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Safety Profile of Aqueous Extract from Traditional Recipes in the Royal Textbook of King Rama V in Albino Wistar Rats

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ABSTRACT

An assessment was conducted on the acute toxicity of an aqueous extract derived from King Rama V's Royal Handbook antidiabetic herbal formula in albino Wistar rats. The extract is composed of six medicinal plants from Thailand. The OECD guidelines specified doses of 5, 50, 300, and 2,000 mg/kg of aqueous extract, with the control group receiving distilled water. Both male and female rats received a single dose and were monitored daily for a period of 14 days. Body weights were recorded during Weeks 0, 1, and 2. Following the studies, we documented the weights of internal organs. Blood biochemistry, including BUN, Cr, AST, ALT, ALP, TG, TC, and HDL, along with liver and kidney histology and hematological parameters such as RBC, Hb, Hct, PLT, MCV, MCH, MCHC, WBC, lymphocytes, and monocytes, were thoroughly examined. The results indicated that there were no fatalities among the rats over a 14-day period and that none of the administered doses of the formulation produced any signs or symptoms of toxicity within the initial 24 hours. All treatments lead to significant weight gain (p<0.05) in rats while not impacting organ weights. The extract did not adversely affect hematological parameters or the morphology of blood cells. Treatment at 300 and 2,000 mg/kg significantly influenced blood biochemical parameters, including AST, ALT, and ALP levels (P<0.05). However, these levels were within acceptable ranges. A close look at tissue sections under a microscope showed that the liver and kidneys had the same histology as the negative control group.

Keywords: Acute toxicity, Aqueous extract, Royal textbook of King Rama, Traditional medicine

Introduction

Despite the persistent challenge of diabetes, an incurable disease with a rising global prevalence, medical experts are actively pursuing alternative treatment methods. Traditional medicine, defined by the World Health Organization (WHO) as practices rooted in indigenous beliefs and experiences, offers a viable option, particularly in the form of herbal treatments.[1] In Thailand, such practices are not only a cornerstone of cultural heritage but also a continuation of methodologies developed since the time of the Buddha. Thai traditional medicine relies on extensive historical knowledge, documented in the Morana Yanana Sutta from the Ayutthaya period and thoroughly preserved on palm-leaf manuscripts, to support the use of herbal remedies for diabetes. King Rama V's reign, during the early Rattanakosin dynasty, revised and officially recorded the texts, highlighting the legitimacy and historical significance of these treatments in the King Rama V Medical Book.[2] Such an enduring tradition accentuates the potential of Thai herbal medicine to contribute to contemporary diabetes management strategies.

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An herbal formula that embodies the anti-diabetic properties of Thai traditional medicine includes the roots of *Momordica cochinchinensis* (Cucurbitaceae), *Tiliacora triandra* (Menispermaceae) and *Schumannianthus dichotomus* (Marantaceae), the rhizome of *Imperata cylindrica* (Poaceae), the roots of *Asparagus racemosus* Willd, (Asparagaceae) and *Calamus rotang* (Palmaearecaceae), along with alum and potassium nitrate.[2] This original composition, prevalent in herbal pharmacies, includes 6 botanicals known for their therapeutic effects and 2 chemical constituents. However, the Codex General Standard for Food Additives (GSFA) warns of the health risks associated with alum and potassium nitrate.[3] Extensive pharmacological research supports the efficacy of these plants. *M. cochinchinensis* is noted for its antioxidative, anticancer, anti-inflammatory, and antiulcer activities,[4] while *T. triandra*'s roots demonstrate antipyretic properties,[5] and its leaf extracts show significant hypoglycemic effects in streptozotocin-induced diabetic and healthy rats.[6-7] This rich historical and scientific backdrop highlights the potential of traditional herbal remedies in diabetes management while stressing the necessity of careful assessment of their safety profiles.

Studies on *I. cylindrica* have shown that it contains many biologically active compounds that have different effects. These include antioxidant properties, anti-complementary activity, and the ability to block quorum sensing, all of which are important for fighting oxidative stress and changing immune responses.[8-10] Compounds from this plant have also been shown to be effective against hepatocellular carcinoma cells and have been used in decoctions to treat IgA nephropathy in rat models, showing that they could be used in therapeutic settings.[11-13] Nonetheless, there is a notable gap in scientific data regarding the effects of *S. dichotomus*, despite its traditional use alongside bitter plants in remedies for fever. Traditional healers attribute germicidal properties to bitter herbs and believe that bland plants can reduce body

temperature by promoting urine flow.[14] This blend of historical usage and emerging scientific evidence emphasizes the need for comprehensive studies to validate the pharmacological claims and optimize the medicinal use of these plants.

Furthermore, research on the root extracts of *A. racemosus* has revealed a diverse spectrum of pharmacological properties, including phytoestrogenic, adaptogenic, cardioprotective, antibacterial, immunological adjuvant, and antitussive effects, demonstrating its versatile therapeutic potential.[15-16] Correspondingly, the roots of *C. rotang* have been traditionally used to treat a variety of ailments, such as piles, burning sensations, coughs, leprosy, and bleeding disorders, and have shown effectiveness in controlling inflammation and modulating the immune system in human studies.[17] Recent investigations into these herbal recipes further reveal their capacity for antioxidation and inhibition of *α*-glucosidase and *α*-amylase *in vitro*, suggesting potential applications in managing metabolic disorders.[2] However, despite these promising results, concerns remain regarding the safety and implementation of these traditional remedies, primarily due to insufficient toxicological data to fully endorse their effects. Such a gap indicates the need for comprehensive toxicological assessments in ensuring the safe use of these potent herbal formulations in clinical settings.

This study employs a rat model to assess the immediate detrimental effects of an aqueous extract from a Thai Traditional Medicine commonly used to treat diabetes, thus addressing concerns about its potential toxicity. In conducting acute oral toxicity tests on both male and female rats, the research adheres to the rigorous standards set by the Organization for Economic Cooperation and Development 420 (OECD 420). This methodology ensures the scientific rigor of the testing process and helps to elucidate the safety profile of these traditional remedies, providing critical data necessary for evaluating their viability and safety in clinical applications.

Materials and Methods

Plant collection and Identification

All raw medicinal substances included in the Thai herbal formula were sourced from various regions across Thailand. *Momordica cochinchinensis* was collected from Nakhon Pathom Province, *Thysanolaena triandra* and *Imperata cylindrica* from Kalasin Province, and *Schizostachyum dichotomum* from Phichit Province. *Asparagus racemosus* was obtained from Prachinburi Province, while *Calamus rotang* was gathered from Sakon Nakhon Province. The botanical identification and verification of all plant specimens were conducted by Assoc. Prof. Dr. Surapon Saensouk, a Plant Taxonomy Specialist from the Conservation Research Unit at Walai Rukhavej Botanical Research Institute, Mahasarakham University. He confirmed the identification of the species—*Momordica cochinchinensis*, *Thysanolaena triandra*, *Imperata cylindrica*, *Schizostachyum dichotomum*, *Asparagus racemosus*, and *Calamus rotang*—through macroscopic evaluation of their morphological characteristics in December 2020 - January 2021. These specimens were thoroughly cataloged and stored at the Faculty of Medicine, Mahasarakham University, with specific codes: MSU.MED-MC0001/ST for *M. cochinchinensis*, MED-TT0001/ST for *T. triandra*, MED-IC0001/ST for *I. cylindrica*, MED-SD001/ST for *S. dichotomus*, MED-AR0001/ST for *A. racemosus*, and MED-CR0001/ST for *C. rotang* as detailed in Table 1. The preparation process involved thoroughly washing and drying the raw materials in a Binder FED115 oven from Germany at 60 °C for 48 h. Following this, they were preserved in a cold, dry place in airtight containers until required for use, ensuring their integrity and efficacy for subsequent medicinal applications.

Table 1: Medicinal plants used in the Thai traditional antidiabetic formula from King Rama V's Royal Handbook

Scientific Name	Plant Family	Part Used	Source from Thailand (Province)	Plant Specimen Code
Momordica cochinchinensis	Cucurbitaceae	Root	Nakhon Pathom (13°48'49.6"N 100°02'06.0"E)	MSU.MED-MC0001/ST
Tiliacora triandra	Menispermaceae	Root	Kalasin (16°33'41.6"N 103°37'04.5"E)	MSU.MED-TT0001/S
Schumannianthus dichotomus	Marantaceae	Root	Phichit (16°12'19.5"N 100°18'51.4"E)	MSU.MED-SD001/ST
Imperata cylindrica	Poaceae	Rhizome	Kalasin (16°33'41.6"N 103°37'04.5"E)	MSU.MED-IC0001/ST
Asparagus racemosus Willd	Asparagaceae	Root	Prachinburi (14°06'08.5"N 101°35'11.3"E)	MSU.MED-AR0001/ST
Calamus rotang	Palmaearecaceae	Root	Sakon Nakhon (17°22'44.8"N 103°40'50.4"E)	MSU.MED-CR0001/ST

These plants are mixed in equal proportions (1:1:1:1:1:1 W/W)

Preparation of aqueous extract

The preparation of the herbal formula involved a comprehensive process utilizing 6 plants mixed in equal proportions (1:1:1:1:1:1 W/W). Initially, the combined plants were ground and sieved through a 60 mesh to ensure uniformity. Subsequently, 300 g of this fine herbal powder were infused in 3,000 mL of distilled water and heated at a temperature range of 80-95 °C for 20 min. After heating, the mixture was filtered through a filter cloth to separate the solid residues. This simmering process was repeated twice to concentrate the solution from 3 to 1 L. Any remaining residue was then removed using Whatman filter

papers from Germany. The clarified liquid was further reduced to a paste using a rotary evaporator (Heidolph Laborota 4000, Germany) and finally freeze-dried to produce a dark brown extract. The extracts were kept in the refrigerator at -4°C until used. This precise and controlled preparation technique ensures the stability and concentration of the herbal constituents, which are critical for subsequent pharmacological evaluations.

Animal model

The acute toxicity investigation adhered to the principles in OECD guideline no. 420,[19] utilizing adult male and female Wistar rats weighing between 150 and 200 g. These animals were housed in the Animal Biosafety Level 3 (ABSL3) facility at the North-Eastern Laboratory Animal Center (NELAC), located at Khon Kaen University in Khon Kaen, Thailand. To acclimatize the animals to consistent experimental conditions, they were maintained in an air-conditioned room at a steady temperature of 23 °C, with a 12-hour light and 12-hour dark cycle, and relative humidity maintained between 30 and 60 %. Throughout the study, the rats had access to normal chow and water *ad libitum.* All experimental procedures involving these animals were approved by the Committee Care and Use of Laboratory Animal Resources, National Research Council of Thailand, and were conducted following the guidelines of the Institutional Animal Care and Use Committee of Khon Kaen University, Thailand (Record No. IACUC-KKU-76/62). This adherence to ethical and methodological standards ensures the reliability and ethical integrity of the toxicity data obtained.

Experimental design

The experimental design adhered to the OECD Guideline No. 420 for acute toxicity testing, employing 50 adult Wistar rats, equally divided by gender into 25 males and 25 females, and further subdivided into 10 groups of 5. Each group was designated for specific doses: Control groups (male and female) received distilled water, while the treatment groups received aqueous extract at doses of 5, 50, 300, and 2,000 mg/kg, respectively, for both genders. Administration was conducted via a single oral dose using a gastric feeding tube, ensuring precise dosage delivery.

Immediately following administration, each rat was observed for signs of regurgitation for the first 5 minutes and then housed in individual metallic cages to prevent cross-contamination and interactive effects. Monitoring continued at 15-minute intervals for the first 4 hours, followed by 30-minute intervals for the next 6 hours, and then once daily for the subsequent 48 hours. This intensive observation period was designed to capture any acute behavioral changes indicative of toxicity, including alterations in skin, hair, eyes, and mucous membranes, as well as changes affecting the respiratory, circulatory, autonomic, and central nervous systems. Additional observations included motor activity, convulsions, tremors, salivation, diarrhea, lethargy, and changes in sleep patterns.

Following the initial intensive monitoring period, the rats were observed for a total of 14 days to evaluate any long-term fatal consequences of the administered extracts. Body weights were recorded on days 1, 7, and 14 to track any significant changes potentially indicative of systemic effects or health deterioration. This comprehensive approach not only ensures adherence to rigorous international standards but also provides a robust data set to assess the acute and potential long-term toxicity of the herbal extracts under study.

Following a 14-day observation period, the rats underwent an 8-hour fasting protocol prior to humane euthanasia, performed under intraperitoneal anesthesia using Thiopental sodium at a dosage of 85 mg/ml/kg. Cardiac blood collection was carried out by accessing the chest cavity and inserting a No. 23 syringe into the left ventricle to withdraw approximately 3 ml of blood for hematological analysis, including red and white blood cell counts as well as platelet levels. Blood chemistry parameters—comprising blood urea nitrogen (BUN), creatinine (Cr), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL)—were analyzed using a Swelab Alfa automated analyzer (Biozen, Sweden) at the Community Clinical Laboratory, Faculty of Associated Medical Science, Khon Kaen University. Major organs, including the liver, lungs, heart, spleen, and kidneys, were subsequently exercised, weighed, and subjected to macroscopic evaluation to assess potential morphological alterations.

Measurement of body and organ weights

The animals were weighed weekly, and the level of weight gain (%) was calculated using the following formula:

Weight gain (%) = (Wf -Wi/Wi)100

Where Wf = final weight; Wi = initial weight.

Blood samples were obtained via cardiac puncture following a 14-day period and transferred into 2 mL collection tubes containing ethylene diamine tetra-acetic acid (EDTA). Blood samples were obtained from the heart and placed into 2 mL vacuum tubes for biochemical analysis. The targeted organs, including the liver, kidneys, heart, lungs, and spleen, were subsequently harvested. A 0.85% sodium chloride solution was employed to remove residual blood from the tissues. The tissues were desiccated and measured to determine their relative weight (g %). Following the acquisition of organ weight, the relative organ weight was determined through a mathematical formula.

The relative organ weight for each harvested organ was calculated utilizing the subsequent formula:

The formula for relative organ weight (%) is (organ weight = body weight) ×100.[20]

Assessment of hematological parameters

Blood samples were collected through cardiac puncture after a 14-day period and placed into a 2 mL blood collection tube. Samples were collected into tubes containing ethylene diamine tetra-acetic acid (EDTA) as an anticoagulant to ensure the integrity of the specimens for accurate analysis. The comprehensive evaluation of hematological para-meters was conducted using a Swelab Alfa automated blood analyzer (Biozen, Sweden) at the Community Clinical Laboratory, Faculty of Associated Medical Science, Khon Kaen University, Thailand. This analysis encompassed a range of crucial blood parameters including red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), white blood cells (WBC), lymphocytes (L), and monocytes (M). These measurements are essential for assessing the impact of various conditions or treatments on blood health and immune function, thereby providing vital data for clinical and research applications.[20]

Assessment of serum biochemical parameters

After the 14-day period and 2 to 3 minutes of anesthesia, blood samples were collected from the heart and transferred into 2 mL vacuum tubes for biochemical analysis. Samples were carefully handled using standard containers to prevent hemolysis and to ensure the preservation of their integrity. The biochemical analysis was performed using a BT 2000 plus automated blood chemical analyzer (Germany) at the Community Clinical Laboratory, Faculty of Associated Medical Science, Khon Kaen University, Thailand. This analysis included a comprehensive set of parameters crucial for assessing metabolic and liver function: blood urea nitrogen (BUN), creatinine (Cr), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL). The handling and advanced analytical techniques employed provide reliable data critical for diagnosing and monitoring various health conditions, thereby underpinning the significance of precise sample management in clinical diagnostics.

Histopathological assessment

The liver and kidney tissues were first preserved in 10 % neutral formalin for histological analysis. Afterward, the tissues were rinsed with normal saline and immediately placed in 10 % formalin for 72

hours, with the fixative solution being changed daily. The tissues were then desiccated using a sequence of alcohols and xylene in the tissue processing equipment. The desiccated tissues were sliced into minute fragments and placed in cassettes. The tissues were immersed in paraffin and sliced into sections with a thickness of 5 - 7 µm using a rotary microtome. The tissue samples coated with ribbon-like paraffin were immersed in a warm water bath to eliminate the paraffin. The tissues were affixed to a microscope slide and subjected to staining with hematoxylin and eosin. The histopathology micrographs were obtained using an Olympus BX51TF light microscope (Olympus Inc., Japan) and a digital camera EP50 (Olympus Inc., Germany).

Statistical analysis

Statistical analysis of the data was thoroughly conducted, with results reported as the mean value plus or minus the standard error of the mean (SEM). To determine the statistical significance of the observed differences, an F-test was applied within the framework of a one-way analysis of variance (ANOVA). This analysis was performed using SPSS software, version 23. Statistical significance was established at a threshold of a p-value ≤ 0.05, ensuring a rigorous assessment of the data to ascertain meaningful conclusions from the study. This methodological approach enhances the reliability and validity of the findings, facilitating a precise understanding of the underlying patterns and relationships within the data.

Results and Discussion

Effect of aqueous extract on acute toxicity studies

The acute toxicity test for the aqueous extract (8.09 % extract) followed the guidelines from OECD 420, using the highest dose of 2,000 mg/kg. Throughout the experiment, both short-term and long-term monitoring showed no mortality among the rats administered the extract up to the highest dose. Observations over the entire 14-day trial period revealed no signs of toxicity, indicating a favorable safety profile for the extract at these doses. Consequently, the lethal dose, the amount estimated to cause death in 50% of the test population when administered, is likely to exceed 2,000 mg/kg. This suggests that the extract is relatively safe for use at and possibly above the doses tested, providing a robust basis for further exploration in therapeutic applications.

Effect of the extract on body weight gain of rats

Over a 14-day period, the impact of the aqueous extract on rat body weight was assessed, revealing statistically significant differences in average weekly weight gain among all groups. Specifically, rats that received the aqueous extract demonstrated a marked variance in weight gain each week compared to the control group, with these differences reaching statistical significance ($p < 0.05$), as detailed in Table 2. This finding suggests that the extract may influence metabolic or physiological processes affecting growth rates, emphasizing the importance of further investigation into its potential effects on body weight regulation.

Table 2: Effects of aqueous extract from a traditional recipe on body weight in treated and controlled male and female rats during a 14-day study

Groups and treatments (mg/kg)		Body weight(g)		
		Week 0	Week 1	Week 2
Male	Control	178.84±3.94[a]	225.67±5.97[a]	277.98±6.12[a]
	5	192.27±8.35[a]	222.39±8.35[a]	268.82±9.64[a]
	50	174.43±2.04[a]	223.26±3.90[a]	279.00±4.98[a]
	300	194.69±2.48[a]	221.03±4.55[a]	266.73±5.36[a]
	2,000	196.71±4.28[a]	223.35±6.85[a]	270.18±6.39[a]
Female	Control	158.29±2.40[a]	173.47±3.41[a]	194.08±4.09[a]
	5	161.52±3.94[a]	169.35±4.63[a]	188.78±3.57[a]
	50	148.06±3.86[a]	167.29±4.50[a]	189.42±5.85[a]
	300	151.60±5.38[a]	168.91±7.16[a]	188.51±8.66[a]
	2,000	141.03±4.04[a]	162.03±7.42[a]	182.69±8.93[a]

Values are (mean ± SEM).
$p < 0.05$ for different letters in the same row

Effect of the extract on relative organ weight

To assess the impact of the extract on relative organ weight, male and female rats were administered varying doses of the extract. After a period of 2 weeks, the analysis of the data showed no significant differences in the average relative weights of key organs, including the liver, lungs, heart, spleen, and kidneys, when compared to the control group ($p < 0.05$) (Table 3). This outcome suggests that the extract does not adversely affect organ weight at the doses administered, supporting its safety with respect to organ health on a short term basis. This finding is crucial for validating the non-toxicological impact of the extract on organ development and maintenance.

Table 3: Effects of aqueous extract from a traditional recipe on relative organ weight in treated and control male and female rats during a 14-day study

Groups and treatments (mg/kg)		Relative organ weight (g)				
		Liver	Lung	Heart	Spleen	Kidney
Male	Control	10.35±0.52[a]	1.24±0.05[a]	0.95±0.06[a]	0.65±0.04[a]	1.10±0.04[a]
	5	10.85±0.45[a]	1.32±0.04[a]	0.96±0.03[a]	0.66±0.03[a]	1.25±0.03[a]
	50	10.50±0.20[a]	1.22±0.02[a]	0.93±0.06[a]	0.67±0.02[a]	1.17±0.03[a]
	300	11.16±0.36[a]	1.24±0.03[a]	0.89±0.03[a]	0.64±0.03[a]	1.17±0.03[a]
	2,000	10.37±0.78[a]	1.25±0.04[a]	0.95±0.02[a]	0.59±0.04[a]	1.14±0.02[a]
Female	Control	6.69±0.14[a]	0.97±0.04[a]	0.68±0.04[a]	0.48±0.03[a]	0.76±0.02[a]
	5	6.89±0.26[a]	0.95±0.04[a]	0.69±0.03[a]	0.49±0.02[a]	0.78±0.03[a]
	50	7.22±0.31[a]	0.93±0.04[a]	0.67±0.03[a]	0.43±0.04[a]	0.79±0.03[a]
	300 mg/kg	6.87±0.36[a]	0.97±0.03[a]	0.68±0.04[a]	0.45±0.02[a]	0.81±0.03[a]
	2,000	7.25±0.23[a]	1.00±0.04[a]	0.69±0.02[a]	0.49±0.02[a]	0.84±0.02[a]

Values are (mean ± SEM). $p < 0.05$ for different letters in the same row

Effect of the extract on hematological parameters

Analysis of the male rats' hematological parameters showed a statistically significant ($p < 0.05$) rise in red blood cell (RBC) and white blood cell (WBC) counts at doses of 300 mg/kg, as well as an increase in mean corpuscular hemoglobin concentration (MCHC) at a dose of 2,000 mg/kg, compared to the control group (Table 3). No significant differences were seen in the other hematological parameters compared to the control group, as shown in Table 4.

The hematological analysis of the female rats showed a noteworthy ($p < 0.05$) rise in lymphocytes at a dose of 2,000 mg/kg, as well as an increase ($p < 0.05$) in Hb, Hct, and MCHC at doses of 300 and 2,000 mg/kg of the extract, in comparison to the control group (Table 4). The other hematological parameters showed no significant differences compared to the control group, as shown in Table 5.

Table 4: The hematological parameters of different doses of aqueous extract from a traditional recipe in male rats during a 14-day study

Hematological parameters	Treatments				
	Control	Aqueous extract			
		5 mg/kg	50 mg/kg	300 mg/kg	2,000 mg/kg
RBC (10^3/µL)	7.10±0.13^{a,b}	7.24±0.18^{a,b}	6.73±0.06^{a}	7.46±0.17^{b}	7.24±0.24^{a,b}
Hb (g/dL)	13.52±0.12^{a}	13.78±0.18^{a,b}	13.40±0.08^{a}	14.32±0.12^{b}	14.00±0.34^{a,b}
Hct (%)	42.70±0.64^{a,b}	42.48±0.57^{a,b}	40.82±0.34^{a}	43.54±0.41^{b}	41.82±1.12^{a,b}
MCV (fL)	60.26±1.49^{a}	58.80±1.19^{a}	60.72±0.17^{a}	58.48±0.95^{a}	57.80±0.62^{a}
MCH (pg)	19.10±0.36^{a}	19.06±0.32^{a}	19.92±0.11^{a}	19.22±0.28^{a}	19.36±0.19^{a}
MCHC (g/dL)	31.66±0.26^{a}	32.46±0.14^{b}	32.82±0.12^{b}	32.90±0.10^{b}	33.50±0.23^{c}
PLT (10^3/µL)	978.60±73.58^{a}	948.40±24.54^{a}	977.25±11.32^{a}	1055.80±106.91^{a}	1076.80±62.83^{a}
WBC (10^3/µL)	4.20±0.35^{a,b}	3.25±0.44^{a}	5.17±0.129^{b,c}	5.86±0.58^{c}	4.86±0.29^{b,c}
L (10^3/µL)	3.60±0.33^{a}	2.72±0.38^{a}	4.60±0.13^{a}	5.20±0.52^{a}	4.44±0.26^{a}
M (10^3/µL)	0.40±0.06^{a}	0.40±0.05^{a}	0.41±0.04^{a}	0.44±0.06^{a}	0.30±0.02^{a}

$^{a, b, c}$ Different letters in the same row indicated significantly difference at $p < 0.05$.
Values are (mean ± SEM).
Red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), platelet (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), lymphocytes (L), and monocytes (M)

Table 5: The hematological parameters of different doses of aqueous extract from a traditional recipe in female rats during a 14-day study

Hematological parameters	Treatments				
	Control	Aqueous extract			
		5 mg/kg	50 mg/kg	300 mg/kg	2,000 mg/kg
RBC (10^3/µL)	7.46±0.09^{a}	7.52±0.16^{a,b}	7.52±0.18^{a,b}	7.92±0.09^{b,c}	8.05±0.14^{c}
Hb (g/dL)	14.14±0.14^{a}	14.24±0.22^{a}	14.10±0.30^{a}	15.06±0.14^{b}	15.26±0.20^{b}
Hct (%)	42.68±0.38^{a}	43.28±0.64^{a,b}	41.58±1.00^{a,b}	43.80±0.40^{b}	44.28±0.59^{b}
MCV (fL)	57.24±0.99^{a}	57.54±0.80^{a}	55.34±1.33^{a}	55.28±0.69^{a}	55.02±0.41^{a}
MCH (pg)	18.92±0.30^{a}	18.94±0.19^{a}	18.76±0.36^{a}	19.00±0.20^{a}	18.96±0.15^{a}
MCHC (g/dL)	33.12±0.18^{a}	32.88±0.14^{a}	33.92±0.17^{b}	34.38±0.08^{c}	34.48±0.15^{c}
PLT (10^3/µL)	939.80±60.78^{a}	959.40±32.40^{a}	973.40±53.72^{a}	788.20±87.09^{a}	824.00±93.27^{a}
WBC (10^3/µL)	2.48±0.32^{a}	2.08±0.23^{a}	2.63±0.27^{a}	2.97±0.23^{a}	2.68±0.34^{a}
L (10^3/µL)	2.07±0.32^{a}	1.81±0.21^{a}	2.34±0.24^{a}	2.55±0.19^{a}	2.35±0.29^{a}
M (10^3/µL)	0.26±0.02^{a}	0.17±0.02^{a}	0.19±0.02^{a}	0.26±0.05^{a}	0.20±0.04^{a}

Values are (mean ± SEM).
$^{a, b}$ Different letters in the same row indicated significantly difference at $p < 0.05$.
Red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), platelet (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), lymphocytes (L), and monocytes (M)

Effect of the extract on serum biochemical parameters

The extract did not negatively impact the clinical chemistry parameters, such as triglycerides (TG) and total cholesterol (TC), in male rats. The Cr levels were comparable between the control group and the rats that received 50 and 300 mg/kg doses. Similarly, there were no notable variations in BUN levels between the control group and rats at 5, 50, and 300 mg/kg doses. Nevertheless, the extract exhibited a substantial ($p < 0.05$) decrease in ALP levels at doses of 5, 50, 300, and 2,000 mg/kg. Furthermore, the extract exhibited a substantial ($p < 0.05$) reduction in AST levels at doses of 300 and 2,000 mg/kg. In addition, the extract had a substantial ($p < 0.05$) effect on reducing HDL levels at a dose of 300 mg/kg, as shown in *Table 6*.

The clinical chemistry values, such as blood urea nitrogen (BUN), creatinine (Cr), alanine transaminase (ALT), alkaline phosphatase (ALP), triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL), were not adversely impacted in the female rats. Nevertheless, all dosages of the extracts exhibited a substantial rise ($p < 0.05$) in AST levels, as shown in Table 7.

Table 6: The biochemical data of different doses of aqueous extract from a traditional recipe in male rats during a 14-day study

Blood chemistry parameters	Treatments				
	Control	Aqueous extract remedy			
		5 mg/kg	50 mg/kg	300 mg/kg	2,000 mg/kg
BUN (mg/dL)	19.60±0.92[b]	18.60±1.12[a]	18.20±1.39[a]	20.20±0.66[b]	19.60±0.81[b]
Cr (mg/dL)	0.38±0.02[a]	0.36±0.04[a]	0.36±0.02[a]	0.36±0.02[a]	0.32±0.02[a]
AST (U/L)	137.80±7.82[b]	109.40±3.55[a]	116.20±8.09[a,b]	122.20±12.33[a,b]	97.20±9.09[a]
ALT (U/L)	35.00±2.28[b]	36.20±4.00[b]	31.00±2.42[a]	30.80±1.65[a]	29.60±2.11[a]
ALP (U/L)	94.20±5.08[b]	88.80±8.69[a,b]	79.00±2.81[a]	75.40±3.35[a]	90.60±7.73[b]
TG (mg/dL)	39.20±4.42[a]	43.80±5.08[b]	41.60±3.93[a,b]	39.20±3.39[a]	49.00±7.55[b]
TC (mg/dL)	30.20±1.56[a]	30.80±1.62[a]	28.60±1.63[a]	34.60±2.69[b]	31.40±1.83[a]
HDL (mg/dL)	24.40±0.60[b]	24.00±0.70[b]	21.40±0.40[a]	23.20±1.28[b]	21.20±0.58[a]

Blood urea nitrogen (BUN), creatinine (Cr), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL)Values are (mean ± SEM)
[a, b] Different letters in the same row indicated significantly difference at $p < 0.05$.

Table 7: The biochemical data of different doses of aqueous extract from a traditional recipe in female rats during a 14-day study

Blood chemistry parameters	Treatments				
	Control	Aqueous extract remedy			
		5 mg/kg	50 mg/kg	300 mg/kg	2,000 mg/kg
BUN (mg/dL)	18.20±0.96[a]	18.20±1.15[a]	18.40±0.87[a]	23.60±0.74[b]	18.60±0.50[a]
Cr (mg/dL)	0.36±0.02[a,b]	0.42±0.02[a]	0.38±0.03[a,b]	0.36±0.02[a,b]	0.26±0.06[b]
AST (U/L)	117.00±11.03[b]	121.20±4.61[b]	120.80±14.36[b]	101.00±5.26[a,b]	88.40±5.81[a]
ALT (U/L)	37.60±0.400[b,c]	42.00±1.70[c]	42.00±1.37[c]	32.40±2.08[a]	34.00±1.14[a,b]
ALP (U/L)	156.00±12.91[b]	152.40±10.01[a,b]	151.20±10.09[a,b]	139.80±6.92[a,b]	125.60±3.66[a]
TG (mg/dL)	110.40±15.40[a]	90.80±9.89[a]	76.00±7.98[a]	89.00±21.37[a]	93.60±10.66[a]
TC (mg/dL)	37.40±1.74[a]	36.00±1.81[a]	34.20±2.20[a]	39.00±2.09[a]	37.40±2.35[a]
HDL (mg/dL)	26.80±0.58[b]	26.20±1.01[a,b]	24.40±0.50[a]	27.20±0.66[b]	25.20±0.48[a,b]

[a, b] Different letters in the same row indicated significantly difference at $p < 0.05$.
Values are (mean ± SEM).
Blood urea nitrogen (BUN), creatinine (Cr), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL)

Morphology of blood cells

The blood cell morphology of all the groups of rats that received the extract at various doses singly was found to be identical with that of the control group. The hemoglobin levels in both male and female rats were in the normal range. The findings indicate that the white blood cells of all groups remain undamaged, as shown in Figures 1 and 2.

Histological characteristics of the liver and kidney

The findings showed that hepatocytes have the characteristic look of a polygonal form and are organized in sheets with a single layer of cells. The results show no detected hepatotoxicity or inflammation in the liver cells of rats at all tested doses of the extract (5, 50, 300, and 2,000 mg/kg), as shown in Figures 3 and 4. The renal histological features were within normal limits. The nephron and interstitium of the normal glomerulus and renal tubule showed no signs of edema, inflammation, or the presence of tubular casts. There is no enlargement or excessive growth of mesangial cells and podocytes in the arteriolar pole of Bowman's space or Bowman's capsule. No abnormal alterations were detected. No tubular cast of any kind was seen in the renal tubules. The tubular cells of both the proximal convoluted tubule and the distal convoluted tubule exhibit typical features.[13.] The Administration of the extract at doses of 5, 50, 300, and 2,000 mg/kg did not result in any adverse effect on the histological features of rat kidneys. There were no apparent differences in the renal histopathology when compared to the control group (Figures 3 and 4).

The acute toxicity assessment conducted in rats, involving the administration of a maximum dose of 2,000 mg/kg of the aqueous extract, showed no toxicological effects within the first 24 hours. Furthermore, no mortality or signs of toxicity were observed over a 14-day monitoring period. The LD_{50}, calculated based on the fixed-dose toxicity test criteria, was found to exceed 2,000 mg/kg body weight.[19] This elevated safety threshold identified in our study may be associated with the comprehensive phytochemical profile of the aqueous extract. Different extraction solvents, such as aqueous, 50% ethanol, and 95% ethanol, produce varying phytochemical compositions; however, we cannot assign safety exclusively to particular compounds without additional research. The extract includes flavonoids recognized for their antioxidative properties; however, it is essential to acknowledge that the safety profile is likely due to a complex interaction of various constituents rather than any one class of compounds. Flavonoids may offer health benefits by neutralizing free radicals and, when ingested in suitable amounts as part of a balanced diet, could be linked to a lower risk of various chronic conditions such as heart disease, cancer, respiratory diseases, and diabetes.[21]

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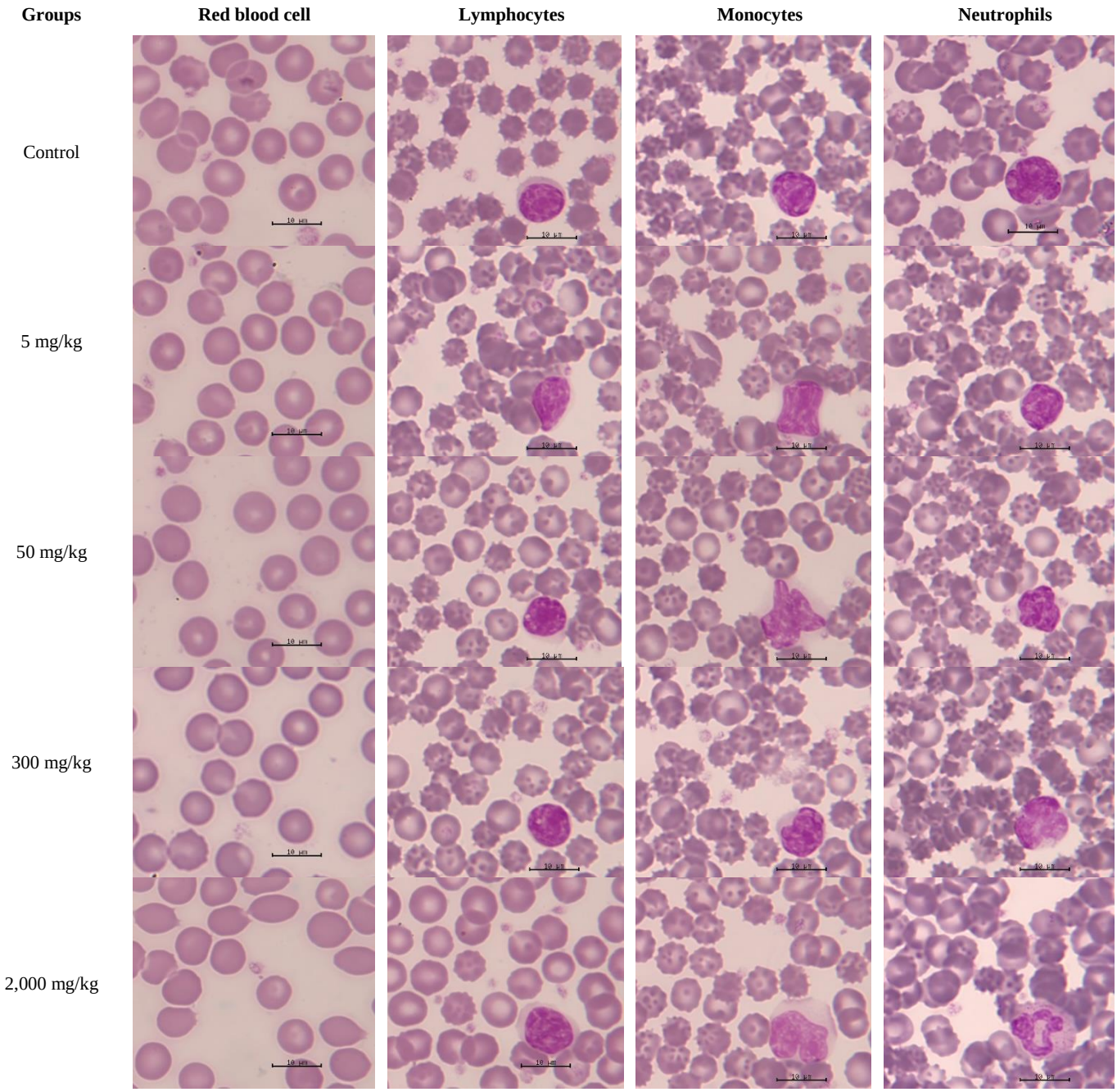

Figure 1: Effect of aqueous extract from a traditional recipe on blood cell morphology in male rats (magnification at 100×).

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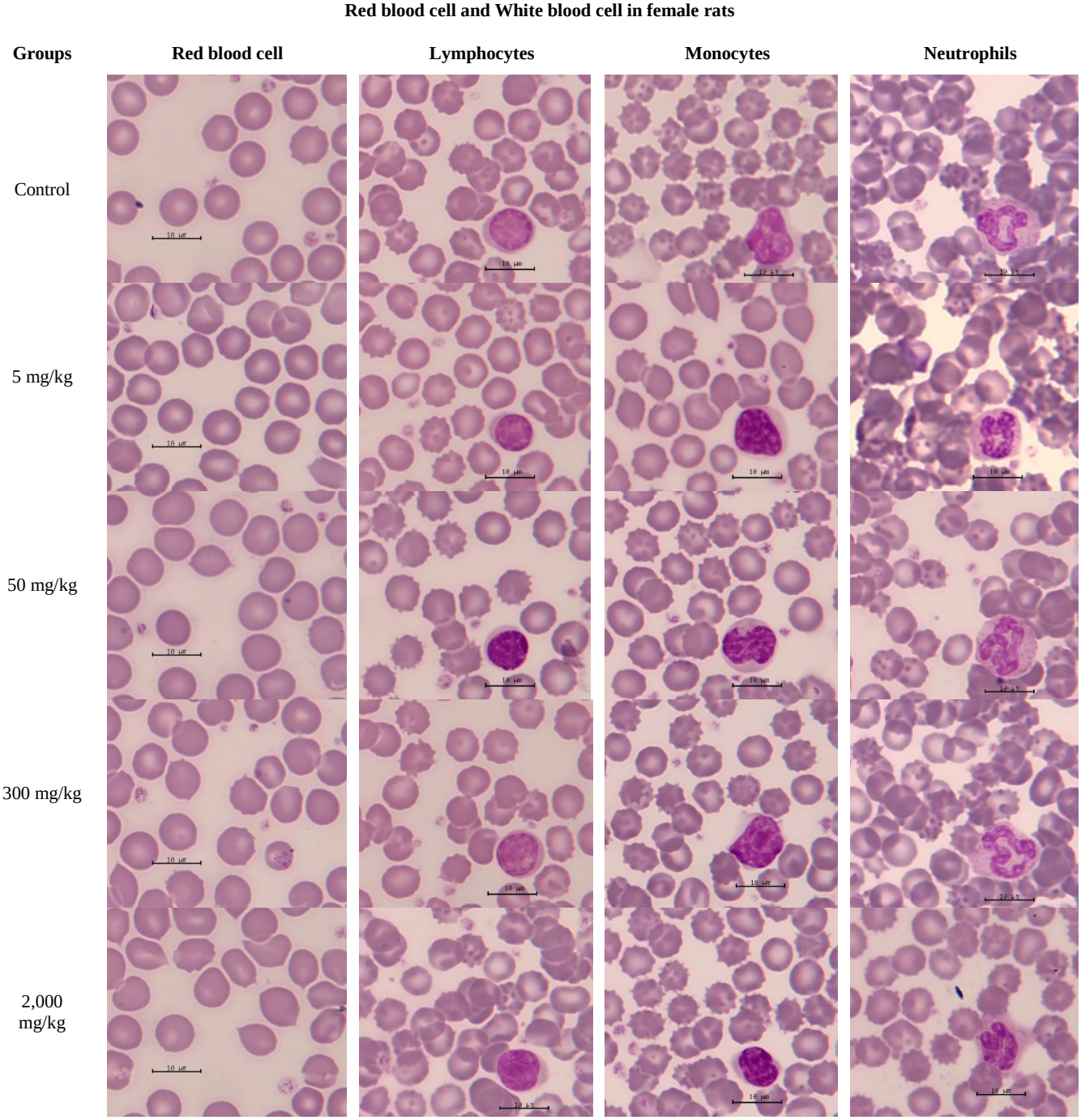

Figure 2: Effect of aqueous extract from a traditional recipe on blood cell morphology in female rats)magnification at 100×(.

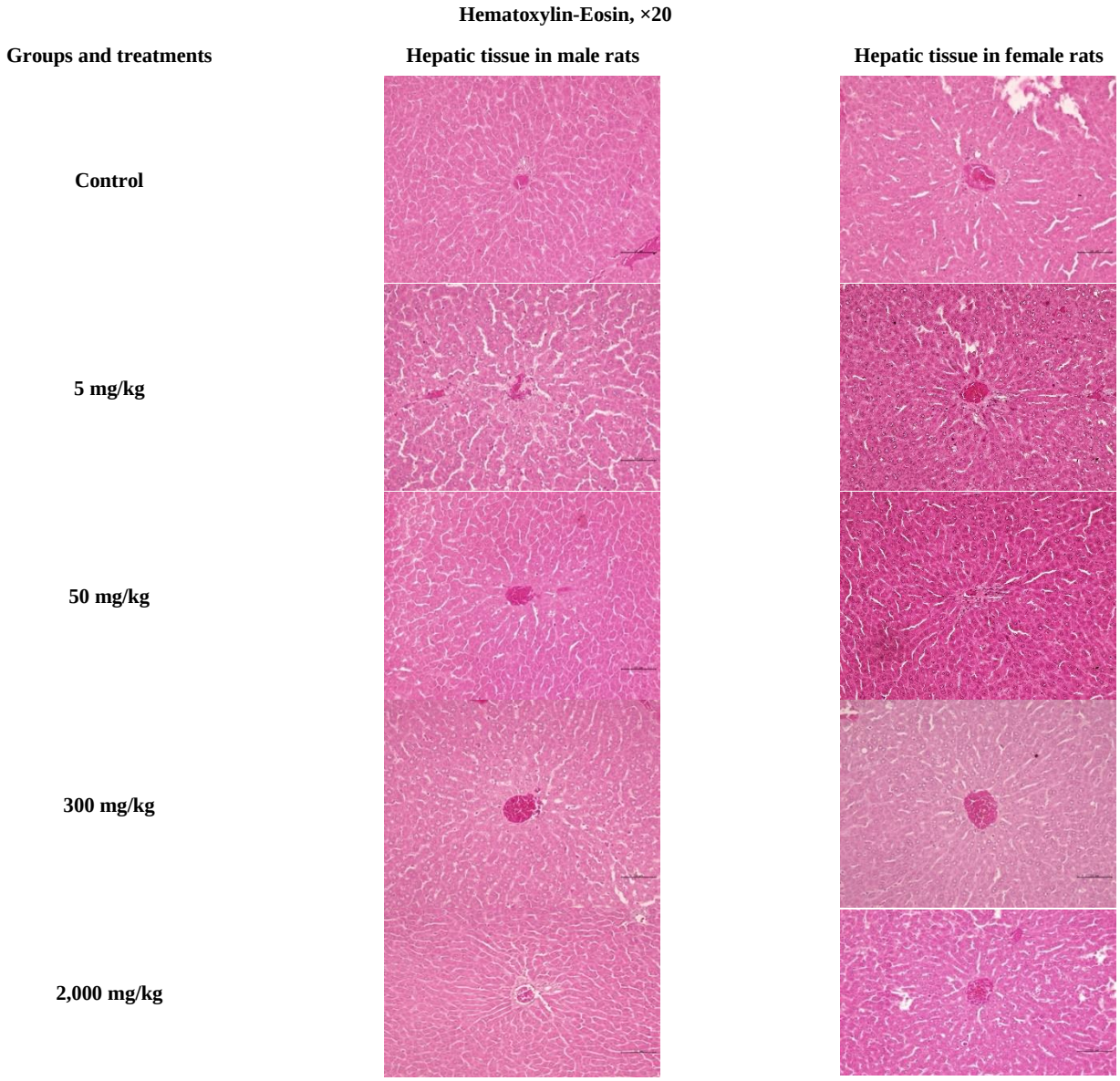

Figure 3: Histopathological illustration of liver in male and female rats treated with aqueous extract from a traditional recipe)magnification = 20×(.

The acute toxicity assessment conducted in rats, involving the administration of a maximum dose of 2,000 mg/kg of the aqueous extract, showed no toxicological effects within the first 24 hours. Furthermore, no mortality or signs of toxicity were observed over a 14-day monitoring period. The LD_{50}, calculated based on the fixed-dose toxicity test criteria, was found to exceed 2,000 mg/kg body weight.[19] This elevated safety threshold identified in our study may be associated with the comprehensive phytochemical profile of the aqueous extract. Different extraction solvents, such as aqueous, 50% ethanol, and 95% ethanol, produce varying phytochemical compositions; however, we cannot assign safety exclusively to particular compounds without additional research. The extract includes flavonoids recognized for their antioxidative properties; however, it is essential to acknowledge that the safety profile is likely due to a complex interaction of various constituents rather than any one class of compounds. Flavonoids may offer health benefits by neutralizing free radicals and, when ingested in suitable amounts as part of a balanced diet, could be linked to a lower risk of various chronic conditions such as heart disease, cancer, respiratory diseases, and diabetes.[21] Additional phytochemical characterization and mechanistic investigations are required to pinpoint the specific compounds that contribute to the safety profile observed in this traditional formulation.[2, 18] This correlation suggests that the extraction method affects the phytochemical composition of the extract; the safety profile is contingent upon various factors, including the plant species, their inherent toxicity, concentrations of bioactive compounds, and possible interactions among constituents. It is crucial to recognize that extraction methods may enhance certain compounds while eliminating others, potentially modifying but not fundamentally altering the toxicity profile of the original plant material. Consequently, the safety observed in this study should be regarded as specific to this particular aqueous extract and not extrapolated to all preparations or forms of these plants.

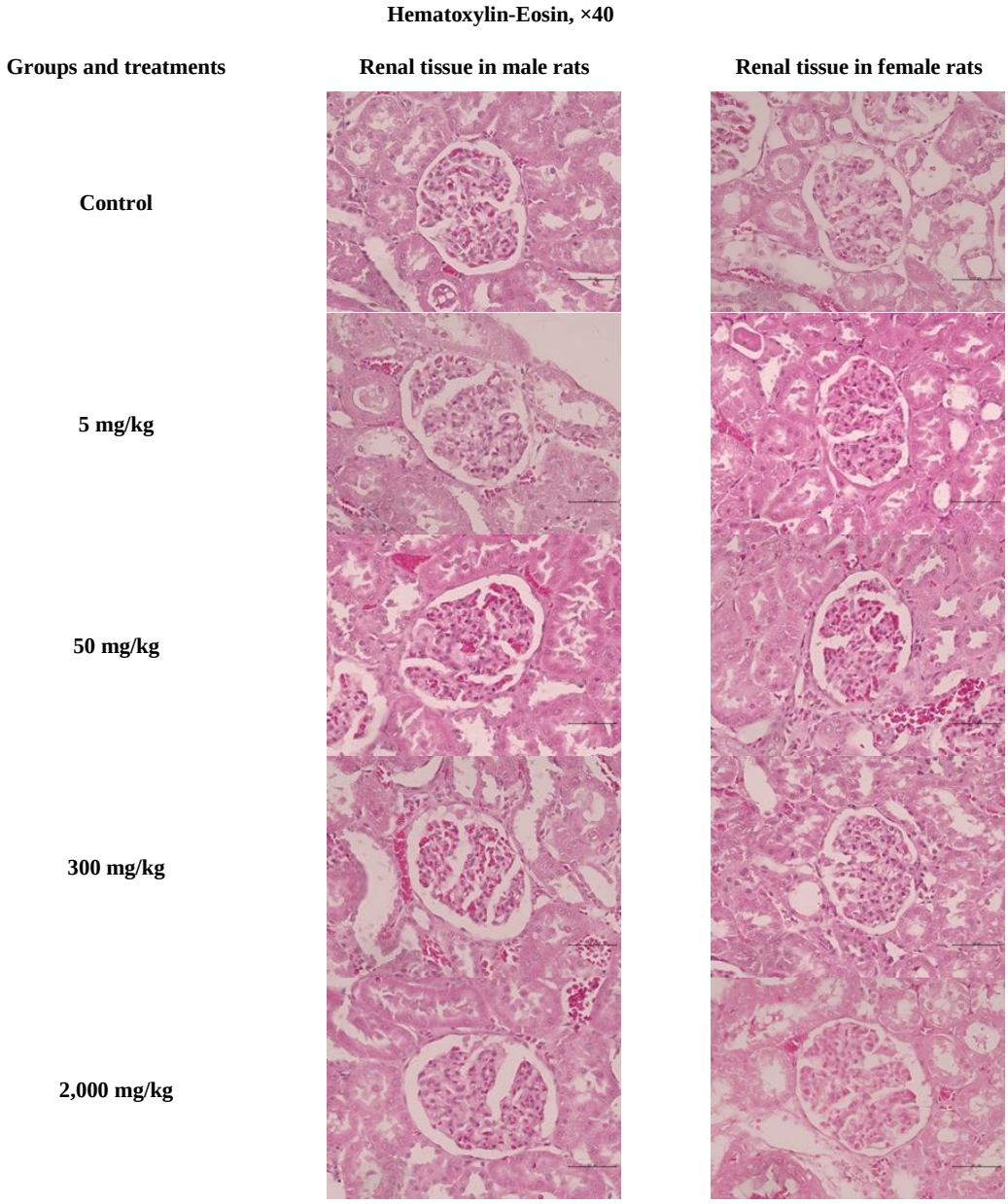

Figure 4: Histopathological illustration of kidney in male and female rats treated with aqueous extract from a traditional recipe)magnification = 40×).

After a 14-day treatment period with the extract, there was no statistically significant change in the average relative weight of critical organs, since inflammatory processes often result in edema, cellular infiltration, and heightened vascular permeability, which commonly present as increased organ weight. Prior research has shown a link between inflammatory responses and alterations in organ weight, especially in organs like the liver and spleen, which exhibit significant sensitivity to systemic inflammation. These results support previous research that emphasized the anti-inflammatory properties of *M. cochinchinensis* extract.[4] Moreover, this result is consistent with pharmacological research on *C .rotang*, which has been explored for its potential to control inflammation and modulate the immune system in human participants.[17] These outcomes collectively highlight the safety of the extract and its potential therapeutic benefits in reducing inflammation, reinforcing the relevance of both *M. cochinchinensis* and *C. rotang* in medical research and treatment strategies.

In assessing the metabolic impact of this aqueous extract, female rats administered a dose of 300 mg/kg exhibited triglyceride (TG) levels similar to those of the control group, indicating no adverse effects on lipid metabolism at this dose. Furthermore, orally administered extract at 5, 50, and 2,000 mg/kg, exhibited the comparable values of total cholesterol (TC) levels with rats in the control group. The high-density lipoprotein (HDL) levels in the groups that received 5 and 300 mg/kg of the extract showed no significant deviations from the control, reinforcing the conclusion that these doses of the extract do not negatively affect lipid profiles. Collectively, these findings suggest that the extract, across a range of doses, maintained a neutral profile on key lipid parameters, supporting its safety and potential utility in therapeutic applications without disrupting normal lipid metabolism.

An analysis of kidney function in male rats administered doses of 5, 50, and 2,000 mg/kg of the extract showed that blood urea nitrogen (BUN) levels were similar to those of the control group, with no statistically significant differences observed. Similarly, creatinine levels in rats that

received 50 and 300 mg/kg of the extract were also comparable to control values, indicating no significant negative impact on the renals. In female rats, doses of 5 and 50 mg/kg resulted in reduced BUN and creatinine levels, mirroring the trends observed in males, with all values remaining within normal clinical ranges. This suggests that the extract did not adversely affect biochemical parameters related to kidney function. Moreover, there was no evidence of hepatotoxicity or adverse histological changes in the liver of the experimental animals, further supporting the extract's safety. However, to fully ascertain the safety profile of the extract, it is crucial to undertake more comprehensive long-term studies and additional toxicological assessments on rats.[20] This approach will help confirm the absence of delayed or cumulative adverse effects, ensuring the extract's suitability for further therapeutic use.

The study's examination of liver histology revealed that both the control and treatment groups exhibited normal hepatocytes with no structural changes observed in the liver cells, indicating that the extract did not induce hepatocellular damage. Similarly, kidney histology across all groups showed no morphological alterations, and the absence of inflammatory cells in the kidney tissue further confirmed the lack of histological toxicity. These findings demonstrate that the extract is non-toxic to both liver and kidney tissues, affirming its safety not causing any adverse structural or cellular modifications in these critical organs. This comprehensive histological assessment supports the potential for safe application of the extract in therapeutic contexts, given its non-detrimental effects on essential organ systems.

The recipe, comprising 6 herbs, exhibits a range of documented pharmacological activities. These include effects on nephropathy[13] and diabetes management,[6] along with properties that inhibit hepatocellular carcinoma cells,[14] and demonstrate anticancer,[4] anti-inflammatory,[4] and antioxidant activities.[12] Notably, *T. triandra*, a key component of the recipe, is celebrated for its multifaceted medicinal properties, including detoxification, anti-inflammatory, anticancer, antibacterial, immune modulation, and antioxidation. This plant's leaf extract is particularly rich in antioxidants such as vitamin E and phytol, which are crucial in neutralizing harmful free radicals, thereby mitigating oxidative stress and maintaining cellular redox balance. The recipe also contains other important bioactive compounds, including lycopene from *M. cochinchinensis*, which is known for its potent antioxidant properties and potential anticancer effects, and shatavarin I-IV from *A. racemosus*, which has demonstrated immunomodulatory and adaptogenic activities in previous studies. It also includes lycopene from *M. cochinchinensis* and shatavarin I-IV from *A. racemosus*. Despite these promising attributes, further research is essential to elucidate the mechanism and efficacy of this herbal concoction.[7]

This aqueous extract under investigation, which is a traditional remedy for diabetes and rich in secondary metabolites, is non-toxic at doses up to 2,000 mg/kg in our acute toxicity study, indicating its potential for therapeutic investigation. This observation from our study offers initial safety data, positioning this traditional formula as a viable candidate for further investigation as a natural alternative for diabetes management. Nonetheless, the long-term safety and clinical effectiveness of this extract necessitate further research, including sub-chronic and chronic toxicity assessments, along with controlled clinical trials, prior to contemplating its incorporation into modern medical practices.

Conclusions

The conclusion of our study on the aqueous extract derived from a traditional recipe documented in the Royal Textbook of King Rama V reveals that at all tested doses, the extract showed no signs or symptoms of toxicity on a short-term basis. Given that these plants are traditionally consumed as herbs for managing diabetes, our findings suggest that they can be safely ingested in amounts not exceeding an extract dose equivalent to 2,000 mg/kg. This supports their continued use in herbal medicine, consistent with decades of medicinal application without reported adverse effects. However, to thoroughly assess the safety of this extract, further studies focusing on sub-chronic and chronic toxicity are necessary. Such studies would provide deeper insights into the implications of repeated-dose administration, thereby solidifying the understanding of the long-term safety profile of these traditional remedies.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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