RESEARCH PAPER

Evaluation of acute and subacute toxicity of hydroethanolic preparation of fruit of Trichosanthes dioica, Trichosanthes cucumerina, Luffa cylendrica, Luffa acutangula and its mixture in Wistar rats: clinical, haematological and biochemical studies.

Maneesh Kumar Pandey[1]* ,Praveen Sharma[2]

[1]*Department of Pharmacy Oriental University, Indore Sanwer Road, Jakhya Opposite Revati Range Gate No.1, Indore, Madhya Pradesh, 453555, India,*
Email : maneyp@gmail.com, https://orcid.org/0000-0003-0790-2077
[2]*Department of Pharmacy Oriental University, Indore Sanwer Road, Jakhya Opposite Revati Range Gate No.1, Indore, Madhya Pradesh, 453555, India*

ABSTRACT
Luffa acutangula, Luffa cylindrica, Trichosanthes dioica, and Trichosanthes cucumerina (family Cucurbitaceae) are widely used in traditional systems of medicine for the management of jaundice, skin disorders, fever, inflammatory conditions, gastrointestinal disturbances, and metabolic disorders. Luffa cylindrica is additionally employed in Panchakarma therapy for emesis and purgation as part of Shodhana Karma. Although these fruits are commonly consumed and therapeutically applied, systematic scientific data on their safety profile remain limited. The present study was designed to evaluate the acute and subacute oral toxicity of hydroethanolic fruit extracts of Trichosanthes dioica (TDHEE), Trichosanthes cucumerina (TCHEE), Luffa cylindrica (LCHEE), Luffa acutangula (LAHEE), and their polyherbal mixture (MAHEE; 1:1:1:1) in wistar rats.

Acute oral toxicity was assessed by administering single doses of 100 and 1000 mg/kg body weight in male and female rats. Subacute toxicity was evaluated through repeated oral administration of the extracts at doses of 100 and 1000 mg/kg for 28 days. Animals were monitored for mortality, clinical signs of toxicity, body weight changes, and alterations in haematological and biochemical parameters.

No mortality or treatment-related toxic effects were observed in the acute toxicity study, indicating that the median lethal dose (LD_{50}) of all extracts exceeds 1000 mg/kg body weight. In the subacute study, repeated administration did not result in significant changes in body weight, haematological indices, or biochemical parameters compared to control groups. These findings suggest that TDHEE, TCHEE, LCHEE, LAHEE, and MAHEE are safe and non-toxic up to 1000 mg/kg and support their traditional medicinal use.

Keywords: Acute toxicity, Subacute toxicity, Cucurbitaceae, plant extract, Polyherbal formulation.
How to cite this article: Pandey MK, Sharma P, Evaluation of acute and subacute toxicity of hydroethanolic preparation of fruit of Trichosanthes dioica, Trichosanthes cucumerina, Luffa cylendrica, Luffa acutangula and its mixture in Wistar rats: clinical, haematological and biochemical studies...Int J Drug Deliv Technol. 2026;16(2s): 429-447; DOI: 10.25258/ijddt.16. 429-447
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Natural products derived from plants, animals, and microorganisms have long contributed to human and animal health. According to the World Health Organization, nearly 80% of populations in developing nations still rely on plant-based traditional medicines for primary healthcare needs (1). Among these natural resources, medicinal plants continue to guide modern pharmacological research due to their rich bioactive profiles and therapeutic versatility. Polyherbal formulations, a hallmark of Ayurvedic and other traditional systems, combine multiple plant extracts to enhance efficacy through synergistic interactions while reducing potential toxicity (2).

The Cucurbitaceae family(3) comprising cucumbers, gourds, melons, and squashes includes over 800 species recognized for their nutritional value and medicinal relevance (4, 5). These vegetables are abundant in flavonoids, phenolic acids, cucurbitacins, saponins, terpenoids, and other secondary metabolites that exhibit antioxidant, anti-inflammatory, antidiabetic, anticancer, and gastroprotective properties.

Trichosanthes dioica, widely cultivated across eastern and northern India(6), is traditionally used for managing metabolic disorders, inflammation, fever, wounds, and hepatic ailments(7, 8). Its fruits contain cucurbitacins, cucurbitane glycosides, flavonoids, vitamins, and triterpenoids, which contribute to diverse pharmacological activities(9).

Trichosanthes cucumerina (snake gourd) holds significant ethnomedicinal value in South Asian systems of medicine(10). Its fruits and aerial parts are used for treating fever, jaundice, respiratory conditions, and gastrointestinal disturbances(11). The plant's cucurbitacins, carotenoids, saponins, and phenolic compounds underpin its antioxidant,

**Author for Correspondence: Maneesh Kumar Pandey*

anti-inflammatory, antidiabetic, and hepatoprotective properties(12).

Luffa cylindrica (sponge gourd) is an important vegetable crop(13) rich in flavonoids, terpenoids, alkaloids, and saponins(14, 15). Traditionally used for inflammation, pain, infections, and wound healing, it also demonstrates strong antioxidant and antimicrobial activities(16, 17).

Luffa acutangula (ridge gourd) is consumed widely across Asia and used in traditional medicine for liver disorders, constipation, diabetes, and inflammatory conditions(18, 19). Its phytochemical constituents—flavonoids, phenolic acids, and triterpenoids are associated with antioxidant, hepatoprotective, and antidiabetic effects(10).

Given their nutritional significance, ethnomedicinal value, and diverse pharmacological potential, these four Cucurbitaceae vegetables offer promising prospects for scientific investigation and therapeutic development(20).

MATERIALS AND METHODS

2.1 Sample Collection, Authentication, and Preparation of Extracts

Fresh fruits of *Trichosanthes dioica* Roxb., *Trichosanthes cucumerina* L., *Luffa cylindrica* (L.) M. Roem., and *Luffa acutangula* (L.) Roxb. were collected during July and November. The plant materials were identified and authenticated under the supervision of Prof. N. K. Dubey, Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi. Voucher specimens were deposited with accession numbers Cucurbita 2022/01 and Cucurbita 2022/02.

Vegetative unripe fruits—*T. dioica* (8.0 kg), *T. cucumerina* (10.0 kg), *L. cylindrica* (10.0 kg), and *L. acutangula* (8.0 kg)—were thoroughly washed, cut into small pieces, and shade-dried under controlled conditions. The dried material was then coarsely powdered for extraction.

Extraction was carried out using 50% v/v hydroethanol. Coarse powders of *T. dioica* (1100 g), *T. cucumerina* (1300 g), *L. acutangula* (850 g), and *L. cylindrica* (900 g) were macerated overnight, followed by centrifugation at 10,000 rpm. The supernatants were filtered through Whatman No. 1 filter paper and concentrated using a rotary vacuum evaporator (IKA RV 10 digital). The semi-solid residues were further dried using a lyophilizer under reduced pressure to obtain the final extracts, which were stored in airtight desiccators for subsequent analyses.

2.2 In Vivo Animal Study

2.2.1 Animals Used

175±25 gm. of Wistar albino rats, male or female around 60 to 70 days aged were received by PBRI, Pinnacle Biomedical Research Institute, Bhopal; M.P, India were stored in the well-equipped animal house mentioned conditions are temp 23 ± 2°C, relative humidity 55 ± 10%, and 12-hours circadian cycle. Meanwhile the research work, animals had ad libitum admittance to typical laboratory food. For PBRI Bhopal, (Reg. No. 1824/PO/Ere/S/15/CPCSEA) approved the experiments with Protocol Approval Reference No. PBRI/IAEC/PN-23194, and the experiments were conducted in accordance with the applicable guidelines for the usage of laboratory animals.

2.3 Toxicological Studies of 50% V/V Ethanolic Extracts of Different Fruits

2.3.1 Experimental design for acute toxicity studies

Acute toxicity of the 50% v/v hydroethanolic fruits extract of *Trichosanthes dioica* Roxb*., Trichosanthes cucumerina* L., *Luffa cylindrica* (L) M., Roem *Luffa acutangula* (L.) Roxb. and Mixture (1:1:1:1) of all fruit hydroethanolic extract was evaluated in A total 33 healthy adult wistar rat both sex male and female using up and down procedure according to The Organization for Economic Cooperation and Development (OECD) guide line 245(21). All the animals were kept at overnight fasting 12 h before experiment with free excess to water. The animals (seventeen males and sixteen females) were randomly allocated into nine groups, each comprising three animals. The first group served as a control, while second and third group was considered as test group which received 50% v/v hydroethanolic extract of *Trichosanthes dioica* (TDHEE), fourth and fifth was considered as test group which received 50% v/v hydroethanolic extract of *Trichosanthes cucumerina* (TCHEE), sixth and seventh group was considered as test group which received 50% v/v hydroethanolic extract of *Luffa cylindrical* (LCHEE), eighth and ninth group was considered as test group which received 50% v/v hydroethanolic extract of *Luffa acutangula* (LAHEE), tenth and eleventh group was considered as test group which received mixture (1:1:1:1) of all fruit hydroethanolic extract (MAHEE) at fixed two doses levels (100 mg/kg and 1000 gm/kg body weight) orally by gavage (20, 22). Control group animals were given 10 ml/kg of 1% carboxy methyl cellulose (CMC) in distilled water by mouth. Prior to administration of the dose, each animal's weight was measured, and the dose was adjusted based on the weight. For 14 consecutive days, the animals were monitored one by one to see any changes in their behaviour and any changes in their physical condition. Special observations were made during the first 4 hours to check for changes in mortality, diarrhoea, respiration, sedation, drowsiness, body posture, skin colour, fur condition, coma, and eye colour (23).

2.3.2 Experimental design for subacute toxicity (28 days)

The subacute oral toxicity was conducted in accordance with the OECD Test Guidelines 407(24). Adult, healthy Wistar rats weighing 150–200 g and aged 8–10 weeks were selected for the study and allocated randomly into multiple experimental groups (n = 10 per group), with each group comprising five males and five females. Animals were housed in stainless-steel cages under standardized laboratory conditions, with males and females maintained separately to avoid physiological interference. The housing environment was stringently regulated, maintaining a 12 h light–dark cycle along with controlled temperature and relative humidity to ensure optimal acclimatization and minimize environmental stress. Throughout the experimental duration, animals had unrestricted access to standard laboratory feed and potable water *ad libitum*.

Test substances were administered once daily for a continuous period of 28 days via the oral route using a calibrated orogastric cannula to ensure accurate dose

delivery. The control group received 1% carboxymethyl cellulose (CMC) prepared in distilled water at a dose volume of 10 ml/kg body weight. The designated test groups were orally administered 50% v/v hydroethanolic fruit extracts of *Trichosanthes dioica* (TDHEE), *Trichosanthes cucumerina* (TCHEE), *Luffa cylindrica* (LCHEE), and *Luffa acutangula* (LAHEE). Additional test groups received a combined formulation consisting of an equal proportion (1:1:1:1) of all four fruit hydroethanolic extracts (MAHEE). These extracts were administered at two graded dose levels, namely 100 mg/kg and 1 g/kg body weight, to evaluate dose-dependent effects.

All animals were monitored on a daily basis for general health status, behavioral alterations, and any overt signs of toxicity or adverse reactions throughout the treatment period. Body weights were recorded periodically to assess growth patterns and detect any treatment-related deviations. In the event of mortality during the study, the affected animal was subjected to pathological examination to ascertain the probable cause of death. At the conclusion of the experimental period, all surviving animals were thoroughly examined to identify any delayed or cumulative toxic manifestations associated with repeated oral administration of the test extracts.(23, 24). At the end of the treatment, the animals were fasted overnight and anesthetized followed by blood collection by cardiac puncture for haematological and biochemical analysis.

2.4 Hematological parameters

On the twenty-ninth day of the experimental schedule, all surviving animals were henceforth subjected to an overnight fast to minimize metabolic variability, after which they were anesthetized under controlled conditions, and blood was carefully withdrawn from the right ventricle to ensure adequate volume and analytical reliability. The collected blood was immediately apportioned into three distinct receptacles: one tube preloaded with 3.2% buffered sodium citrate, one tube containing heparin as an anticoagulant, and one dry, non-heparinized centrifuge tube. This strategic division was carried out in accordance with standard laboratory protocols to aggrandize the accuracy and scope of subsequent hematological, coagulation, and biochemical evaluations. The citrated samples were specifically reserved for coagulation profiling, wherein prothrombin time (PT) and partial thromboplastin time (PTT) were determined to assess the functional integrity of the coagulation cascade, which represents the physiological carapace safeguarding the vascular intima against hemorrhagic insult. The heparinized blood was utilized for comprehensive hematological investigations, including quantification of red blood cell count, hemoglobin concentration, hematocrit value, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, total white blood cell count, and differential leukocyte profile. These parameters collectively reflect the cellular rind of the circulatory system, providing insight into oxygen transport capacity, immune competence, and hemostatic balance. The non-heparinized samples were processed for serum separation to facilitate biochemical analysis, thereby allowing correlation between cellular indices and metabolic status. Throughout this procedure, meticulous handling was maintained to prevent hemolysis or exsiccation of samples, as such preanalytical errors could contumaciously distort results and lead to interpretative ignominy. The immanence of systemic homeostasis was evaluated through the integrated analysis of these parameters, as alterations in blood composition often signify underlying tissue stress or adaptive amelioration in response to experimental intervention. Rapid processing of samples quickened analytical turnaround and reduced the risk of cellular degradation, ensuring data integrity. Any deviation from these carefully controlled steps could enfetter the reliability of findings; therefore, strict adherence to protocol was maintained to ensure that the collected hematological and coagulation data faithfully represented the physiological status of the animals at the termination of the study.(24)

Following collection, the blood portion allocated to the non-anticoagulated vessel was allowed to undergo spontaneous coagulation, after which the clotted mass was subjected to controlled centrifugation to disengage the clear serum from the cellular rind. The recovered serum, representing the soluble biochemical intima of the circulatory compartment, was henceforth employed for an extensive panel of clinical chemistry estimations. Serum glucose was quantified to appraise metabolic homeostasis, while creatinine and blood urea nitrogen were measured to evaluate renal functional integrity and nitrogenous waste clearance. Hepatic status was assessed through determination of the transaminase enzymes—aspartate transaminase and alanine transaminase—whose elevations signify hepatocellular perturbation and compromise of the hepatic carapace. In parallel, serum electrolytes, including sodium, potassium, and chloride, were analyzed to reflect ionic equilibrium and neuromuscular stability, as disturbances in these parameters may quicken systemic dysfunction. Additional estimations encompassed calcium and phosphorus to assess mineral balance and skeletal metabolism, alongside conjugated and total bilirubin to evaluate hepatic excretory efficiency and biliary patency. Lipid metabolism was profiled through measurement of total cholesterol, high-density lipoprotein, and triglycerides, parameters that collectively aggrandize understanding of cardiovascular risk and metabolic adaptation. Furthermore, total protein and albumin concentrations were determined as indices of synthetic liver capacity and nutritional status, providing insight into plasma oncotic pressure and transport functions. Throughout the analytical workflow, meticulous handling was maintained to avert hemolysis or exsiccation of serum constituents, as such artifacts could contumaciously distort biochemical readouts and lead to interpretative ignominy. Nevertheless, adherence to standardized protocols ensured that the resulting data faithfully reflected the immanence of systemic physiological balance, while deviations from normal ranges were interpreted as markers of either pathological insult or adaptive amelioration in response to experimental conditions.(25)

2.5 Statistical Analysis

Total mathematical interpretation considered as mean ± standard error of the mean (SEM). Statistical ANOVA test was performed with probability value ($p \leq 0.05$) was

accepted significant. Software used GraphPad Prism (Version 10.)

RESULTS

3.1 Acute Toxicity Study

Neither mortality nor some short of adverse signs were observed in rats during 14 days of observation following acute treatment by oral route with *T.dioica, T. cucumirina, L. acutangula,L. cylendrica* and Mixture (1:1:1:1) of all fruit hydroethanolic extract. Further TDHEE, LCHEE, TCHEE, LAHEE and MAHEE did not produce any behavioral changes during study period (table 1) and no mortality was recorded throughout two weeks. There were no significant ($p>0.05$) differences in the body weight changes between the control and TDHEE, LCHEE, TCHEE, LAHEE, MAHEE treated rats (chart1). The food consumption of animals receiving TDHEE, LCHEE, TCHEE, LAHEE, MAHEE was generally similar to that of the control. The results of food consumption show increasing body weight of all animal associated with increased food consumption. The quantity of water consumption by control group was similar to that of all test groups

Table 1 Cageside observations for 14 days of control and *T.dioica, T. cucumirina, L. acutangula and L. cylendrica* hydroethanolic extract treated wistar rats at a 100mg/kg and 1000mg/kg bw.

Parameter	Day 0 Observation	Day 7 Observation	Day 14 Observation
Ocular appearance	Unaltered	Unaltered	Unaltered
Body alignment and posture	Physiological	Physiological	Physiological
Cutaneous changes	Absent	Absent	Absent
Fur texture and appearance	Healthy	Healthy	Healthy
Diarrheal signs	None detected	None detected	None detected
Respiratory pattern	Within normal	Within normal	Within normal
Sedative effects	Not observed	Not observed	Not observed
Drowsy behavior	Not evident	Not evident	Not evident
Comatose state	Not observed	Not observed	Not observed
Survival status	All survived	All survived	All survived

3.1.1 Effect of Plant Extracts on Body Weight Changes

The acute toxicity assessment of hydroethanolic extracts of *Trichosanthes dioica* (TDHEE), *Luffa cylindrica* (LCHEE), *Trichosanthes cucumerina* (TCHEE), *Luffa acutangula* (LAHEE), and Mixture of all hydroethanolic extracts (MAHEE) was carried out at a dose of 100 mg/kg body weight in Wistar albino rats. The results of body weight monitoring over 14 days are presented in Table 1.

Throughout the experimental period, none of the treated groups exhibited any signs of mortality, abnormal posture, altered grooming behavior, or tremors, indicating the absence of acute toxicity. A gradual increase in body weight was observed in the control and extract-treated animals, suggesting normal metabolic functioning and healthy physiological growth. Among the extracts, MAHEE produced the highest body weight gain (from 168.6 ± 3.38 g on day 0 to 171.47 ± 1.69 g on day 14), followed by LCHEE (165.07 ± 6.82 g to 169.90 ± 4.55 g) and TCHEE (163.44 ± 5.47 g to 166.86 ± 3.66 g). TDHEE and LAHEE also showed slight but consistent increases in weight, comparable to the control group.

The absence of any significant reduction or erratic changes in body weight indicates that none of the tested hydroethanolic extracts exerted toxic effects on the metabolic or physiological status of the rats. Therefore, all the extracts can be considered safe at the tested dose of 100 mg/kg body weight, with no adverse effects observed during the acute exposure period

Table 2 Acute toxicity study effects of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on body weight changes in wistar albino rats at dose 100 mg/kg, bw.

Days	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Day0	160.37±5.86	159.03±4.61	165.07±6.82	163.44±5.47	161.75±3.09	168.6±3.38
Day7	163.37±3.99	160.23±4.32	166.83±5.62	164.43±4.84	163.01±2.40	170.73±1.56
Day14	166.21±2.32	160.37±1.29	169.9±4.55	166.86±3.66	165±1.73	171.47±1.69

The acute oral toxicity study of hydroethanolic extracts of *Trichosanthes dioica* (TDHEE), *Luffa cylindrica* (LCHEE), *Trichosanthes cucumerina* (TCHEE), *Luffa acutangula* (LAHEE), and Mixture of all hydroethanolic extracts (MAHEE)

was further evaluated at a higher dose level of 1000 mg/kg body weight in Wistar albino rats. The data on body weight variations recorded over a period of 14 days are summarized in Table 2.

Throughout the experimental period, all animals remained active and healthy with no signs of mortality, abnormal gait, convulsions, piloerection, or behavioral alterations. A consistent and gradual increase in body weight was observed across all treatment groups, comparable to that of the control group. Among the treated groups, LCHEE exhibited the highest increase in body weight, rising from 172.13 ± 4.90 g on day 0 to 175.36 ± 2.42 g on day 14, followed closely by TDHEE and MAHEE, which also showed steady growth patterns. The TCHEE and LAHEE groups demonstrated similar trends, with slight but regular increases in body weight over the observation period.

The absence of significant body weight loss or behavioral toxicity suggests that none of the tested extracts produced deleterious physiological effects at the administered 1000 mg/kg bw dose. The observed weight gain pattern indicates normal metabolic activity and good tolerability of the extracts

Table 3 Acute toxicity study effects of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on body weight changes in wistar albino rats at dose 1000 mg/kg, bw.

Days	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Day0	166.17±5.38	168.22±3.06	172.13±4.90	166.4±5.19	164.4±4.43	169.89±5.27
Day7	168.1±3.08	170.65±1.20	173.544.08	169.28±3.34	165.74±3.52	171.20±3.90
Day14	170.79±3.16	172.03±0.82	175.36±2.42	170.15±2.58	167.39±2.92	172.84±2.302

Note: D0 = day zero, D7 = day 7, and D14 = day 14. Comparisons were made with a control group before and after the experiment. The data illustrated the mean ±SEM of 3 rats per treatment groups (n = 3).

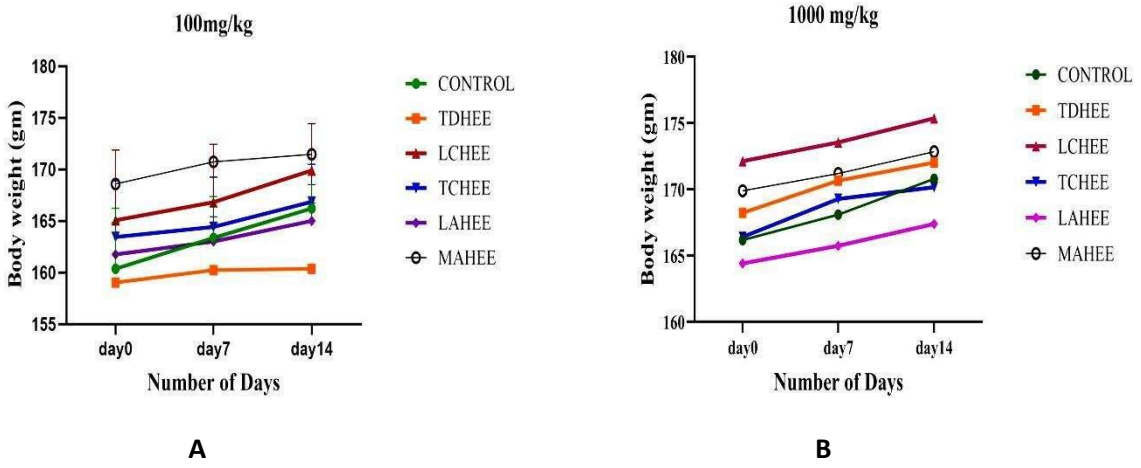


Chart 1 Acute toxicity effects of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on body weight changes wistar albino rats at dose (A) 100 mg/kg, and (B) 1000 mg/kg, bw

3.2 Subacute toxicity Study

3.2.1 Effect of Plant Extracts on Body Weight Changes in Female and Male Wistar Albino Rats at 100 mg/kg b.w.

The subacute oral toxicity evaluation of hydroethanolic extracts of *Trichosanthes dioica* (TDHEE), *Luffa cylindrica* (LCHEE), *Trichosanthes cucumerina* (TCHEE), *Luffa acutangula* (LAHEE), and Mixture of all hydroethanolic extracts (MAHEE) was conducted in both female and male Wistar albino rats at a dose of 100 mg/kg body weight for 28 consecutive days. The variations in body weight recorded weekly are presented in Table 4 (female) and Table 5 (male).

3.2.1.1 Body Weight Changes in Female Rats

Throughout the 28-day experimental period, no mortality or signs of clinical toxicity such as piloerection, tremors, diarrhea, altered food intake, or lethargy were observed in any group. A gradual and consistent increase in body weight was noted among both control and extract-treated animals, indicating normal growth and the absence of extract-induced metabolic disturbances. Among the treated groups, MAHEE demonstrated the highest increase in body weight, from 182.39 ± 5.89 g on day 0 to 183.91 ± 5.15 g on day 28, followed by LCHEE (177.77 ± 10.16 g to 181.04 ± 7.41 g) and TCHEE (176.71 ± 9.26 g to 182.04 ± 7.41 g). The TDHEE and LAHEE groups also showed marginal but steady increases comparable to the control.

The consistent upward trend across all groups suggests that administration of the extracts at 100 mg/kg did not interfere with nutrient absorption, energy metabolism, or general health status. The findings confirm that the test extracts were well tolerated in female rats and did not induce any observable toxic effects under subacute exposure conditions.

3.2.1.2 Body Weight Changes in Male Rats

A similar pattern was observed in male Wistar rats. No mortality or abnormal behavioral symptoms were recorded during the 28-day treatment period. A gradual increase in body weight was evident in all experimental groups, confirming the absence of systemic toxicity. The MAHEE-treated group exhibited the most pronounced weight gain, from 193.08 ± 9.60 g at baseline to 208.65 ± 5.89 g on day 28, indicating good tolerability. The TCHEE and LAHEE groups also showed significant progressive increases (189.14 ± 7.37 g to 201.39 ± 4.84 g and 193.19 ± 7.21 g to 203.94 ± 5.26 g, respectively). LCHEE and TDHEE showed moderate but stable weight increments throughout the study as shown in table 4 & 5.

No significant reduction in body weight was detected in any of the extract-treated groups when compared to the control, confirming that the test substances did not exert anorexic or catabolic effects represented in chart 2.

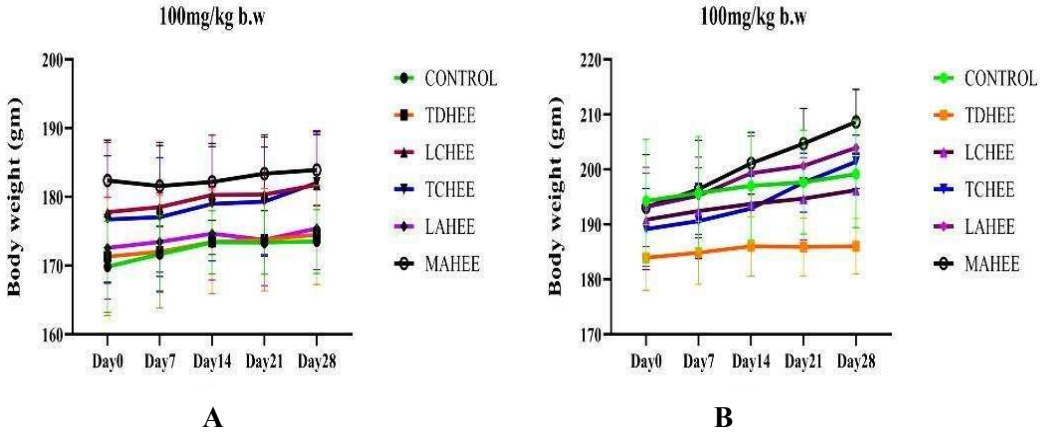
Overall, the steady increase in body weight across both sexes indicates normal physiological development, stable metabolic function, and the absence of subacute toxicity for all tested hydroethanolic extracts at the administered dose.

Table 4 Subacute toxicity study effects of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on body weight changes in female wistar albino rats at dose 100 mg/kg, bw.

Days	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Day0	169.83± 6.64	171.32± 8.57	177.77± 10.16	176.71± 9.26	172.56± 7.42	182.39± 5.89
Day7	171.66± 5.54	172.02± 8.19	178.5± 9.45	177.06± 8.65	173.44± 7.12	181.58± 5.88
Day14	173.39± 4.59	173.44± 7.53	180.29± 8.67	178.99± 8.31	174.63± 6.72	182.16± 5.60
Day21	173.35± 4.59	173.78± 7.47	180.39± 8.71	179.32± 7.92	172.33± 6.73	183.37± 5.34
Day28	173.5± 4.66	174.47± 7.20	181.04± 7.41	182.04± 7.41	172.75± 6.56	183.91± 5.15

Table 5 Subacute toxicity study effects of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on body weight changes in male wistar albino rats at dose 100 mg/kg, bw.

Days	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Day0	194.28± 11.25	183.95± 5.99	190.84± 8.46	189.14± 7.37	193.19± 7.21	193.08± 9.60
Day7	195.7± 10.28	184.86± 5.74	192.44± 7.87	190.62± 6.86	195.21± 7.01	196.41± 8.84
Day14	197.02± 9.85	186.02± 5.39	193.72± 7.51	192.88± 5.92	199.32± 6.76	201.09± 5.59
Day21	197.67± 9.43	185.9± 5.24	194.67± 7.48	197.6± 5.36	200.66± 6.0	204.69± 6.43
Day28	199.13± 9.70	186.01± 5.03	196.22± 6.80	201.39± 4.84	203.94± 5.26	208.65± 5.89



A **B**

Chart 2 Subacute toxicity effects of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE 100 mg/kg, bw on body weight changes wistar albino Male (A) & Female (B)Rats

3.2.2 Effect on Body Weight in Female and Male Wistar Albino Rats at 1000 mg/kg b.w.

The subacute oral toxicity evaluation of hydroethanolic extracts of Trichosanthes dioica (TDHEE), Luffa cylindrica (LCHEE), Trichosanthes cucumerina (TCHEE), Luffa acutangula (LAHEE), and their mixture (MAHEE) was carried out at a dose of 1000 mg/kg body weight in Wistar albino rats of both sexes for 28 consecutive days. Body weight changes were recorded weekly to assess the general health and growth pattern of the animals. The results are presented in Table 6 &7 and Chart 3.

3.2.2.1 Body Weight Changes in Female Rats

All extract-treated and control animals remained healthy throughout the experimental period with no mortality or visible signs of toxicity such as tremors, piloerection, diarrhea, or decreased food intake. A steady increase in body weight was observed across all groups, indicating normal physiological growth and the absence of treatment-related adverse effects. Among the treated groups, TDHEE and MAHEE (the combined extract) demonstrated the highest gain in body weight, from 166.6 ± 4.42 g to 187.2 ± 3.28 g and from 187.4 ± 6.74 g to 188.9 ± 4.49 g, respectively. LCHEE, TCHEE, and LAHEE also showed consistent increments in weight comparable to the control group, confirming that none of the individual or combined extracts exerted any inhibitory effect on metabolic function or growth rate as shown in table 6. The normal progression of body weight in all groups suggests that oral administration of the extracts at 1000 mg/kg body weight was well tolerated by female rats and did not induce any apparent subacute toxicity.

3.2.2.2 Body Weight Changes in Male Rats

A similar pattern was observed in male Wistar albino rats. None of the treated animals exhibited signs of toxicity, morbidity, or behavioral alterations during the study period. All groups displayed a regular increase in body weight, indicating the maintenance of normal metabolic and physiological function. The TCHEE, LCHEE, and TDHEE groups showed notable weight gains, reaching 214.53 ± 6.67 g, 211.68 ± 5.55 g, and 209.98 ± 5.13 g, respectively, by day 28. The MAHEE-treated group also showed a healthy weight progression (195.28 ± 10.44 g to 208.38 ± 5.63 g), closely comparable to the control animals. The consistent pattern of growth throughout the study confirms the absence of any cumulative or delayed toxic effect due to repeated administration of the extracts as shown in table 7

Table 6 Subacute toxicity study effects of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on body weight changes in female wistar albino rats at dose 1000 mg/kg, bw.

Days	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Day0	163.32±7.41	166.6±4.42	165.8±2.76	170.6±2.12	176.6±1.64	187.4±6.74
Day7	165.6±9.89	175.9±4.12	166.4±3.45	173.7±3.64	177.3±2.74	189.6±5.72
Day14	169.03±5.13	178.3±4.79	167.7±3.28	175.5±1.51	179.4±8.21	188.9±4.46
Day21	169.46±7.17	184.5±3.97	169.4±3.74	176.4±1.66	182.1±3.36	187±6.81
Day28	172.3±8.17	187.2±3.28	171.7±1.38	177.52±2.76	183.7±3.12	188.9±4.49

Table 7 Subacute toxicity study effects of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on body weight changes in male wistar albino rats at dose 1000 mg/kg, bw.

Days	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Day0	200.26±8.93	191.61±7.43	193.52±8.06	195.57±11.52	192.12±7.60	195.28±10.44
Day7	202.34±10.11	196.16±8.56	200.46±7.22	199.36±10.18	193.9±6.92	201.88±7.69
Day14	206.42±8.79	198.51±7.21	204.38±5.34	203.16±10.56	196.98±5.89	206.29±7.41
Day21	212.17±8.96	201.17±6.23	210.55±6.21	205.69±11.03	200.60±5.78	207.29±6.54
Day28	220.83±5.42	209.98±5.13	211.68±5.55	214.53±6.67	201.3±6.40	208.38±5.63

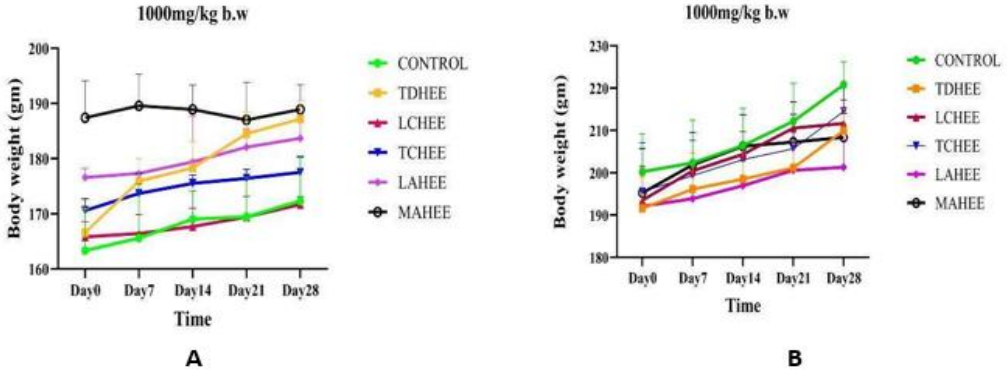


Chart 3 Subacute toxicity effects of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE 1000 mg/kg, bw on body weight changes wistar albino Male(A) & Female (B) Rats

3.2.3 Effect of Plant Extracts on Food Intake at 100 mg/kg Body Weight in Female and Male Wistar Albino Rats

The subacute oral toxicity study of hydroethanolic extracts of *Trichosanthes dioica* (TDHEE), *Luffa cylindrica* (LCHEE), *Trichosanthes cucumerina* (TCHEE), *Luffa acutangula* (LAHEE), and their mixture (MAHEE) was conducted at a dose of 100 mg/kg body weight for 28 consecutive days. The average daily food intake was monitored weekly to assess the influence of the extracts on feeding behavior, appetite, and metabolic balance. The recorded data are summarized in Table 8 & 9 and represent in chart 4.

3.2.3.1 Food Intake in Female Rats

Throughout the 28-day period, all female rats maintained normal feeding behaviour with no significant differences in food consumption between the control and treated groups. The mean food intake increased progressively with time in all experimental sets, indicating normal appetite and physiological adaptation. At the beginning of the study, food intake ranged between 18.86 ± 0.87 g (LCHEE) and 21.21 ± 1.98 g (TDHEE). By day 28, consumption increased to 22.08 ± 2.08 g (TDHEE), 21.68 ± 2.71 g (LCHEE), and 22.27 ± 2.18 g (TCHEE and LAHEE), values comparable to the control group (23.36 ± 1.75 g). The MAHEE-treated group (mixture of all extracts) showed a stable pattern of food consumption, ranging from 20.48 ± 2.28 g to 20.99 ± 1.53 g by day 28, without any sign of appetite suppression.

The stable or slightly increased food intake observed across all groups indicates that the tested hydroethanolic extracts did not produce anorexic or gastrointestinal effects in female rats and were well tolerated throughout the subacute exposure period as shown in table 8.

3.2.3.2 Food Intake in Male Rats

Similar results were observed in male Wistar rats. The mean food intake in all extract-treated groups gradually increased with time and remained comparable to the control group throughout the study. On day 0, the food intake varied from 25.6 ± 2.42 g (control) to 30.3 ± 2.11 g (MAHEE). By the end of the 28-day period, the values increased to 33.19 ± 2.51 g (TCHEE), 32.57 ± 1.62 g (control), and 33.06 ± 0.99 g (MAHEE), suggesting normal appetite and healthy metabolic activity. Other groups such as TDHEE, LCHEE, and LAHEE also exhibited consistent food intake patterns without any indication of reduced consumption as shown in table 9.

No significant variation in food intake between treatment and control groups was observed, demonstrating that repeated administration of these extracts at 100 mg/kg did not adversely affect feeding behaviour, digestion, or palatability of food in male rats

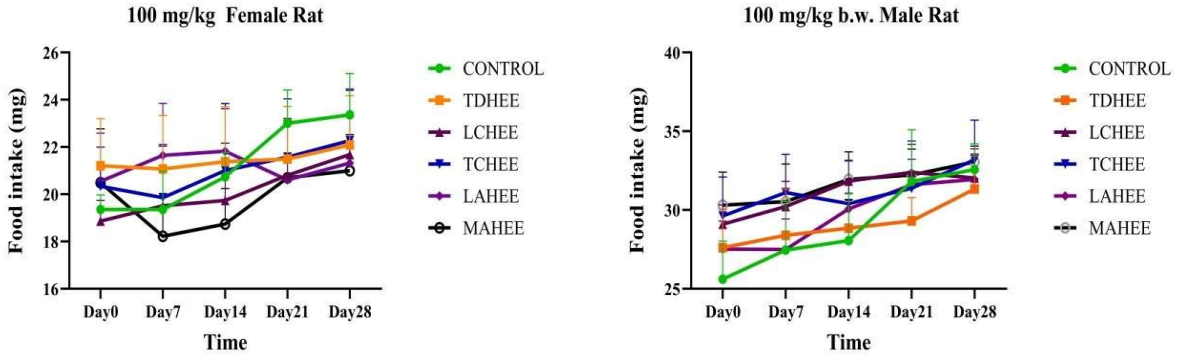
Table 8 Subacute toxicity study effect of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on food intakes in female wistar albino rats at dose 100 mg/kg, bw.

Days	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Day0	19.35±0.61	21.21±1.98	18.86±0.87	20.34±1.65	20.34±1.65	20.48±2.28
Day7	19.36±1.62	21.07±2.25	19.51±2.58	19.85±2.18	19.85±2.18	18.22±1.21
Day14	20.74±1.03	21.38±2.31	19.74±2.42	21.0±2.84	21.0±2.84	18.74±1.49
Day21	23.0±1.41	21.49±2.22	20.8±2.40	21.56±2.46	21.56±2.46	20.7±2.49
Day28	23.36±1.75	22.08±2.08	21.68±2.71	22.27±2.18	22.27±2.18	20.99±1.53

Table 9 Subacute toxicity study effect of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on food intakes in male wistar albino rats at dose 100 mg/kg, bw.

Days	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Day0	25.6±2.42	27.60±2.42	29.08±1.23	29.62±2.47	27.51±1.77	30.3±2.11

Day7	27.45±3.09	28.4±2.93	30.22±1.58	31.10±2.41	27.49±1.93	30.52±2.38
Day14	28.06±2.97	28.84±1.69	31.82±1.28	30.40±2.75	30.06±1.00	31.93±1.75
Day21	31.81±3.27	29.30±1.47	32.38±1.78	31.37±2.99	31.60±1.61	32.21±1.65
Day28	32.57±1.62	31.32±2.08	32.03±1.53	33.19±2.51	31.95±1.92	33.06±0.99



A B

Chart 4 Subacute toxicity effects of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE 100 mg/kg, bw on food intake in wistar albino Male (A) & Female (B) Rats

3.2.4 Effect on Food Intake at 1000 mg/kg bw. in Female and Male Wistar Albino Rats

To evaluate the potential influence of high-dose administration of hydroethanolic extracts on feeding behavior, food consumption was recorded weekly for 28 days in both female and male Wistar albino rats treated with *Trichosanthes dioica* (TDHEE), *Luffa cylindrica* (LCHEE), *Trichosanthes cucumerina* (TCHEE), *Luffa acutangula* (LAHEE), and their mixture (MAHEE). The results obtained at a dose of 1000 mg/kg body weight are presented in Tables 10 & 11 and chart 5.

3.2.4.1 Food Intake in Female Rats

The food intake pattern among all treated female groups remained comparable to the control throughout the 28-day treatment period, indicating normal appetite and absence of treatment-related anorexia or metabolic disturbance.

At the initiation of treatment, the average food intake varied between 18.97 ± 1.15 g (MAHEE) and 25.42 ± 3.18 g (LAHEE), while the control group recorded 23.4 ± 3.81 g. Progressive increases were observed over the experimental period, with day-28 values ranging from 24.37 ± 1.47 g (MAHEE) to 27.44 ± 2.66 g (LAHEE). The TDHEE (26.8 ± 2.13 g) and LCHEE (27.04 ± 2.39 g) groups exhibited a similar trend of gradual increase, reflecting normal feeding behavior as shown in table 10. Overall, the increase in food consumption across all treated female groups was consistent with physiological growth and comparable to the control, suggesting that oral administration of extracts up to 1000 mg/kg did not interfere with appetite, palatability, or gastrointestinal function.

3.2.4.2 Food Intake in Male Rats

A similar pattern was observed in male rats. The mean food intake in all treatment groups exhibited gradual increases during the 28-day exposure, comparable to the control group, and no significant changes indicative of toxicity were noted. On day 0, the control group recorded an intake of 27.3 ± 4.20 g, while treated groups ranged from 25.13 ± 3.02 g (TDHEE) to 29.22 ± 3.40 g (LAHEE). By day 28, food intake increased to 29.28 ± 3.06 g (control) and ranged from 25.52 ± 3.29 g (TDHEE) to 33.14 ± 3.72 g (MAHEE) among the treated groups. The MAHEE-treated rats, representing the mixture of all extracts, showed a steady increase throughout the study, from 27.90 ± 4.58 g on day 0 to 33.14 ± 3.72 g on day 28, reflecting a normal appetite and tolerance at the high-dose level as shown in table 11.

No statistically significant reduction in food intake was noted in any extract-treated group, confirming that repeated administration at 1000 mg/kg body weight did not produce adverse effects on feeding patterns or induce any behavioural or metabolic abnormalities

Table 10 Subacute toxicity study effect of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on food intakes in female wistar albino rats at dose 1000 mg/kg, bw.

Days	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Day0	23.4±3.81	24±2.73	23.89±2.95	21.15±3.24	25.42±3.18	18.97±1.15
Day7	24.04±3.62	24±2.73	24.13±2.61	21.17±2.18	25.72±3.04	19.46±1.29
Day14	24.56±2.85	24.8±2.65	24.33±2.69	22.4±2.54	25.4±3.23	20.72±1.25

Day21	24.86±2.37	26.4±1.96	25.97±2.71	25.0±2.1	26.6±2.89	22.58±2.28
Day28	25.46±2.13	26.8±2.13	27.04±2.39	25.0±2.1	27.44±2.66	24.37±1.47

Table 11 Subacute toxicity study effect of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on food intakes in male wistar albino rats at dose 1000 mg/kg, bw.

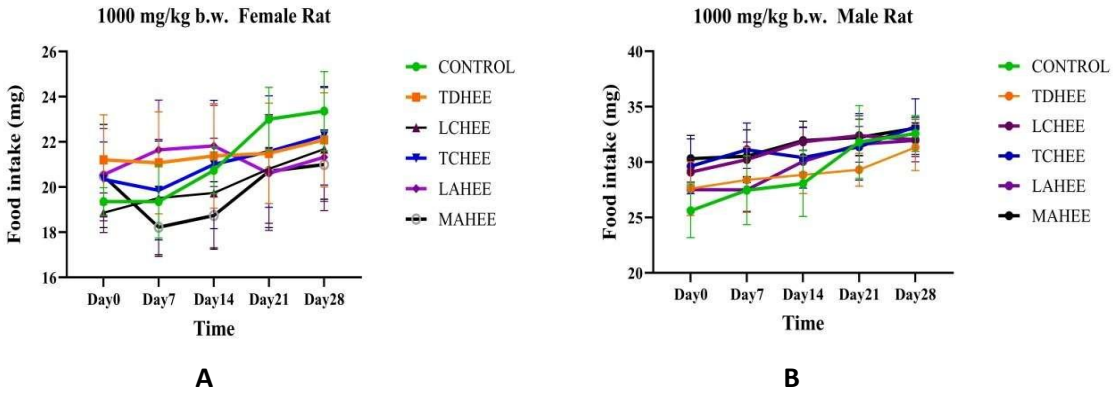
Days	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Day0	27.3±4.20	25.13±3.02	27.62±3.35	26.34±3.24	29.22±3.4	27.90±4.58
Day7	28.29±3.46	25.72±2.85	26.6±2.71	26.31±2.64	29.02±4.18	28.19±3.72
Day14	28.1±3.31	23.65±2.00	27.82±3.22	27.96±2.82	30.64±3.06	33.99±2.58
Day21	28.78±2.93	25.19±4.10	29.94±3.62	27.68±4.24	30.64±3.06	32.02±3.34
Day28	29.28±3.06	25.52±3.29	30.78±3.16	29.03±3.72	31.88±1.94	33.14±3.72

Chart 5 Subacute toxicity effects of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE 1000 mg/kg, bw on food intake in wistar albino Male(A) & Female (B) Rats

3.2.5 Effect of Plant Extracts on Water Intake in Wistar Albino Rats

The effect of repeated oral administration of hydroethanolic extracts of *Trichosanthes dioica* (TDHEE), *Luffa cylindrica* (LCHEE), *Trichosanthes cucumerina* (TCHEE), *Luffa acutangula* (LAHEE), and their combination (MAHEE) on water consumption was evaluated in both female and male Wistar albino rats at doses of 100 mg/kg and 1000 mg/kg body weight for a period of 28 days. The results are summarized in Tables 12&13 and chart 6.

3.2.5.1 Water Intake at 100 mg/kg Body Weight in Female Rats



At the lower dose (100 mg/kg), all treated female groups exhibited a normal pattern of water consumption comparable to the control throughout the 28-day study. On day 0, the water intake ranged between 28.0 ± 0.63 mL (LAHEE) and 30.8 ± 1.85 mL (LCHEE), which closely matched the control value of 29.4 ± 1.06 mL.

During the study, a gradual rise in intake was noted across all groups, corresponding to normal physiological adaptation and growth. By day 28, the water intake increased slightly, ranging from 31.6 ± 1.7 mL (LAHEE) to 35.2 ± 1.95 mL (TCHEE). The MAHEE-treated group, representing the combined extract, showed a consistent increase from 30.4 ± 1.20 mL to 34.8 ± 1.4 mL, which was similar to individual extract-treated groups. Overall, no significant deviation from control was observed, indicating that oral administration of the extracts at 100 mg/kg did not cause dehydration, excessive thirst (polydipsia), or other disturbances in water balance in female rats as shown in table10.

3.2.5.2 Water Intake at 100 mg/kg Body Weight in Male Rats

A comparable trend was observed in the male groups. The control animals consumed 38.2 ± 1.56 mL on day 0, while treated groups ranged between 34.0 ± 1.78 mL (LCHEE) and 37.4 ± 2.63 mL (MAHEE). Over the 28 days, water consumption showed a mild and steady increase in all groups. By day 28, the control group reached 42.0 ± 0.63 mL, while treated groups recorded 39.0 ± 1.41 mL (MAHEE) to 40.8 ± 1.82 mL (TCHEE). The minor variations observed were within physiological limits and did not indicate any adverse or treatment-related effects. Thus, the hydroethanolic extracts, even when administered repeatedly for 28 days at 100 mg/kg, did not alter the normal hydration status or metabolic function in male rats as shown in table 11 and chart 6B

Table 12 Subacute toxicity study effect of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on water intakes in female wistar albino rats at dose 100 mg/kg, bw.

Days	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Day0	29.4±1.06	29.4±1.7	30.8±1.85	29.8±1.68	28.0±0.63	30.4±1.20
Day7	28.0±0.89	29.4±0.97	32.6±1.88	33.2±1.85	31.8±1.35	32.8±2.08
Day14	31.2±0.8	30.6±1.46	32.0±1.54	32.4±1.46	32.2±2.15	33.6±1.6
Day21	33.4±1.07	33.0±1.67	33.8±1.62	32.8±1.88	32.4±1.93	33.6±1.72
Day28	32±1.09	32.4±1.6	34.6±1.66	35.2±1.95	31.6±1.7	34.8±1.4

Table 13 Subacute toxicity study effect of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on water intakes in male wistar albino rats at dose 100 mg/kg, bw.

Days	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Day0	38.2±1.56	37.4±2.2	34.0±1.78	36.4±2.4	35.6±2.31	37.4±2.63
Day7	37.8±2.28	36.4±1.83	42.0±1.74	37.2±1.85	35.6±2.24	37.6±2.03
Day14	38.4±2.31	36.8±1.62	39.6±0.74	40.0±1.09	38.0±1.67	39.2±1.49
Day21	40.0±1.78	37.2±1.35	40.4±1.46	39.4±2.95	38.0±1.26	38.8±1.35
Day28	42.0±0.63	39.6±0.74	39.6±0.97	40.8±1.82	38.2±1.28	39.0±1.41

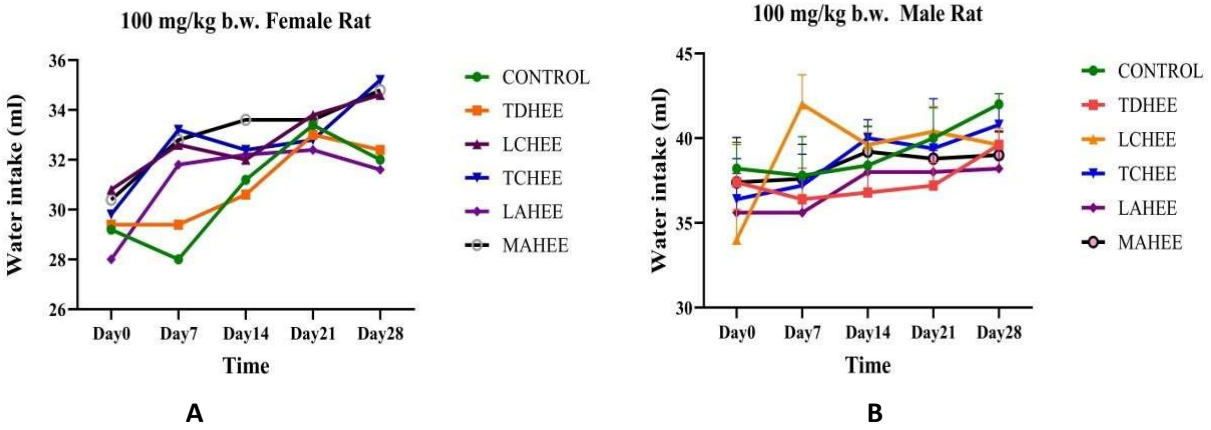


Chart 6 Subacute toxicity effects of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE) 100 mg/kg, bw on water intake in wistar albino Male (A) & Female (B) Rats

3.2.5.3 Water Intake at 1000 mg/kg Body Weight in Female Rats

At the higher dose level (1000 mg/kg), the mean daily water intake in female rats remained largely comparable to the control throughout the experimental period, with no statistically significant differences. The control group recorded 32.4 ± 1.46 mL on day 0, while treated groups ranged from 30.0 ± 0.63 mL (MAHEE) to 32.2 ± 1.35 mL (TDHEE). A gradual increase in intake was observed across all groups over the study duration, with day-28 values ranging between 33.0 ± 0.63 mL (LAHEE) and 35.2 ± 0.8 mL (control). The extracts neither caused excessive fluid consumption nor any reduction suggestive of reduced metabolic demand. This indicates that the hydroethanolic extracts were well tolerated and did not interfere with fluid balance at higher doses as shown in table 14 and chart 7A.

3.2.5.4 Water Intake at 1000 mg/kg Body Weight in Male Rats

The male rats also showed normal patterns of water intake during the 28-day subacute toxicity study. On day 0, consumption ranged between 35.0 ± 1.26 mL (TCHEE) and 36.8 ± 1.77 mL (control). A gradual increase was observed over time in all treatment groups, with final values on day 28 ranging from 35.2 ± 1.01 mL (TDHEE) to 38.8 ± 1.01 mL (MAHEE). The MAHEE-treated rats displayed a slight increase in water intake throughout the study, remaining within normal limits. This observation suggests that even at 1000 mg/kg, the combination extract did not exert diuretic, antidiuretic, or toxic effects on renal or systemic hydration mechanisms as shown in table 15 and chart 7 B

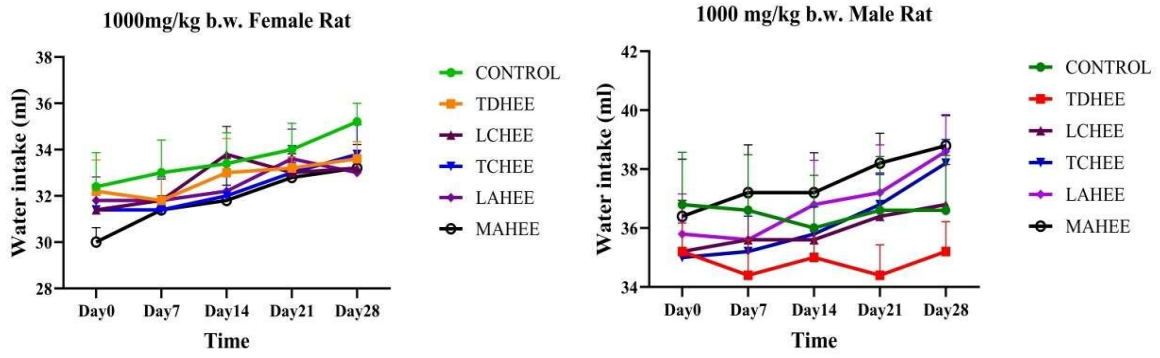
Table 14 Subacute toxicity study effect of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on water intakes in female wistar albino rats at dose 1000 mg/kg, bw.

Days	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Day0	32.4±1.46	32.2±1.35	31.4±0.87	31.4±1.07	31.8±1.01	30.0±0.63
Day7	33.0±1.41	31.8±1.2	31.8±1.01	31.4±1.32	31.8±1.01	31.4±0.6
Day14	33.4±1.32	33.0±1.48	33.8±1.2	32.0±1.09	32.2±0.8	31.8±0.66
Day21	34.0±1.14	33.2±0.48	33.0±.44	33.0±1.18	33.6±1.28	32.8±1.01
Day28	35.2±0.8	33.6±0.74	33.2±.48	33.8±1.28	33.0±0.63	33.2±1.01

Table 15 Subacute toxicity study effect of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE on water intakes in male wistar albino rats at dose 1000 mg/kg, bw.

Days	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Day0	36.8±1.77	35.2±0.96	35.2±1.74	35.0±1.26	35.8±1.35	36.4±1.93
Day7	36.6±1.88	34.4±1.20	35.6±1.72	35.2±1.2	35.6. ±0.97	37.2±1.62
Day14	36.0±1.78	35.0±0.89	35.6±1.72	35.8±0.91	36.8±1.49	37.2±1.35
Day21	36.6±1.83	34.4±1.02	36.4±1.46	36.8±1.01	37.2±1.62	38.2±1.09
Day28	36.6±1.86	35.2±1.01	36.8±1.35	38.2±0.8	38.6±1.24	38.8±1.01

Chart 7 Subacute toxicity effects of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE 100 mg/kg, bw on food intake in wistar albino Male (A) & Female (B) Rats.



A B

3.2.6 Effect of Extracts on Haematological Parameters in Wistar Albino Rats

The haematological profiles of male and female Wistar albino rats treated orally with hydroethanolic extracts of *Trichosanthes dioica* (TDHEE), *Luffa cylindrica* (LCHEE), *Trichosanthes cucumerina* (TCHEE), *Luffa acutangula* (LAHEE), and their combination (MAHEE) for 28 days were evaluated at two dose levels 100 mg/kg and 1000 mg/kg body weight. The mean ± SEM values for various haematological parameters are presented in Tables 16 to 19.

3.2.6.1 Haematological Profile in Female Rats at 100 mg/kg b.w.

At a dose of 100 mg/kg, no statistically significant alterations were observed in the haematological indices of treated female rats compared with the control group ($P > 0.05$). The haemoglobin (Hb) levels ranged from 14.71 ± 0.29 g/dL (LCHEE) to 15.25 ± 0.33 g/dL (TCHEE), remaining within normal physiological limits. Similarly, haematocrit (Ht) values varied slightly between 48.98 ± 1.54 % and 49.85 ± 1.97 %, showing no evidence of anaemia or haemoconcentration.

The RBC counts (8.43 – 9.20 × 10^6/mm³) and WBC counts (3.98 – 5.93 × 10^3/mm³) were comparable to controls, suggesting the absence of bone marrow suppression or immune hyperactivation. Differential leukocyte counts (lymphocytes, eosinophils, monocytes, and segmented cells) exhibited minor variations but stayed within reference ranges, indicating normal immune homeostasis.

Platelet counts (PLT) showed mild elevation in extract-treated groups (1097–1267 × 10^3/mm³) without statistical significance, suggesting no hematotoxin effects on thrombopoiesis. The erythrocytic indices—MCV (54–56 fL), MCH (16.4–17.2 pg), and MCHC (30–31 g/dL)—remained stable, signifying normal erythrocyte morphology and haemoglobinization. Overall, treatment with TDHEE, LCHEE, TCHEE, LAHEE, and MAHEE at 100 mg/kg b.w. did not induce any deleterious effects on haematological parameters in female rats as shown in table 16.

3.2.6.2 Haematological Profile in Male Rats at 100 mg/kg b.w.

In male rats, exposure to the same dose (100 mg/kg) produced no significant deviations in haematological values from control levels. The haemoglobin content ranged from 16.68 ± 1.04 g/dL (MAHEE) to 17.48 ± 0.94 g/dL (TDHEE), while haematocrit values were between 49.94 ± 2.23 % and 53.68 ± 1.75 %, indicating normal oxygen-carrying capacity. The RBC counts (8.83–9.47 × 10^6/mm³) and WBC counts (6.35–7.52 × 10^3/mm³) remained within reference ranges, confirming that none of the extracts affected erythropoiesis or leukopoiesis. Minor, non-significant increases in MCV and MCH were observed in treated groups, suggesting adequate erythrocyte integrity and haemoglobin synthesis. Platelet counts (≈920–956 × 10^3/mm³) and leukocyte differentials (lymphocytes ≈ 76–79 %, eosinophils ≈ 0.9–1.9 %) were consistent with normal physiological ranges as shown in table 17.

Thus, oral administration of hydroethanolic extracts at 100 mg/kg was haematologically safe in both sexes.

3.2.6.3 Haematological Profile in Female Rats at 1000 mg/kg b.w.

At the higher dose (1000 mg/kg), the treated female groups did not show significant differences in any haematological indices relative to control. The haemoglobin concentration slightly increased from 15.12 ± 0.29 g/dL (control) to 16.08 ± 0.18 g/dL (MAHEE), while haematocrit values remained stable around 48–49 %. The RBC counts (7.70–8.50 × 10^6/mm³) and WBC counts (6.43–7.46 × 10^3/mm³) reflected a normal physiological range, confirming the absence of haemolytic or myelotoxic effects. Differential leukocyte counts remained unaltered, with lymphocytes constituting approximately 83–84 % of total WBCs. Slight increases in eosinophil and monocyte percentages in TDHEE-treated

animals were within physiological limits and likely of no toxicological relevance. Platelet levels (≈997–1017 × 10^3/mm³) and red cell indices (MCV ≈ 59–63 fL; MCH ≈ 18–19 pg; MCHC ≈ 30–31 g/dL) remained consistent, suggesting maintenance of normal erythrocyte morphology and hematinic balance even at high-dose exposure.

3.2.6.4 Haematological Profile in Male Rats at 1000 mg/kg b.w.

Male rats administered 1000 mg/kg b.w. of the plant extracts also exhibited haematological values within the normal physiological range. Haemoglobin concentrations were between 16.98 ± 0.47 g/dL (TCHEE) and 17.60 ± 0.58 g/dL (MAHEE), accompanied by proportionate haematocrit levels (49.46–56.58 %). The RBC counts (8.95–9.74 × 10^6/mm³) and WBC counts (6.13–6.92 × 10^3/mm³) were comparable to control, implying normal hematopoietic activity. The differential counts showed no extract-related alterations in lymphocytes, eosinophils, or monocytes, and platelet counts (986–1086 × 10^3/mm³) were slightly higher but not statistically significant. Red cell indices (MCV ≈ 55–56 fL, MCH ≈ 17 pg, MCHC ≈ 30 g/dL) demonstrated that erythrocyte size and haemoglobin content remained unaffected. Hence, no adverse haematological effects were detected even at 10-fold higher dose, indicating good haematological tolerance to the extracts

Table 16 Effect on hematological parameters of female rats at dose 100 mg/kg after 28 days of the oral administration of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE in subacute toxicity study.

Parameter	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Hb[a]	14.8 ± 0.27	15.10 ± 0.47	14.71 ± 0.29	15.25 ± 0.33	15.11 ± 0.29	14.92 ± 0.31
Ht[b]	49.24 ± 1.54	49.85 ± 1.97	48.98 ± 1.54	49.38 ± 1.47	49.34 ± 1.64	49.34 ± 1.54
RBC[c]	8.43 ± 0.49	9.16 ± 0.46	9.2 ± 0.49	8.97 ± 0.51	8.83 ± 0.49	8.63 ± 0.49
WBC[d]	5.93 ± 1.83	3.98 ± 0.54	5.33 ± 1.83	4.34 ± 0.53	5.13 ± 1.33	5.23 ± 1.43
Lymp.[e]	83.17 ± 2.13	86.00 ± 1.08	83.17 ± 2.13	86.74 ± 1.73	84.17 ± 1.53	84.77 ± 1.33
Eosi.[f]	0.64 ± 0.27	1.00 ± 0.41	0.64 ± 0.27	1.16 ± 0.53	0.63 ± 0.37	0.74 ± 0.47
Mono.[g]	2.18 ± 1.28	1.25 ± 0.25	2.18 ± 1.32	1.94 ± 0.36	2.38 ± 1.28	2.28 ± 1.18
PLT.[h]	1097.31 ± 97.43	1242.4 ± 63.75	1267.31 ± 97.43	1198.42 ± 92.81	1187.31 ± 97.43	1197.31 ± 93.43
MCV[i]	56.74 ± 2.73	54.50 ± 0. 87	55.94 ± 2.13	54.93 ± 1.07	54.94 ± 1.73	54.04 ± .73
MCH[j]	17.13 ± 0.65	16.48 ± 0.31	16.83 ± 0.35	16.53 ± 0.43	17.13 ± 0.85	17.21 ± 0.61
MCHC[k]	30.74 ± 0.53	30.33 ± 0.27	30.64 ± 0.53	30.13 ± 0.32	31.74 ± 0.83	30.14 ± 0.32
Seg.[l]	13.04 ± 0.53	12.00 ± 0.82	13.14 ± 0.23	12.12 ± 1.73	12.24 ± 0.83	12.04 ± 0.97

Data is expressed as mean ± S.E.M., n=5, No statistical difference between control and plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE (P>0.05).

a Haemoglobin concentration (g/dl), b Haematocrit (%), c Red blood cell (× 106 mm-3), d White blood cell (× 103 mm-3), e Lymphocyte (%), f Eosinophilic leukocyte (%), g Monocyte (%), h Platelets (× 103 mm-3), i Mean corpuscular volume (FL), j Mean corpuscular haemoglobin (pg), k Mean corpuscular haemoglobin concentration (g/dl), l Segmented leukocyte (%).

Table 17 Effect on hematological parameters of male rats at dose 100 mg/kg after 28 days of the oral administration of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE in subacute toxicity study.

Parameter	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Hb[a]	15.02 ± 0.98	17.48 ± 0.94	16.98 ± 0.74	16.97 ± 0.68	17.08 ± 0.54	16.68 ± 1.04
Ht[b]	49.94 ± 2.23	53. 68 ± 1.75	51. 68 ± 1.82	52.03 ± 2.03	53. 18 ± 1.25	50. 48 ± 1.95
RBC[c]	08.83 ± 0.46	09.27 ± 0.41	09.47 ± 0.41	09.02 ± 0.43	09.07 ± 0.41	08.97 ± 0.36
WBC[d]	06.35 ± 1.26	07.50 ± 2.32	07.52 ± 2.02	07.43 ± 1.98	07.50 ± 2.22	07.50 ± 2.81
Lymp.[e]	79.96 ± 4.48	76. 75 ± 3.52	76. 45 ± 3.12	76.24 ± 3.12	78. 75 ± 2.28	77. 45 ± 3.02
Eosi.[f]	00.89 ± 0.29	01.75 ± 1.03	01.75 ± 1.03	01.56 ± 1.07	01.95 ± 1.03	01.25 ± 0.94
Mono.[g]	03.94 ± 1.21	03.25 ± 1.32	03.15 ± 1.02	03.27 ± 1.48	03.45 ± 0.92	03.19 ± 1.18
PLT.[h]	926.7 ± 94.30	924.3 ± 105.8	927.3 ± 95.8	956.31 ± 101.34	919.1 ± 115.8	924.4 ± 100.8
MCV[i]	55.74 ± 1.03	58.00 ± 2.27	56.00 ± 2.17	57.23 ± 1.74	57.00 ± 1.25	58.00 ± 2.19
MCH[j]	16.23 ± 0.58	17.48 ± 0.53	17.48 ± 0.49	18.13 ± 0.63	17.58 ± 0.23	16.18 ± 0.47
MCHC[k]	31.04 ± 0.22	30.03 ± 0.33	30.43 ± 0.32	30.78 ± 0.43	31.03 ± 0.13	30.83 ± 0.63
Seg.[l]	16.23 ± 4.63	18.25 ± 3.66	18.45 ± 3.62	17.93 ± 4.03	17.95 ± 3.79	18.01 ± 3.27

Data is expressed as mean ± S.E.M., n=5, No statistical difference between control and plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE (P>0.05).

a Haemoglobin concentration (g/dl), b Haematocrit (%), c Red blood cell (× 106 mm-3), d White blood cell (× 103 mm-3), e Lymphocyte (%), f Eosinophilic leukocyte (%), g Monocyte (%), h Platelets (× 103 mm-3), i Mean corpuscular volume (FL), j Mean corpuscular haemoglobin (pg), k Mean corpuscular haemoglobin concentration (g/dl), l Segmented leukocyte (%).

Table 18 Effect on hematological parameters of female rats at dose 1000 mg/kg after 28 days of the oral administration of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE in subacute toxicity study

Parameter	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Hb[a]	15.12 ± 0.29	15.60 ± 0.29	15.40 ± 0.34	15.46 ± 0.28	15.60 ± 0.27	16.08 ± 0.18
Ht[b]	49.34 ± 1.54	48.73 ± 1.38	49.23 ± 1.38	49.16 ± 1.48	48.73 ± 1.45	48.53 ± 1.21
RBC[c]	8.43 ± 0.49	7.70 ± 0.80	7.89 ± 0.70	8.12 ± 0.63	8.39 ± 0.63	8.50 ± 0.64
WBC[d]	6.43 ± 1.83	7.46 ± 2.57	7.46 ± 2.57	7.24 ± 1.98	7.06 ± 1.97	6.46 ± 2.17
Lymp.[e]	83.17 ± 2.13	83. 00 ± 2.68	83. 00 ± 2.68	84.14 ± 2.32	83. 00 ± 2.68	83. 00 ± 2.68
Eosi.[f]	0.68 ± 0.27	2.00 ± 0.71	1.80 ± 0.38	1.98 ± 0.87	1.88± 0.41	2.00 ± 0.81
Mono.[g]	2.18 ± 1.28	3.25 ± 1.03	3.21 ± 1.23	2.92 ± 1.07	2.85 ± 0.93	3.15 ± 0.94
PLT.[h]	1015.31 ± 98.43	1017.2 ± 93.4	997.2 ± 91.4	998.2 ± 75.74	1005.2 ± 91.4	1011.2 ± 86.4
MCV[i]	59.74 ± 2.73	61.50 ± 3.38	62.50 ± 3.28	61.34 ± 1.93	63.50 ± 2.28	61.20 ± 2.38
MCH[j]	18.02 ± 0.75	19.18 ± 1.22	19.09 ± 1.43	18.97 ± 1.42	19.28 ± 1.18	19.18 ± 1.02
MCHC[k]	30.64 ± 0.43	30.74 ± 0.41	31.15 ± 0.51	30.82 ± 0.25	30.85 ± 0.71	30.65 ± 0.40
Seg.[l]	13.14 ± 0.53	12.20 ± 2.13	12.40 ± 2.03	12.43 ± 2.13	12.29 ± 1.83	13.07 ± 0.83

Data is expressed as mean ± S.E.M., n=5, No statistical difference between control and plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE (P>0.05).

a Haemoglobin concentration (g/dl), b Haematocrit (%), c Red blood cell (× 106 mm-3), d White blood cell (× 103 mm-3), e Lymphocyte (%), f Eosinophilic leukocyte (%), g Monocyte (%), h Platelets (× 103 mm-3), i Mean corpuscular volume (FL), j Mean corpuscular haemoglobin (pg), k Mean corpuscular haemoglobin concentration (g/dl), l Segmented leukocyte (%).

Table 19 Effect on hematological parameters of male rats at dose 1000 mg/kg after 28 days of the oral administration of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE in subacute toxicity study

Parameter	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
Hb[a]	15.24 ± 1.14	17.50 ± 0.56	17.26 ± 0.51	16.98 ± 0.47	17.30 ± 0.48	17.60 ± 0.58
Ht[b]	49.46 ± 2.46	56.58 ± 3.05	52.58 ± 3.45	55.74 ± 2.56	50.58 ± 3.15	54.58 ± 3.25
RBC[c]	8.82 ± 0.36	9.23 ± 1.12	9.65 ± 1.02	9.74 ± 0.93	9.15 ± 0.96	08.95 ± 1.02
WBC[d]	6.65 ± 1.55	6.50 ± 0.99	6.50 ± 0.59	6.92 ± 1.18	6.13 ± 0.69	6.26 ± 0.92
Lymp.[e]	79.04 ± 5.55	76.79 ± 5.74	79.05 ± 4.74	77.13 ± 4.40	76.16 ± 4.34	77.34 ± 4.74
Eosi.[f]	0.80 ± 0.37	0.25 ± 0.25	0.45 ± 0.25	0.87 ± 0.38	0.65 ± 0.43	0.76 ± 0.21
Mono.[g]	3.97 ± 1.58	3.00 ± 0.71	2.90 ± 0.71	02.23 ± 0.84	02.72 ± 0.71	02.80 ± 0.26
PLT.[h]	986.2 ± 101	1086.3 ± 27.18	1067.3 ± 26.38	1044.6 ± 62.77	1057.3 ± 25.38	1067.3 ± 27.07
MCV[i]	55.28 ± 1.24	55.00 ± 1.23	54.60 ± 1.23	56.45 ± 1.45	55.90 ± 1.13	56.00 ± 1.00
MCH[j]	16.84 ± 0.48	17.20 ±0.98	17.10 ±0.98	17.73 ±0.87	17.40 ±0.78	17.40 ±0.88
MCHC[k]	30.52 ± 0.37	29.78 ± 0.64	30.68 ± 0.57	30.13 ± 0.73	29.98 ± 0.64	29.38 ± 0.84
Seg.[l]	15.20 ± 5.49	20.70 ± 4.51	20.50 ± 5.31	19.93 ± 4.14	20.50 ± 5.21	21.08 ± 5.04

3.2.7 Effect of Plant Extracts on Biochemical Parameters in Female and Male Wistar Albino Rats

The biochemical parameters of female and male Wistar rats treated orally for 28 days with hydroethanolic extracts of *Trichosanthes dioica* (TDHEE), *Luffa cylindrica* (LCHEE), *Trichosanthes cucumerina* (TCHEE), Luffa acutangula (LAHEE), and their combined formulation (MAHEE) were analyzed to assess hepatic, renal, and metabolic functions.

All data are expressed as mean ± SEM (n = 5), and statistical comparison with the control group revealed no significant differences (P > 0.05), indicating biochemical safety at both tested dose levels (Tables 20-23).

3.2.7.1 Biochemical Profile in Female Rats at 100 mg/kg b.w.

At 100 mg/kg b.w., no significant alterations were observed in liver function markers among extract-treated female rats when compared to the control. The total bilirubin (T.B.) and direct bilirubin (C.B.) values remained within physiological limits (0.19–0.24 mg/dl and 0.029–0.036 mg/dl, respectively), suggesting normal hepatic conjugation and excretory activity. The enzymatic markers of hepatic integrity—aspartate transaminase (AST) and alanine transaminase (ALT)—ranged between 129.8 ± 13.68 and 162.2 ± 7.69 U/L for AST and 31.9 ± 2.03 to 36.1 ± 5.41 U/L for ALT, with no evidence of hepatocellular damage.

The total protein (7.18–7.78 g/dl) and albumin (2.54–2.93 g/dl) concentrations showed minimal variation, indicating unimpaired hepatic synthetic function. Renal parameters such as blood urea nitrogen (BUN) and creatinine were comparable to control, confirming the absence of nephrotoxicity.

Lipid profiles, including cholesterol (56.3–58.13 mg/dl), HDL (39.16–41.80 mg/dl), and triglycerides (34.2–47.4 mg/dl), exhibited only minor fluctuations without statistical relevance. Electrolyte values for sodium (158–164 mEq/L), potassium (5.64–5.88 mEq/L), and chloride (95–102 mEq/L) were within normal physiological limits, confirming electrolyte stability.

Calcium (9.98–10.25 mg/dl) and phosphorus (7.82–8.16 mg/dl) levels, along with prothrombin time (PT: 10.6–12.1 s) and partial thromboplastin time (PTT: 26.2–28.1 s), did

not deviate significantly, indicating intact coagulation profiles. Glucose levels (239–243 mg/dl) were stable across all treatment groups.

Overall, the results indicated normal hepatic, renal, and metabolic function following administration of the extracts at 100 mg/kg in female rats.

3.2.7.2 Biochemical Profile in Male Rats at 100 mg/kg b.w.

Biochemical assessment of male rats at 100 mg/kg revealed similar non-significant changes. The bilirubin levels (T.B.: 0.17–0.23 mg/dl; C.B.: 0.024–0.028 mg/dl) remained within reference ranges, while AST (168–173 U/L) and ALT (37–46 U/L) activities showed no treatment-related increases, indicating no hepatotoxic effect.

The total protein (7.40–7.91 g/dl) and albumin (2.42–2.94 g/dl) contents were unaffected, signifying preserved hepatic synthesis. BUN (19.68–21.14 mg/dl) and creatinine (0.88–0.99 mg/dl) levels confirmed normal renal function.

Lipid parameters remained stable with cholesterol ranging from 52.8–59.0 mg/dl, HDL from 38.5–41.0 mg/dl, and triglycerides from 43.8–49.2 mg/dl. The values of sodium (155–157 mEq/L), potassium (5.2–5.9 mEq/L), and chloride (98–102 mEq/L) also showed no significant variation.

Calcium (9.94–11.08 mg/dl) and phosphorus (7.84–8.43 mg/dl) remained within normal limits, while coagulation times (PT: 9.98–11.70 s; PTT: 23.5–26.5 s) and glucose (230–238 mg/dl) values were unaltered.

Hence, oral administration of plant extracts at 100 mg/kg produced no biochemical abnormalities in male rats.

3.2.7.3 Biochemical Profile in Female Rats at 1000mg/kg b.w.

At the higher dose of 1000 mg/kg, all biochemical indices of treated female rats remained comparable to control. The bilirubin levels (T.B.: 0.21–0.23 mg/dl; C.B.: 0.024–0.027 mg/dl) and hepatic enzyme activities (AST: 179.7–186.0 U/L; ALT: 35.4–37.1 U/L) demonstrated no significant elevation, confirming the absence of hepatic injury.

Total protein (7.31–7.89 g/dl) and albumin (2.78–3.04 g/dl) concentrations were within normal ranges, suggesting intact liver function. The renal function markers—BUN (19.1–21.2 mg/dl) and creatinine (0.87–0.97 mg/dl)—remained stable across all groups. Lipid parameters also showed no remarkable differences, with cholesterol ranging from 49.2–56.3 mg/dl, HDL between 37.8–39.6 mg/dl, and triglycerides from 39.2–48.6 mg/dl. The electrolytes (Na^+: 153–157 mEq/L, K^+: 5.6–6.3 mEq/L, Cl^-: 98–101 mEq/L) and minerals (Ca^{2+}: 9.8–10.7 mg/dl, P: 7.4–8.4 mg/dl) did not deviate from control values. The coagulation parameters (PT: 12.78–15.08 s; PTT: 36.38–41.53 s) and glucose levels (243–258 mg/dl) were comparable to controls, implying that high-dose exposure did not compromise hepatic, renal, or coagulative function in female rats.

3.2.7.4 Biochemical Profile in Male Rats at 1000 mg/kg b.w.

At 1000 mg/kg, biochemical profiles of male rats also indicated no extract-induced toxicity.

The bilirubin concentrations (T.B.: 0.19–0.20 mg/dl; C.B.: 0.025–0.038 mg/dl) were consistent with normal hepatic function, and AST (139–178 U/L) and ALT (31–46 U/L) activities did not reveal hepatocellular damage.

Protein metabolism markers—total protein (7.28–7.81 g/dl) and albumin (2.45–3.45 g/dl)—were unaffected, confirming normal hepatic synthesis.

Renal function indices (BUN: 17–21.2 mg/dl; creatinine: 0.91–0.96 mg/dl) also remained within physiological limits. Serum lipid profile showed non-significant variation among treated groups with HDL between 36.1–40.8 mg/dl, cholesterol between 52.4–58.1 mg/dl, and triglycerides between 34.2–41.9 mg/dl.

Electrolyte and mineral balance was maintained as Na^+ ranged from 151–158 mEq/L, K^+ from 5.4–5.9 mEq/L, Cl^- from 95–98 mEq/L, Ca^{2+} from 9.7–10.2 mg/dl, and P from 8.0–8.4 mg/dl.

Coagulation parameters (PT: 10.18–10.9 s; PTT: 23.4–28.1 s) were within physiological limits. Glucose levels (232–245 mg/dl) did not show significant variation from the control group.

Overall, biochemical analyses demonstrated that administration of TDHEE, LCHEE, TCHEE, LAHEE, and MAHEE up to 1000 mg/kg did not produce hepatotoxic, nephrotoxic, or metabolic disturbances in male rats

Table 20 Effect on biochemistry parameters of female rats at dose 100 mg/kg after 28 days of the oral administration of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE in subacute toxicity study.

Parameter	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
TB[a]	0.24 ± 0.018	0.23 ± 0.023	0.22 ± 0.025	0.19 ± 0.031	0.21 ± 0.024	0.20 ± 0.016
CB[b]	0.031 ± 0.004	0.035 ± 0.002	0.036 ± 0.002	0.032 ± 0.003	0.031 ± 0.002	0.029 ± 0.005
AST[c]	162.2 ± 7.69	158.4 ± 14. 1	149.4 ± 13. 28	129.8 ± 13.68	156.4 ± 14. 13	152.2 ± 07.04
ALT[d]	36.1 ± 5.41	31.9 ± 2.03	33.6 ± 2.13	32.12 ± 2.12	32.00 ± 2.23	34.0 ± 5.41
Total proteins (g/dl)	7.62 ± 0.15	7.68 ± 0.07	7.58 ± 0.07	7.18 ± 0.09	7.78 ± 0.06	7.52 ± 0.15
Albumin (g/dl)	2.83 ± 0.07	2.93 ± 0.08	2.79 ± 0.09	2.93 ± 0.07	2.63 ± 0.08	2.54 ± 0.08
BUN[e]	19.90 ± 1.780	17.00 ± 01.32	17.00 ± 01.32	18.12 ± 01.45	17.00 ± 01.32	19.90 ± 1.780
Creatinine (mg/dl)	0.89 ± 0.06	0.93 ± 0.02	0.98 ± 0.02	0.97 ± 0.01	0.94 ± 0.02	0.87 ± 0.06

HDL[f]	39.16 ± 01.48	40.60 ± 01.24	41.80 ± 01.23	40.33 ± 1.53	39.98 ± 1.24	39.28 ± 1.38
Cholesterol mg/dl)	56.60 ± 07.56	57.80 ± 2.28	57.70 ± 2.19	58.13 ± 3.21	57.72 ± 2.49	56.30 ± 4.56
TG[g]	47.40 ± 10.21	37.20 ± 03.46	39.20 ± 03.46	38.29 ± 04.61	34.20 ± 03.46	42.40 ± 8.21
Potassium (mequiv. / L)	05.88 ± 0.34	05.78 ± 0.24	05.78 ± 0.14	05.64 ± 0.24	05.76 ± 0.14	05.78 ± 0.35
Sodium (mequiv. / L)	164.6 ± 1.69	161.2 ± 1.16	159.2 ± 01.16	158.4 ± 01.46	159.2 ± 01.96	159.6 ± 02.69
Chloride (mequiv. / L)	99.86 ± 01.41	95.50 ± 2.22	95.40 ± 2.12	96.71 ± 2.52	95.60 ± 2.22	102.0 ± 1.81
Calcium (mg/dl)	09.98 ± 00.38	10.25 ± 0.16	10.08 ± 0.57	10.11 ± 0.32	10.21 ± 0.27	10.14 ± 00.19
Phosphorus (mg/dl)	07.94 ± 00.54	08.13 ± 00.60	08.16 ± 00.50	08.16 ± 00.53	08.06 ± 00.61	07.82 ± 00.24
PT[h]	11.59 ± 00.87	10.60 ± 1.73	10.90 ± 01.91	11.14 ± 01.31	10.90 ± 01.51	12.10 ± 0.87
PTT[i]	26.20 ± 02.53	27.20 ± 04.46	27.10 ± 04.26	28.10 ± 03.76	27.30 ± 04.76	26.20 ± 3.53
Glucose (mg/dl)	239.6 ± 24.80	240.40 ± 23.72	243.40 ± 24.72	242.52 ± 25.71	242.40 ± 24.32	241.6 ± 23.50

Data is expressed as mean S.E.M., n=5, No statistical difference between control and TDHEE, LCHEE, TCHEE, LAHEE and MAHEE (P >0.05). a Total bilirubin (mg/dl), b Direct bilirubin (mg/dl), c Aspartate transaminase (U/l), d Alanine transaminase (U/l), e Bloodurea nitrogen (mg/dl), f High density lipoproteins (mg/dl), g Triglycerides (mg/dl), h Prothrombin time, i Thromboplastin partial time.

Table 21 Effect on biochemistry parameters of male rats at dose 100 mg/kg after 28 days of the oral administration of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE in subacute toxicity study.

Parameter	**Control**	**TDHEE**	**LCHEE**	**TCHEE**	**LAHEE**	**MAHEE**
TB[a]	0.19 ± 0.021	0.21 ± 0.019	0.17 ± 0.024	0.22 ± 0.018	00.23 ± 0.017	00.22 ± 0.022
CB[b]	0.024 ± 0.007	0.025 ± 0.006	0.027 ± 0.005	0.028 ± 0.008	0.028 ± 0.004	0.024 ± 0.006
AST[c]	173.4 ± 22.38	168.2 ± 7.57	170.2 ± 8.04	168.4 ± 07.72	169.8 ± 8.26	173.2 ± 18.40
ALT[d]	46.20 ± 4.42	38.00 ± 5.41	40.00 ± 6.21	38.60 ± 4.51	42.00 ± 6.11	37.00 ± 3.91
Total proteins (g/dl)	7.40 ± 0.28	7.50 ± 0.19	07.82 ± 0.27	7.91 ± 0.28	7.64 ± 0.18	7.59 ± 0.19
Albumin (g/dl)	2.42 ± 0.19	2.72 ± 0.10	2.54 ± 0.12	2.81 ± 0.12	2.94 ± 0.06	2.71 ± 0.13
BUN[e]	20.40 ± 1.38	19.70 ± 1.92	19.68 ± 1.78	21.14 ± 1.45	20.48 ± 1.83	20.28 ± 1.52
Creatinine (mg/dl)	0.95 ± 0.04	0.89 ± 0.08	0.88 ± 0.07	0.91 ± 0.08	0.97 ± 0.12	0.99 ± 0.10
HDL[f]	39.0 ± 2.12	40.0 ± 1.38	41.0 ± 1.98	38.53 ± 1.28	41.0 ± 1.34	40.56 ± 2.01
Cholesterol mg/dl)	52.80 ± 2.87	54.20 ± 6.36	53.60 ± 3.56	54.94 ± 04.96	55.21 ± 6.49	59.02 ± 8.56
TG[g]	43.80 ± 12.94	45.95 ± 11.28	48.40 ± 12.01	44.16 ± 10.38	48.24 ± 9.78	49.24 ± 11.51
Potassium (mequiv. / L)	5.87 ± 0.35	5.91 ± 0.39	5.79 ± 0.38	5.84 ± 0.29	5.28 ± 0.32	5.68 ± 0.28
Sodium (mequiv. / L)	156.3 ± 1.67	155.6 ± 1.68	156.4 ± 1.69	156.1 ± 01.54	155.9 ± 1.29	157.6 ± 1.56
Chloride (mequiv. / L)	98.42 ± 3.57	99.3 ± 1.81	101.0 ± 1.21	99.87 ± 2.01	102.0 ± 1.08	99.87± 1.05
Calcium (mg/dl)	10.80 ± 0.26	10.09 ± 0.28	11.05 ± 0.24	11.08 ± 0.25	9.94 ± 0.31	10.45 ± 0.32
Phosphorus (mg/dl)	8.43 ± 0.72	7.84 ± 0.24	7.94 ± 0.28	7.89 ± 0.32	8.14 ± 0.62	8.27 ± 0.81
PT[h]	09.98 ± 00.72	11.70 ± 00.87	11.70 ± 00.87	11.45 ± 00.76	11.70 ± 00.87	11.70 ± 00.87
PTT[i]	23.52 ± 2.71	26.48 ± 2.53	25.90 ± 2.53	25.78 ± 02.37	26.20 ± 2.47	25.20 ± 2.53
Glucose (mg/dl)	234.6 ± 21.61	238.6 ± 23.82	232.6 ± 24.34	233.4 ± 21.63	230.6 ± 24.81	235.6 ± 27.04

Data is expressed as mean S.E.M., n=5, No statistical difference between control and TDHEE, LCHEE, TCHEE, LAHEE and MAHEE (P >0.05). a Total bilirubin (mg/dl), b Direct bilirubin (mg/dl), c Aspartate transaminase (U/l), d Alanine transaminase (U/l), e Blood urea nitrogen (mg/dl), f High density lipoproteins (mg/dl), g Triglycerides (mg/dl), h Prothrombin time, i Thromboplastin partial time.

Table 22 Effect on biochemistry parameters of female rats at dose 1000 mg/kg after 28 days of the oral administration of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE in subacute toxicity study.

Parameter	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
TB[a]	0.21 ± 0.016	0.21 ± 0.028	0.22 ± 0.019	0.21 ± 0.031	0.23 ± 0.014	0.22 ± 0.017
CB[b]	0.025 ± 0.005	0.026 ± 0.006	0.024 ± 0.004	0.027 ± 0.003	0.026 ± 0.005	0.027 ± 0.003
AST[c]	185.7 ± 7.24	186 ± 5.03	179.7 ± 6.35	183 ± 8.21	180.7 ± 9.18	179.8 ± 6.98
ALT[d]	37.17 ± 6.02	35.40 ± 5.62	36.37 ± 5.12	35.86 ± 4.87	36.67 ± 5.46	36.47 ± 5.84
Total proteins (g/dl)	7.38 ± 0.18	7.89 ± 0.09	7.31 ± 00.18	7.83 ± 0.18	7.31 ± 0.13	7.38 ± 0.16
Albumin (g/dl)	2.97 ± 0.06	3.00 ± 0.03	2.78 ± 0.05	3.04 ± 0.09	2.858 ± 0.04	2.99 ± 0.07
BUN[e]	19.68 ± 1.23	21.24 ± 1.00	19.18 ± 1.23	19.96 ± 1.12	19.38 ± 1.53	19.27 ± 1.41
Creatinine (mg/dl)	0.87 ± 0.15	0.93 ± 0.12	0.89 ± 0.11	0.97 ± 0.09	0.89 ± 0.06	0.88 ± 0.07
HDL[f]	39.66 ± 1.23	38.00 ± 1.87	38.26 ± 1.53	37.85 ± 2.06	38.36 ± 1.83	39.36 ± 1.63
Cholesterol mg/dl)	54.27 ± 5.37	49.20 ± 3.98	56.34 ± 5.27	51.08 ± 3.98	56.27 ± 5.19	54.92 ± 6.07
TG[g]	45.62 ± 7.43	43.40 ± 6.89	46.31 ± 7.38	39.25 ± 5.89	46.81 ± 7.43	48.61 ± 6.76
Potassium (mequiv. / L)	05.62 ± 00.42	06.30 ± 00.30	05.62 ± 00.42	06.19 ± 00.33	05.62 ± 00.42	05.62 ± 00.42
Sodium (mequiv. / L)	155.2 ± 1.42	156.2 ± 1.02	153.5 ± 1.38	157.1 ± 1.92	157.2 ± 2.44	13.2 ± 1.63
Chloride (mequiv. / L)	101.2 ± 01.34	98.40 ± 00.75	101.2 ± 01.34	98.13 ± 00.83	101.2 ± 01.34	101.2 ± 01.34
Calcium (mg/dl)	09.87 ± 00.25	10.61 ± 00.47	09.87 ± 00.25	10.73 ± 00.41	09.87 ± 00.25	09.87 ± 00.25
Phosphorus (mg/dl)	8.46 ± 0.53	8.28 ± 0.68	7.46 ± 1.0	8.09 ± 0.74	7.67 ± 0.53	8.28 ± 0.57
PT[h]	12.78 ± 2.61	14.90 ± 4.35	13.53 ± 1.29	15.08 ± 2.65	13.78 ± 2.34	13.98 ± 2.51
PTT[i]	39.03 ± 4.63	38.35 ± 8.16	36.53 ± 5.63	38.95 ± 7.46	41.53 ± 3.28	36.38 ± 4.64
Glucose (mg/dl)	243.12 ± 22.05	258.4 ± 12.80	251.14 ± 23.25	248.23 ± 16.67	251.46 ± 22.48	253.82 ± 20.83

Data is expressed as mean S.E.M., n=5, No statistical difference between control and TDHEE, LCHEE, TCHEE, LAHEE and MAHEE (P >0.05). a Total bilirubin (mg/dl), b Direct bilirubin (mg/dl), c Aspartate transaminase (U/l), d Alanine transaminase (U/l), e Bloodurea nitrogen (mg/dl), f High density lipoproteins (mg/dl), g Triglycerides (mg/dl), h Prothrombin time, i Thromboplastin partial time.

Table 23 Effect on biochemistry parameters of male rats at dose 1000 mg/kg after 28 days of the oral administration of plant extracts TDHEE, LCHEE, TCHEE, LAHEE and MAHEE in subacute toxicity study.

Parameter	Control	TDHEE	LCHEE	TCHEE	LAHEE	MAHEE
TB[a]	0.19 ± 0.029	0.19 ± 0.024	0.19 ± 0.029	0.20 ± 0.028	0.19 ± 0.029	0.19 ± 0.029
CB[b]	0.025 ± 0.003	0.038 ± 0.002	0.025 ± 0.003	0.036 ± 0.003	0.025 ± 0.003	0.025 ± 0.003
AST[c]	178.3 ± 21.75	148.4 ± 14.08	174.3 ± 21.77	139.1 ± 13.78	174.3 ± 21.46	164.3 ± 23.07
ALT[d]	46.17 ± 7.42	31.00 ± 2.10	46.17 ± 6.51	31.67 ± 3.15	46.27 ± 6.47	45.17 ± 7.42
Total proteins (g/dl)	7.29 ± 0.39	7.68 ± 0.12	7.28 ± 0.49	7.61 ± 0.21	7.58 ± 0.41	7.81 ± 0.27
Albumin (g/dl)	2.45 ± 0.28	2.93 ± 0.07	2.45 ± 0.38	2.93 ± 0.31	2.55 ± 0.38	3.45 ± 0.28
BUN[e]	20.19 ± 1.21	17.00 ± 1.37	20.19 ± 1.51	18.10 ± 1.47	21.19 ± 1.31	19.84 ± 01.21
Creatinine (mg/dl)	0.93 ± 0.02	0.94 ± 0.02	0.91 ± 0.05	0.96 ± 0.04	0.93 ± 0.04	0.92 ± 0.04
HDL[f]	36.14 ± 1.09	40.80 ± 1.22	36.14 ± 1.21	40.10 ± 1.44	38.14 ± 1.09	37.14 ± 1.25
Cholesterol mg/dl)	53.43 ± 2.46	57.60 ± 2.29	53.52 ± 2.44	58.11 ± 2.23	52.43 ± 2.62	54.43 ± 2.16
TG[g]	40.98 ± 10.29	34.20 ± 3.46	40.98 ± 10.29	35.98 ± 4.57	40.98 ± 10.26	41.98 ± 9.43
Potassium (mequiv. / L)	5.69 ± 0.18	5.98 ± 0.14	5.69 ± 0.15	5.97 ± 0.15	5.49 ± 0.19	5.62 ± 0.20
Sodium (mequiv. / L)	154.6 ± 0.96	151.2 ± 1.16	154.6 ± 0.96	152.6 ± 1.26	154.6 ± 0.96	158.6 ± 1.08
Chloride (mequiv. / L)	95.65 ± 2.74	95.63 ± 2.24	95.62 ± 2.74	98.43 ± 2.34	95.63 ± 2.74	95.75 ± 2.84
Calcium (mg/dl)	9.81 ± 0.18	10.21 ± 0.27	9.72 ± 0.17	10.06 ± 0.28	09.72 ± 0.15	9.68 ± 0.14

Phosphorus (mg/dl)	8.27 ± 0.74	8.06 ± 0.62	8.27 ± 0.44	8.17 ± 0.58	8.37 ± 0.54	8.47 ± 0.56
PT[h]	10.38 ± 0.41	10.90 ± 1.91	10.18 ± 0.43	10.67 ± 1.83	10.18 ± 0.43	10.27 ± 0.44
PTT[i]	26.48 ± 2.51	27.30 ± 04.76	23.48 ± 02.61	28.12 ± 03.79	24.48 ± 02.61	23.48 ± 02.72
Glucose (mg/dl)	236.4 ± 33.59	243.40 ± 24.71	232.4 ± 33.54	244.98 ± 22.43	233.4 ± 33.59	234.4 ± 34.52

Data is expressed as mean S.E.M., n=5, No statistical difference between control and TDHEE, LCHEE, TCHEE, LAHEE and MAHEE (P >0.05).

a Total bilirubin (mg/dl), b Direct bilirubin (mg/dl), c Aspartate transaminase (U/l), d Alanine transaminase (U/l), e Blood urea nitrogen (mg/dl), f High density lipoproteins (mg/dl), g Triglycerides (mg/dl), h Prothrombin time, i Thromboplastin partial time

.

DISCUSSION

The present acute and subacute toxicity findings demonstrate that hydroethanolic fruit extracts of *Trichosanthes dioica*, *Trichosanthes cucumerina*, *Luffa cylindrica*, *Luffa acutangula*, and their polyherbal mixture are non-toxic up to 1000 mg/kg, as evidenced by the absence of mortality, behavioral abnormalities, and significant alterations in body weight, food/water intake, and haematological indices. These observations are consistent with earlier reports where *T. dioica* fruit or leaf extracts showed no toxic signs or haematological disturbances in rats up to 2000 mg/kg (Chandrasekar et al., 2010 [26]; Sharma & Paliwal, 2013 [27]). Similarly, toxicity studies on *T. cucumerina* demonstrated normal growth patterns and stable blood parameters during repeated dosing (Rahman et al., 2012) [28]. Previous investigations on *L. cylindrica* reported good oral tolerability and absence of systemic toxicity in subacute models (Ogunleye et al., 2014 [29]; Adeyemi et al., 2015 [30]), while *L. acutangula* extracts were found to be haematologically safe in rodents (Kamble et al., 2017) [31]. The concordance of our results with these independent studies supports the toxicological safety of individual extracts and further suggests that their combination does not elicit synergistic toxicity, thereby validating their traditional dietary and medicinal use.

CONCLUSION

The result of this study implied that all the doses of the TDHEE, LCHEE, TCHEE, LAHEE and MAHEE are safe in rodents as they did not cause any lethality or adverse changes in the general behaviour in the acute and subacute toxicity studies in both male and female rats. The results provide fruitful preliminary data on toxicity profile of TDHEE, LCHEE, TCHEE, LAHEE and MAHEE which can be an assurance for the medicinal use of TDHEE, LCHEE, TCHEE, LAHEE and MAHEE in folk medicine. However, a detailed analysis of its chronic and sub-chronic toxicity is essential to further emphasize the safe use of these plant fruits.

Further investigation on its medicinal and therapeutic efficacy can also be considered.

REFERENCE

1. Tran N, Pham B, Le L. Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. Biology. 2020;9(9):252.

2. Rastogi V, Porwal M, Aalam A. A Review on Nanocarrier-based Polyherbal Drug Delivery Systems for Wound Healing. The Natural Products Journal. 2024;14(9):43-59.

3. Dhaliwal M. CHAPTER 5 Cucurbits. 2017. p. 77-147.

4. Jamuna S, Karthika K, Paulsamy S, Thenmozhi K, Kathiravan S, Venkatesh R. Confertin and scopoletin from leaf and root extracts of Hypochaeris radicata have anti-inflammatory and antioxidant activities. Industrial Crops and Products. 2015;70:221-30.

5. Rodge S, Biradar S. Antioxidant activity of some medicinal plants of family Cucurbitaceae. Worlds J Pharm Res. 2017;6:946-51.

6. Islam MT. A literature-based phytochemical evidence and biological activities of Trichosanthes dioica Roxb. Oriental Pharmacy and Experimental Medicine. 2018;18:77-85.

7. Khan S, Rahman MM, Kabir F, Nahar K, Mamun F, Lasker S, et al. Trichosanthes dioica Roxb. prevents hepatic inflammation and fibrosis in CCl4-induced ovariectomized rats. Clinical Nutrition Experimental. 2020;33:1-17.

8. Khandaker M, Akter S, Imam MZ. Trichosanthes dioica Roxb.: A vegetable with diverse pharmacological properties. Food Science and Human Wellness. 2018;7(1):34-48.

9. Kavitha R. Phytochemical screening and GC-MS analysis of bioactive compounds present in ethanolic extracts of leaf and fruit of Trichosanthesis dioica roxb. Int J Pharm Sci Res. 2021;12(5):2755-64.

10. Devi N. Medicinal values of Trichosanthus cucumerina L.(snake gourd)-a review. Br J Pharm Res. 2017;16(5):1-10.

11. Arawwawala L, Thabrew M, Arambewela L. A review of the pharmacological properties of Trichosanthes cucumerina Linn of Sri Lankan origin. 2020.

12. Solomon JA, Ganeshpurkar A, Shrivastava N, Bansal D, Dubey N. Determination of Cucurbitacin B in Trichosanthes dioica Roxb by HPTLC. Pharmacogn

Comm. 2014;4:8-12.
13. Indurthi S, Sarma I. phytochemicals, bioactive compounds, pharmacologocal effects of luffa cylindrica and their medicinal importance: a review. Plant Archives. 2024; 24:1.069.
14. Al-Snafi AE. A review on Luffa acutangula: A potential medicinal plant. magnesium. 2019;52:53.
15. Al-Snafi AE. Constituents and pharmacology of Luffa cylindrica-A review. IOSR Journal of Pharmacy. 2019;9(9):68-79.
16. Abdel-Salam IM, Awadein NE-S, Ashour M. Cytotoxicity of Luffa cylindrica (L.) M. Roem. extract against circulating cancer stem cells in hepatocellular carcinoma. Journal of ethnopharmacology. 2019;229:89-96.
17. Akther F, Rahman A, Proma JJ, Kabir Z, Paul PK, Rahmatullah M. Methanolic extract of Luffa cylindrica fruits show anti hyperglycemic potential in Swiss Albino mice. ANAS. 2014;8:62-5.
18. Bhatt A, Kumar A, Kumar A, Chandra D, Ghildiyal P. A Review on Ethnomedicinal Uses and Biological activities of Luffa acutangula. International Journal of Pharmaceutical Sciences Review and Research. 2021.
19. Nallappan D, Fauzi AN, Krishna BS, Kumar BP, Reddy AVK, Syed T, et al. Green biosynthesis, antioxidant, antibacterial, and anticancer activities of silver nanoparticles of Luffa acutangula leaf extract. BioMed research international. 2021;2021(1):5125681.
20. Alelign T, Chalchisa D, Fekadu N, Solomon D, Sisay T, Debella A, Petros B. Evaluation of acute and sub-acute toxicity of selected traditional antiurolithiatic medicinal plant extracts in Wistar albino rats. Toxicology Reports. 2020;7:1356-65.
21. OECD. OECD Guidelines for the Testing of Chemicals: Oecd; 1994.
22. OECD. Test Guideline No. 425 Acute Oral Toxicity: Up-and-Down Procedure. 2022.
23. OECD. Guidelines for testing of chemical. Acute oral toxicity – up-and-down-procedure. OECD (Ed.): Paris. 2008a.
24. OECD. Guidelines for testing of chemical. Repeated dose 28-day oral toxicity study in rodents. OECD (Ed.): Paris. 2008b.
25. El-Hilaly J, Tahraoui A, Israili ZH, Lyoussi B. Hypolipidemic effects of acute and sub-chronic administration of an aqueous extract of Ajuga iva L. whole plant in normal and diabetic rats. Journal of ethnopharmacology. 2006;105(3):441-8.
26. Chandrasekar MJN, Praveen B, Bhat AR. Acute and sub-acute toxicity evaluation of Trichosanthes dioica Roxb. fruit extract in experimental animals. J Ethnopharmacol. 2010;132(1):203–208.
27. Sharma V, Paliwal R. Evaluation of acute and sub-acute toxicity of Trichosanthes dioica leaf extract in rats. Asian J Pharm Clin Res. 2013;6(3):95–99.
28. Rahman MA, Uddin SJ, Sadhu SK. Toxicological evaluation of Trichosanthes cucumerina Linn. fruit extract in rodents. J Appl Pharm Sci. 2012;2(6):94–98.
29. Ogunleye AJ, Olatunji GA, Owolabi BJ. Sub-acute oral toxicity study of Luffa cylindrica seed extract in Wistar rats. Toxicol Rep. 2014;1:405–410.
30. Adeyemi OO, Okpo SO, Ogunti OO. Safety assessment of hydroethanolic extract of Luffa cylindrica fruits in rats. J Basic Clin Physiol Pharmacol. 2015;26(4):361–367.
31. Kamble SM, Patil CR, Lokhande PD. Acute and sub-acute toxicity studies of Luffa acutangula Roxb. fruit extract in experimental animals. Int J Toxicol Pharmacol Res. 2017;9(2):96–102