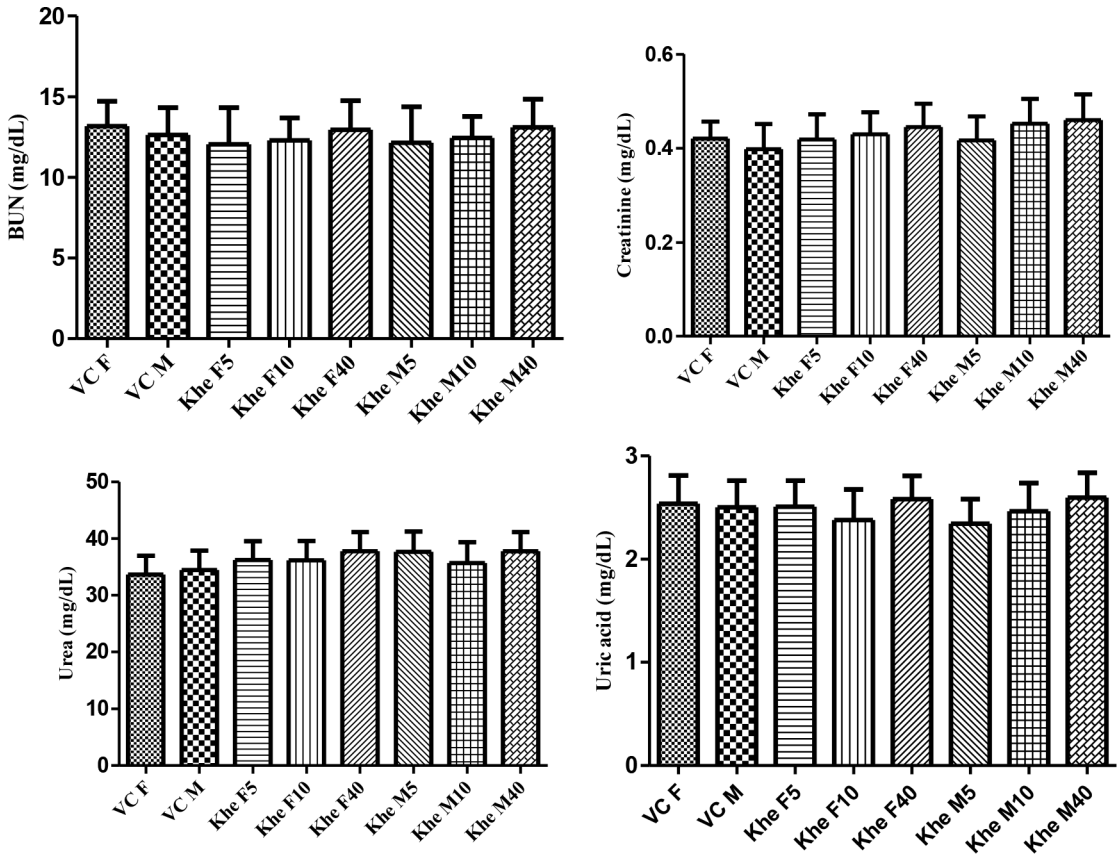


Figure 6. Effect of Khe (5, 10 and 40 mg/kg) on kidney parameters (BUN, Creatinine, Urea and Uric acid) in sub-acute toxicity in rats.

Khe F-40: Khe Female High Dose, Khe M-5: Khe Male Low Dose, Khe M-10: Khe Male Medium Dose, Khe M-40: Khe Male High Dose. There was no significant change in lipid parameters (CH, TG, HDL and LDL)in Sub-acute toxicity in Khe-treated and VC group ($P < 0.05$).

3.2.6 *Effect of Khe on Histopathological Analysis in Sub-acute Toxicity Study in Rats*

The histological structure of the brain in Khe-treated rats (5, 10, and 40 mg/kg) was comparable to the normal pattern of the organ in VC animals. No modifications to the normal brain structural architecture were identified (Figure 8). The histological structure of the heart in Khe (5, 10, and 40 mg/kg) treated rats was similar to the normal pattern of the organ in VC animals.

No alterations in cardiac muscle fibres in the myocardium were found (Figure 9). The histological structure of the colon in Khe (5, 10, and 40 mg/kg) treated rats was similar to the normal pattern of the organ in VC animals. No modifications to the usual colon structural architecture were found (Figure 10). Histopathological examination of colon tissue revealed normal glandular stomach mucosa with intact epithelial layers, as well as normal submucosa and serosa. The histological structure of the kidney in Khe (5, 10, and 40 mg/kg) treated rats was similar to the normal pattern of the organ in VC animals. There were no alterations to the morphology of the glomeruli or renal tubules (Figure 11). The histological structure of the liver in all Khe (5, 10, and 40 mg/kg) treated rats was similar to the normal pattern of the organ in VC rats. Hepatic lobular architecture and hepatocytes remained unchanged (Figure 12). The histological structure of the spleen in all Khe (5, 10, and 40 mg/kg) treated rats was similar to the normal pattern of the organ in VC rats. There were no modifications identified in the red and white pulps of spleen tissue with an appropriate lymphoid cellular population (Figure 13). Histopathological

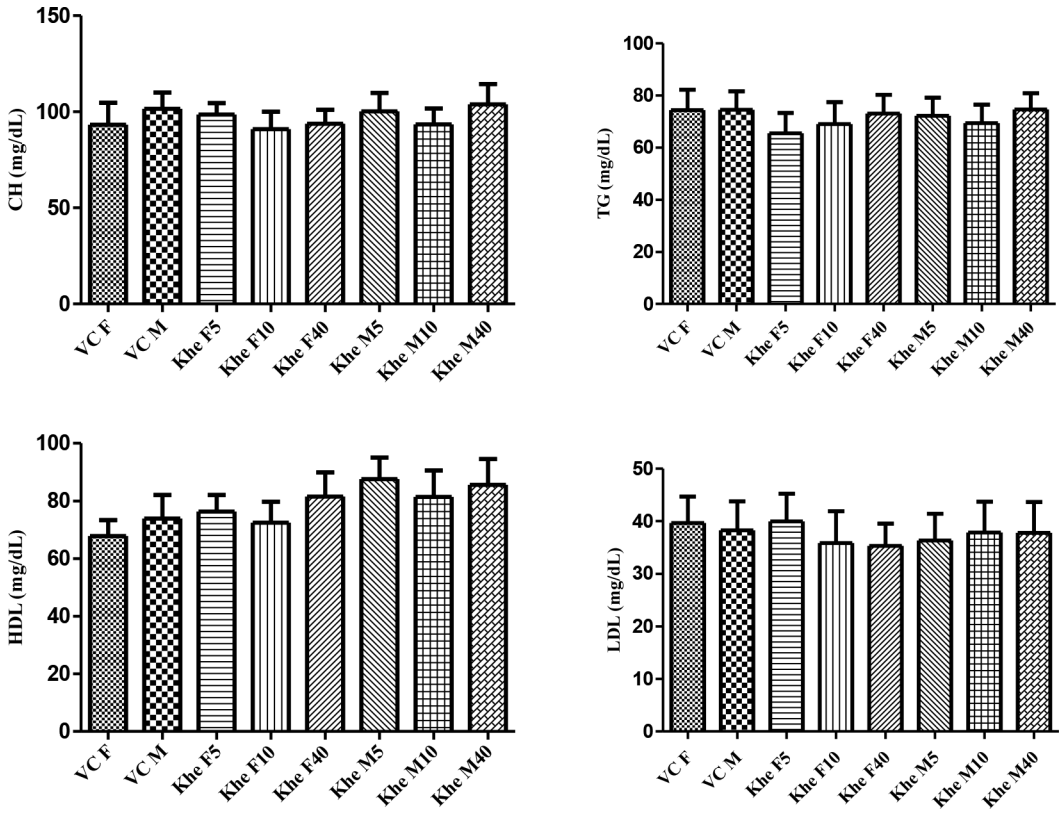


Figure 7. Effect of Khe (5, 10 and 40 mg/kg) on lipid parameters (CH, TG, HDL and LDL) in sub-acute toxicity in rats.

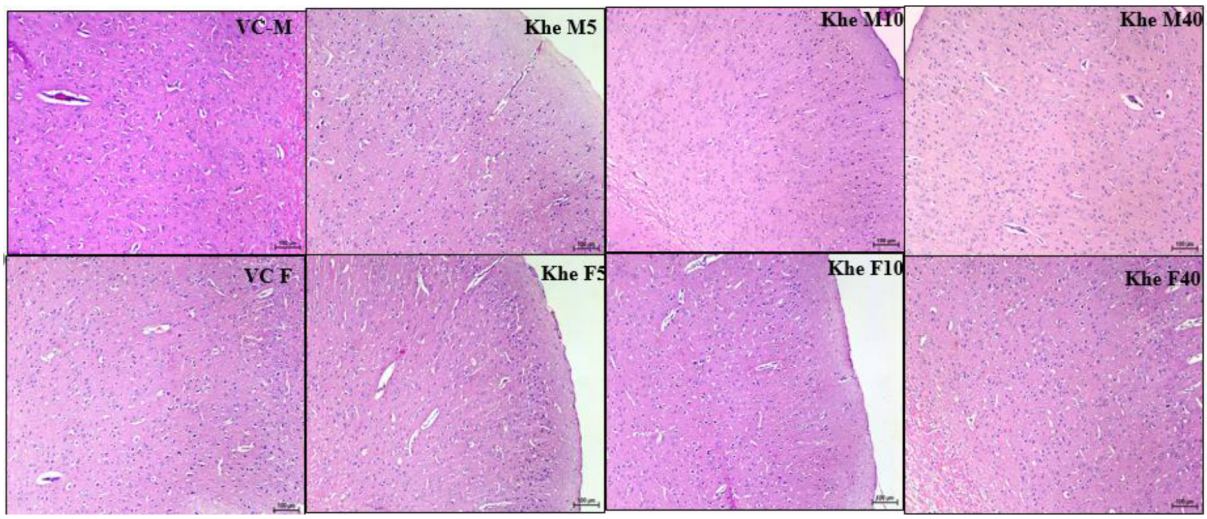


Figure 8. Effect of Khe (5, 10 and 40 mg/kg) on brain histopathology in sub-acute toxicity in rats stained with H and E, examined at 10x (100 µm). F: Female and M: Male.

evaluation of the testes and ovaries revealed normal cellular architecture without any significant pathological alterations. The testes showed intact seminiferous tubules with normal spermatogenic cells, while the ovaries exhibited differentiated cortex and medulla was observed along with healthy primordial follicle, primary follicle and secondary follicle and well-arranged stromal cells (Figure 14).

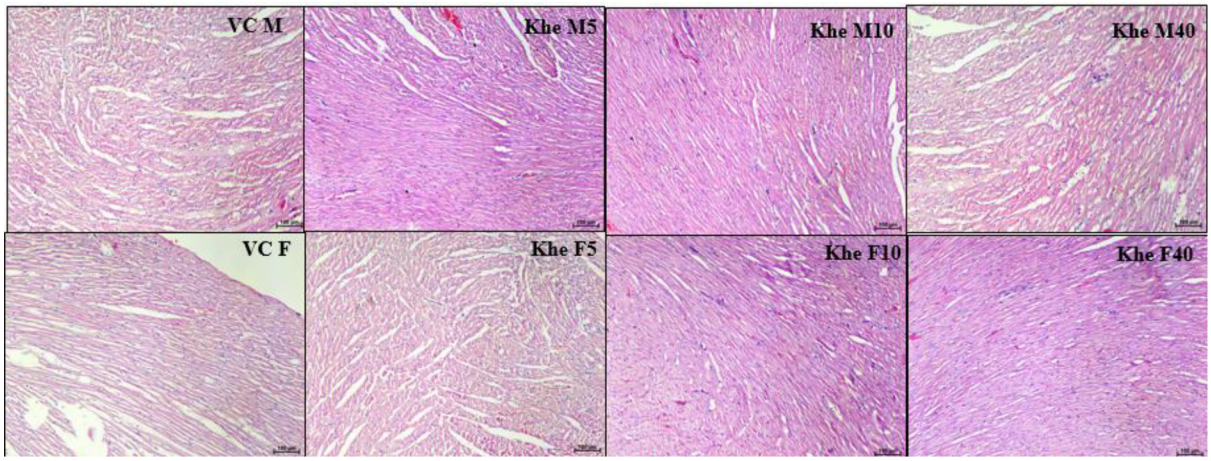


Figure 9. Effect of Khe (5, 10 and 40 mg/kg) on heart histopathology in sub-acute toxicity in rats stained with H and E, examined at 10x (100 µm). F: Female and M: Male.

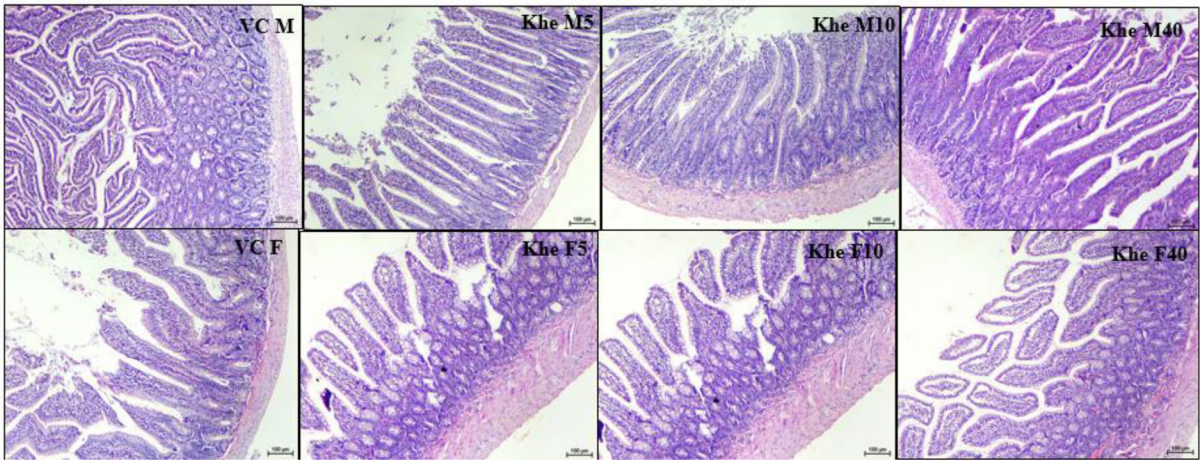


Figure 10. Effect of Khe (5, 10 and 40 mg/kg) on colon histopathology in sub-acute toxicity in rats stained with H and E, examined at 10x (100 µm). F: Female and M: Male.

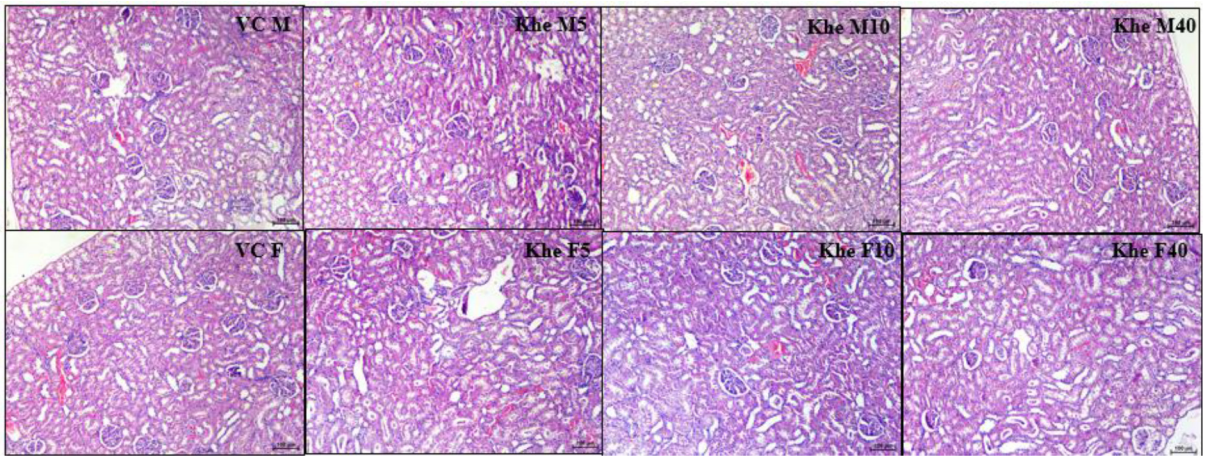


Figure 11. Effect of Khe (5, 10 and 40 mg/kg) on kidney histopathology in sub-acute toxicity in rats stained with H and E, examined at 10x (100 µm). F: Female and M: Male.

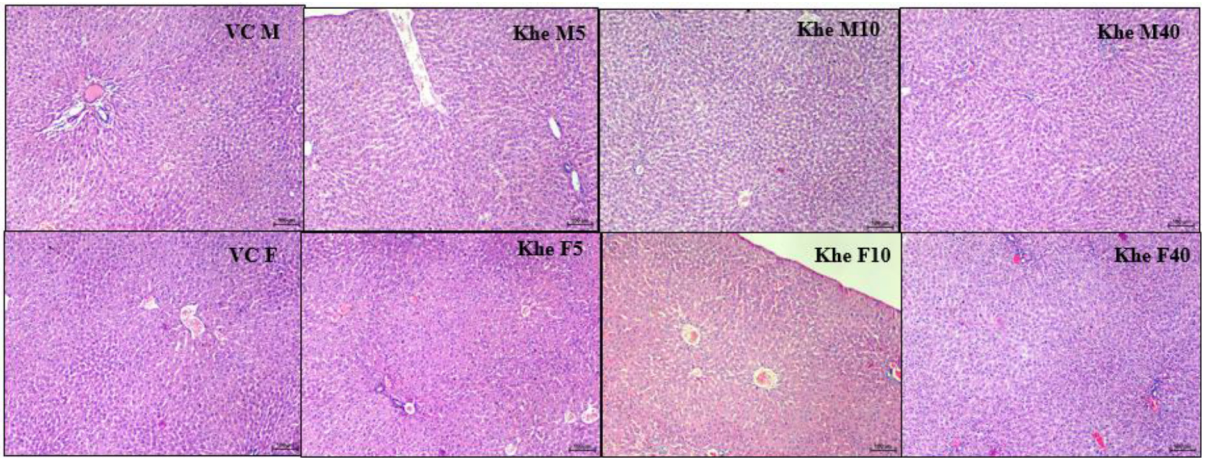


Figure 12. Effect of Khe (5, 10 and 40 mg/kg) on liver histopathology in sub-acute toxicity in rats stained with H and E examined at 10x (100 µm). F: Female and M: Male.

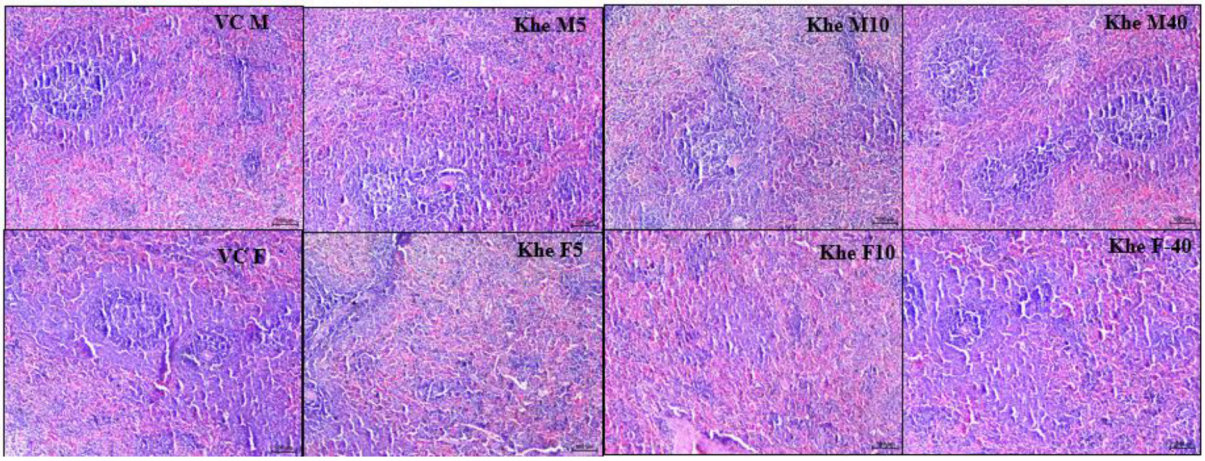


Figure 13. Effect of Khe (5, 10 and 40 mg/kg) on spleen histopathology in sub-acute toxicity in rats stained with H and E, examined at 10x (100 µm). F: Female and M: Male.

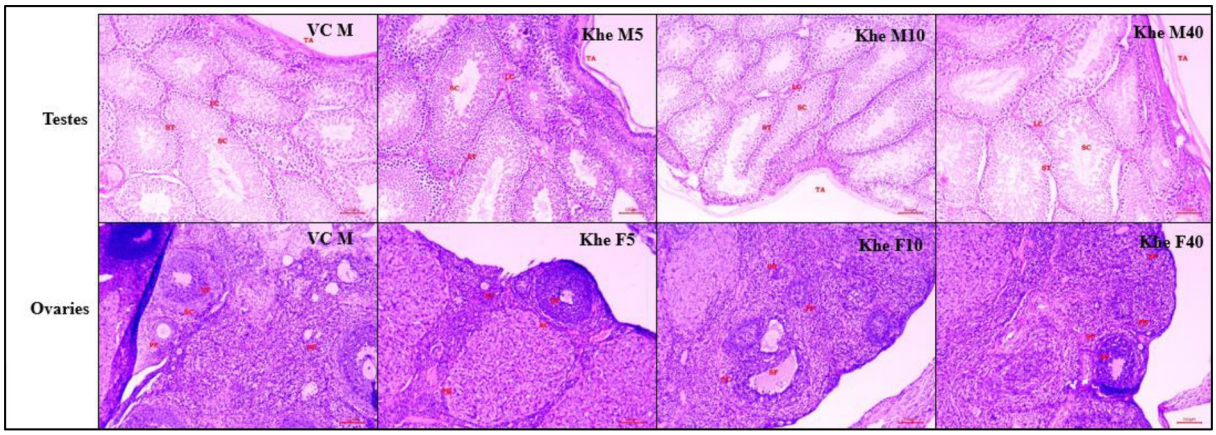


Figure 14. Effect of Khe (5, 10 and 40 mg/kg) on testes and ovaries histopathology in sub-acute toxicity in rats stained with H and E, examined at 10x (100 µm). F: Female and M: Male.

4. Discussion

Medicinal plants are becoming recognised for their various pharmacological characteristics; yet, there is limited evidence on the possible toxicity of their physiologically active compounds[15,16]. Acute toxicity studies are intended to investigate adverse effects occurring quickly after the delivery of a single dosage of a test chemical. These studies are typically conducted in rodents and represent an essential initial step in the safety assessment of new compounds[17]. In the present study, Khe did not induce mortality or observable behavioural alterations in rats at a dose of 50 mg/kg. According to the OECD's toxicity guidelines, Khe poses no acute hazardous risks. To further assess Khe's safety profile, a 28-day sub-acute toxicity research in rats was performed. Sub-acute toxicity studies are crucial for assessing cumulative toxicity, potential target-organ damage, and physiological or metabolic alterations resulting from prolonged exposure to low doses of a test compound. In the present investigation, oral administration of Khe at doses of 5 mg/kg, 10 mg/kg, and 40 mg/kg did not result in any mortality or observable signs of toxicity during the 28-day study period. Body weight variation is a sensitive and generally established indication for determining the side effects of medications and chemicals[18,19]. In this study, mean BW was normal in both the VC and Khe-treated groups. Furthermore, statistically significant differences in weight improvement were observed between the VC and Khe-treated groups at low (5 mg/kg), medium (10 mg/kg), and high (40 mg/kg) doses, showing normal growth patterns without harmful influence. Organ weights are excellent indicators of the physiological and pathological conditions of experimental animals[20]. Herbal products may have a harmful effect on key organs such as the heart, liver, kidney, spleen, brain, and colon because of their important metabolic and physiological processes. The present investigation found no significant variations in the relative weights of the heart, liver, kidney, spleen, brain, and colon in Khe-treated rats compared to the VC group, demonstrating the absence of organ-specific toxicity.

Haematological measurements provide important information on the systemic effects of xenobiotics. According to Wu *et al*., toxic compound-induced anaemia can be caused by hemolysis or hematopoiesis inhibition, resulting in haematological indices being reduced[21]. Because blood is the principal carrier of nutrients and foreign chemicals, RBC, Hb, WBC, and PLT are especially vulnerable to hazardous exposure[22,23]. In this investigation, subacute Khe treatment resulted in normal RBC, Hb, WBC, LYM, MID, NEUT, HCT, MCV, and PLT levels in all Khe-treated and VC groups. These findings suggest that Khe may exert a renal potential consistent with previous reports indicating that elevated leukocyte counts are associated with enhanced immune responses[24]. Importantly, no significant changes in RBC counts or Hb levels were found, demonstrating that Khe does not cause nematotoxicity.

Biochemical analysis indicated substantial differences in liver, kidney, and lipid characteristics. Elevated liver enzymes AST, ALT, and ALP are commonly associated with hepatocellular damage or decreased bile flow. ALT is a liver-specific enzyme that is produced after hepatocellular injury, whereas AST is found in a variety of tissues, including red blood cells, cardiac muscle, skeletal muscle, and kidneys[25,26]. In the current investigation, the absence of an aberrant enzyme increase confirms Khe hepatopathy. Furthermore, hepatic enzyme markers such as Ab, Gb, TP, and TB showed no signs of pathogenic increase after Khe administration at dosages of 5, 10, and 40 mg/kg. Furthermore, the liver is responsible for cholesterol synthesis and breakdown; thus, the minor and non-pathological changes in CH and TG levels indicate that Khe has no deleterious impact on lipid metabolism. These findings are similar to previous studies that found no significant changes in biochemical markers after Khe treatment[27]. Renal function indicators such as BUN, creatinine, urea, and uric acid remained within normal ranges in Khe-treated groups when compared to VC. In the current investigation, BUN, creatinine, urea, and uric acid levels in Khe-treated groups remained within normal limits as compared to VC. These studies imply that Khe is not nephrotoxic and may potentially have a beneficial effect on kidney function[28,29].

Histopathological evaluation provides critical supportive evidence for biochemical and haematological findings[30]. Microscopic examination of the brain, Heart, Colon, Kidney, Liver, and Spleen tissues from rats treated with Khe at doses of (5, 10, and 40 mg/kg) orally for 28 days revealed normal histoarchitectural features. The histopathological study demonstrated normal brain architecture, intact neuronal morphology, and no inflammatory or degenerative alterations. The heart exhibited normal myocardial fibre organisation with no signs of inflammation. The colon showed intact mucosal architecture and typical epithelial layers.

Kidney sections revealed typical kidney tubules and glomeruli in both the cortex and the medulla. Liver tissues from high-dose groups included well-preserved hepatocytes arranged normally around the major vein in both male and female rats. The spleen had typical red and white pulp architecture and appropriate lymphoid cellularity. The testes and ovaries showed preserved tissue morphology with no observable toxicological changes. These findings imply that Khe is safe within the measured dose range and has the potential to be studied further in a variety of therapeutic applications, including neuroprotection, hepatoprotection, cardioprotection, and nephroprotection.

5. Conclusion

The acute and subacute toxicity profile of Khe in male and female Wistar rats is thoroughly demonstrated in this work. During the sub-acute study, repeated oral administration of Khe at doses of 5, 10 and 40 mg/kg BW did not result in any treatment-related adverse effects or mortality. Furthermore, when compared to VC rats in the 28-day oral toxicity investigation, Khe did not significantly change clinical symptoms, haematological indices, serum biochemical markers, and histological architecture.

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7. References

1. Vakili T, *et al*. Safety evaluation of auraptene in rats in acute and subacute toxicity studies. Regul Toxico Pharmacol. 2017; 91:159–164. https://doi.org/10.1016/j.yrtph.2017.10.025 PMid: 29080847.
2. Kumar R, *et al*. Phytonutrients: harnessing anti-inflammatory and anti-cancer potential for health benefits. Curr Res Nutr Food Sci. 2025; 13:53-71. https://doi.org/10.12944/CRNFSJ.13.Special-Issue-July.04
3. Aiyalu R, Ramasamy A. Acute and sub-acute toxicity study of aqueous extracts of *Canscora heteroclita* (L) Gilg in rodents. Pharmacogn J. 2016; 8(4):399-410. https://doi.org/10.5530/pj.2016.4.15
4. Odoh AE, *et al*. Toxicités orale aiguë et subaiguë de l'extrait aqueux des feuilles de *Holarrhena Floribunda* chez les rongeurs. Rev Ivoir Sci Technol. 2021; 38:206–216.
5. Li Y, *et al*. *In vivo* acute and subacute toxicities of phenolic extract from rambutan (*Nephelium lappaceum*) peels by oral administration. Food Chem. 2020; 320:126618. https://doi.org/10.1016/j.foodchem.2020.126618 PMid: 32229397.
6. Al-Snafi AE. Chemical constituents and pharmacological activities of *Ammi majus* and *Ammi visnaga*. A review. *Int J Pharm Ind Res*. 2013; 3(3):257-265.
7. Ahmed SS, *et al*. Chemical and biological potential of *Ammi visnaga* (L.) Lam. and *Apium graveolens* L.: a review (1963-2020). *J Adv Biomed Pharm Sci*. 2021; 4(3):160-176. https://doi.org/10.21608/jabps.2021.55949.1115
8. Ez-Zahir A, *et al*. Immuno-modulatory, anti-psoriatic effects and furanochromone (khellin and visnagin) contents of *Ammi visnaga* (L.) hydeoethanolic extract. Biomed Pharmacol J. 2022; 19(3):1623-1635. https://doi.org/10.13005/bpj/2500
9. Guideline PB. OECD guideline for the testing of chemicals. The Hershberger. 2001; 601:858.
10. Kennedy MC, Stock JP. The bronchodilator action of khellin. Thorax. 1952; 7(1):43. https://doi.org/10.1136/thx.7.1.43 PMid: 14913500 PMCid: PMC1019140.
11. Sharma R, *et al*. Khellinoflavanone, a semisynthetic derivative of khellin, overcomes benzo pyrene toxicity in human normal and cancer cells that express CYP1A1. ACS Omega. 2018; 3(8):8553-8566. https://doi.org/10.1021/acsomega.8b01088 PMid: 31458985 PMCid: PMC6645225.
12. Vanachayangkul P, *et al*. Prevention of renal crystal deposition by an extract of *Ammi visnaga* L. and its constituents khellin and visnagin in hyperoxaluric rats. Urol Res. 2011; 39(3):189-195. https://doi.org/10.1007/s00240-010-0333-y PMid: 21069311 PMCid: PMC3663934.
13. Babu AH, *et al*. Antiparkinson potential of khellin on rotenone-induced Parkinson's disease in a zebrafish model: targeting MAO, inflammatory, and oxidative stress markers with molecular docking, MD simulations, and histopathology evidence. Comp Biochem Physiol C Toxicol Pharmacol. 2024; 284:109997. https://doi.org/10.1016/j.cbpc.2024.109997 PMid: 39103133.
14. Lee JK, *et al*. Anti-inflammatory effect of visnagin in lipopolysaccharide-stimulated BV-2 microglial cells. Arch Pharm Res. 2010; 33(11):1843-1850. https://doi.org/10.1007/s12272-010-1117-1 PMid: 21116788.
15. Ouahchia C, *et al*. Toxicité aiguë et subaiguë des extraits méthanoliques d'*Inula viscosa* L. (*Dittrichia viscosa* L.). Agrobiologia. 2017; 7(2):562-573.
16. Benrahou K, *et al*. Acute toxicity, phenol content, antioxidant and postprandial anti-diabetic activity of *Echinops spinosus*

extracts. Int J Second Metab. 2022; 9(1):91-102. https://doi.org/10.21448/ijsm.1031208
17. Yang M, *et al*. Acute and subacute toxicity evaluation of ethanol extract from aerial parts of *Epigynum auritum* in mice. Food Chem Toxicol. 2019; 131:110534. https://doi.org/10.1016/j.fct.2019.05.042 PMid: 31150781.
18. Vahalia MK, *et al*. Chronic toxicity study for *tamra bhasma* (a generic *Ayurvedic* mineral formulation) in laboratory animals. Recent Res Sci Technol. 2011; 3(11):76-79.
19. Ukpabi-Ugo JC, *et al*. Evaluation of subacute toxicity of methanol extract of *Justicia carnea* leaves in Wistar albino rats. *Anim Res Int*. 2023; 20(1):4862.
20. Raina P, *et al*. Evaluation of subacute toxicity of methanolic/aqueous preparation of aerial parts of *O. sanctum* in Wistar rats: clinical, haematological, biochemical and histopathological studies. *J Ethnopharmacol*. 2015; 175:509-517. https://doi.org/10.1016/j.jep.2015.10.015 PMid: 26456329.
21. Wu Z, *et al*. Acute and subchronic toxicities of the ethanol and hot-water extracts from Chinese sumac (*Rhus chinensis* Mill.) fruits by oral administration in rats. Food Chem Toxicol. 2018; 119:14-23. https://doi.org/10.1016/j.fct.2018.06.009 PMid: 29886233.
22. Ed-Dahmani I, *et al*. Phytochemical, antioxidant activity, and toxicity of wild medicinal plant of *Melitotus albus* extracts, *in vitro* and *in silico* approaches. ACS Omega. 2024; 9(8):9236-9246. https://doi.org/10.1021/acsomega.3c08314 PMid: 38434823 PMCid: PMC10905593.
23. Li X, *et al*. Acute and subacute toxicity of ethanol extracts from *Salvia przewalskii* Maxim in rodents. J Ethnopharmacol. 2010; 131(1):110-115. https://doi.org/10.1016/j.jep.2010.06.012 PMid: 20561576.
24. Atsamo AD, *et al*. Acute and subchronic oral toxicity assessment of the aqueous extract from the stem bark of *Erythrina senegalensis* DC (*Fabaceae*) in rodents. J Ethnopharmacol. 2011; 134(3):697-702. https://doi.org/10.1016/j.jep.2011.01.023 PMid: 21256951.
25. Adewale OB, *et al*. Evaluation of acute and subacute toxicity of aqueous extract of *Crassocephalum rubens* leaves in rats. J Ethnopharmacol. 2016; 188:153-158. https://doi.org/10.1016/j.jep.2016.05.003 PMid: 27154407.
26. Ozer J, *et al*. The current state of serum biomarkers of hepatotoxicity. Toxicology. 2008; 245(3):194-205. https://doi.org/10.1016/j.tox.2007.11.021 PMid: 18291570 PMCid: PMC13211875.
27. Mrabti HN, *et al*. Phenolic content, antibacterial, antioxidant, and toxicological investigations of *Erodium guttatum* (*Geraniaceae*) collected from the Northeast of Morocco. Turk J Bot. 2021; 45(8):739-749. https://doi.org/10.3906/bot-2107-29
28. Wasan E, *et al*. Review of novel oral amphotericin B formulations for the treatment of parasitic infections. Pharmaceutics. 2022; 14(11):2316. https://doi.org/10.3390/pharmaceutics14112316 PMid: 36365135 PMCid: PMC9697626.
29. Taghizadeh M, *et al*. Sub-chronic oral toxicity of *Cuminum cyminum* L.'s essential oil in female Wistar rats. Regul Toxicol Pharmacol. 2017; 88:138-143. https://doi.org/10.1016/j.yrtph.2017.06.007 PMid: 28634146.
30. Traesel GK, *et al*. Acute and subacute (28 days) oral toxicity assessment of the oil extracted from *Acrocomia aculeata* pulp in rats. Food Chem Toxicol. 2014; 74:320-325. https://doi.org/10.1016/j.fct.2014.10.026 PMid: 25445758.

Author Contribution

SB and DM authors were equally responsible for literature research, review work and drafting of the manuscript. All authors have read and approved the manuscript.

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List of Abbreviations:

Abbreviations	Full Form	Abbreviations	Full Form
mg/kg	Milligram per kilogram	**HCT**	Haematocrit
Khe	Khellin	**PLT**	Platelet count
L	Liter	**RPM**	Rotation per minute
mL	Milliliter	**min**	Minute
OECD	Organisation for Economic Co-operation and Development	**AST**	Aspartate aminotransferase
CCSEA	Committee for the Control and Supervision of Experiments on Animals	**ALT**	Alanine aminotransferase
IAEC	Institutional Animal Ethical Committee	**ALP**	Alanine Phosphatase
RBC	Red blood cells	**Ab**	Albumin
Hb	Haemoglobin	**Gb**	Globulin
MCV	Mean corpuscular volume	**TP**	Total Protein
WBC	White blood cell	**TB**	Total Bilirubin
LYM%	Lymphocyte percentage	**CH**	Cholesterol
MID%	Mid-sized cell percentage	**TG**	Triglyceride
NEUT%	Neutrophil percentage	**HDL**	High Density Lipoprotein
ANOVA	Analysis of variance	**LDL**	Low Density Lipoprotein
µL	Microliter	**VC F**	Vehicle Control Female
BUN	Blood urea nitrogen	**VC M**	Vehicle Control Male
P	Probability	**Khe F10**	Khe Female Medium Dose
Khe F5	Khe Female Low Dose	**Khe M10**	Khe Male Medium Dose
Khe F40	Khe Female High	**Khe M40**	Khe Male High Dose
Khe M5	Khe Male Low	**Khe F40**	Khe Female High
Khe F5	Khe Female Low	**Khe M5**	Khe Male Low
Khe F10	Khe Female Medium	**Khe M40**	Khe Male High
Khe M10	Khe Male Medium	**PEG**	Polyethylene glycol