Assessment of Phytochemical Constituents and Toxicity Profile of Trema Orientalis Leaves Extract.

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ABSTRACT

This study was conducted to evaluate the phytochemical composition and toxicological safety of the ethanolic extract of Trema orientalis leaves. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, glycosides, steroids, terpenoids, and carbohydrates, indicating that the plant is rich in bioactive constituents. Acute oral toxicity was evaluated in Swiss albino mice at doses ranging from 250 to 3000 mg/kg body weight. No mortality, behavioral changes, or signs of toxicity were observed, indicating that the extract is safe up to 3000 mg/kg. Sub-acute toxicity was assessed by administering 500 mg/kg of the extract for 14 consecutive days. The treatment did not produce any significant changes in body weight, liver enzymes (SGPT, SGOT, ALP), bilirubin, creatinine, lipid profile, or HDL levels when compared to the control group. These findings suggest that the ethanolic extract of Trema orientalis leaves is non-toxic under both acute and short-term repeated exposure. The presence of multiple phytochemicals along with its favorable safety profile supports the potential of this plant for further pharmacological and therapeutic investigations.

Keyword: Trema orientalis, Phytochemical screening, Ethanolic leaf extract, Acute and sub-acute toxicity, Medicinal plants.

INTRODUCTION

Medicinal plants represent one of the oldest and most reliable sources of therapeutic agents for humankind. The World Health Organization (WHO) has reported that approximately 80% of the population in developing countries continues to depend on herbal medicines for their primary healthcare needs. This reliance highlights the significance of medicinal plants as reservoirs of bioactive compounds, many of which serve as templates or precursors for modern drug development. Alkaloids, flavonoids, tannins, saponins, and phenolic compounds are among the key secondary metabolites that play important roles in antioxidant, antimicrobial, anti-inflammatory, and anticancer activities.

Trema orientalis (Linn.) Blume, belonging to the family Ulmaceae, is a fast-growing deciduous tree widely distributed across Asia, Africa, and tropical regions. In traditional medicine, different parts of this plant are used to manage diverse conditions such as malaria, cough, wounds, dysentery, asthma, and hypertension. The leaves are particularly noted in ethnomedicine for their wound-healing and anti-inflammatory properties. Preliminary studies have indicated that Trema orientalis contains phytochemicals including flavonoids, glycosides, tannins, and sterols, which may be responsible for its biological activities.

Despite its wide traditional use, scientific validation regarding its phytochemical composition and toxicological safety is still limited. Phytochemical screening provides essential insights into the types of active compounds present, while toxicological evaluation ensures the safety of plant extracts for further pharmacological application. As natural products can possess both therapeutic benefits and potential risks, systematic studies are crucial before their integration into modern healthcare.

In this context, the present study was designed to evaluate the phytochemical profile of Trema orientalis leaves extract and to assess its acute and sub-acute toxicological effects in experimental models. The findings are expected to provide baseline data for future pharmacological research and potential drug development.

Plant Preview:

Botanical Name: Cyclea barbata Miers

Family: Cannabaceae

Vernacular (Local) Name: Noyal, Nalita, or Pakur

Growth Habit: Shrub

Used Part: Leaves

Figure 2.1: Trema Orientalis

Taxonomy of T. Orientalis

Rank: Taxon

Kingdom: Plantae

Division: magnoliophyta

Class: Magnoliopsida

Order: Rosales

Family: Cannabaceae (Formerly Ulmaceae)

Genus: Trema

Species: Trema orientalis (L.) Blume

Morphology

Trema orientalis (L.) Blume is a deciduous, fast-growing small to medium-sized tree that belongs to the family Cannabaceae. It is characterized by its distinct vegetative and reproductive features:

Habit: A small to medium tree, generally reaching 10–20 meters in height. It has a straight, cylindrical trunk with a spreading crown and slender branches.

Bark: The bark is greyish-brown to dark brown, rough or slightly fissured, with a fibrous inner layer. The inner bark yields strong fibers often used for making ropes and mats.

Leaves: Leaves are simple, alternate, and spirally arranged, with ovate to lanceolate shapes. They are 6–12 cm long and 2–6 cm wide, having serrated margins and acute tips. The upper surface is rough and dark green, while the lower surface is pale green and hairy. The petioles are slender and about 0.5–1.5 cm long.

Flowers: The flowers are small, greenish-yellow, and unisexual, occurring in axillary or terminal clusters. The plant is dioecious, meaning male and female flowers are borne on separate trees. The male flowers are more numerous and arranged in clusters, while female flowers are fewer and solitary.

Fruit: The fruit is a small, round drupe about 3–5 mm in diameter, turning purple or blackish when ripe. Each fruit contains a single seed, which is an important food source for birds and small mammals.

Root System: The plant develops a deep taproot system with well-developed lateral roots, enabling it to survive in poor and dry soils.

Traditional Uses

Trema orientalis has been widely used in traditional medicine across Asia and Africa for centuries. Different parts of the plant — leaves, bark, roots, and fruits — possess various medicinal and practical applications:

Leaves: Used as a decoction or poultice for treating fever, cough, asthma, wounds, and diabetes. The leaves are also used as fodder for livestock.

Bark: Applied for toothache, dysentery, and diarrhea. The inner bark fibers are used for making ropes, mats, and paper.

Roots: Used traditionally as a diuretic and to treat stomach pain, ulcers, and infections.

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Fruits: The small drupes are edible and serve as food for birds and wildlife; sometimes used in local remedies for digestive problems.

Wood: Soft and lightweight, often used for fuelwood, charcoal, and tool handles. In ethnomedicine, Trema orientalis is believed to possess antimicrobial, anti-inflammatory, antidiabetic, analgesic, antioxidant, and cytotoxic properties, which have been supported by several pharmacological studies.

LITERATURE REVIEW:

Authors, Year	Plant Part & Extract Type	Assay / Experimental Model	Main Findings	Limitations
Mashiar Rahman et al., 2018	Leaves, methanol extract	Antibacterial (disc diffusion & MIC); Brine shrimp lethality	Moderate antibacterial activity; LC50 ~170 µg/mL for cytotoxicity	High MIC values; crude cytotoxicity; lacks in vivo data
Anonymous, 2006	Stem bark, aqueous extract	OGTT in diabetic rats	Reduced blood glucose in diabetic rats; improved glucose tolerance	Short term; limited doses; mechanism not explored
Jiji K. N., 2016	Leaves, ethanolic extract	Type-2 diabetic rat model; OGTT; lipid profile; histopathology	Reduced fasting blood glucose; improved lipid profile; partially restored β-cell histology	Limited comparison with standard drugs; sample size limited; long-term toxicity not addressed
Masnoon Kabir et al., 2019	Leaves, crude methanol extract	In vivo anticancer (EAC) in mice; in vitro MTT; apoptosis assessment	59% tumor inhibition; apoptotic features; IC50 ~29.95 µg/mL in vitro	Only one tumor model; crude extract; mechanisms not fully explored
Babatunde et al., 2015	Leaves, acetone extract	In vivo antiplasmodial (*P. berghei*)	Significant suppression of parasitemia; active fractions identified	Only rodent model; doses/toxicity not fully characterized; human relevance unclear
Eniola et al., 2016	Leaves & bark, aqueous extract	In vivo antimalarial (*P. berghei*)	Suppressed parasitemia; improved survival	Only rodent model; active compounds not isolated; safety profile limited
Hemalatha et al., 2018-2020	Aerial parts, methanol extract	Acute & sub-acute toxicity in rats	No major changes in body weight or biochemical parameters	Chronic, reproductive toxicity not explored; limited organs checked
Open Chemistry Study	Aerial parts, methanol extract	In vitro antioxidant, antimicrobial, cytotoxicity	Strong radical scavenging; IC50 ~2.26 µg/mL on HCT116; variable antimicrobial	In vivo activity not tested; selectivity/toxicity to normal cells not fully assessed
Fabowale et al., 2020	Leaves, methanol & petroleum ether extracts	Antibacterial & antifungal	Zones of inhibition 22-32 mm; MIC 3.125-25 mg/mL	High MICs; toxicity not assessed

Extraction

The abundance of medicinally useful chemicals found in plants is a major consideration. In these bioactive compounds lies the possibility of creating new medicines with broad therapeutic applicability. Crucial to the procedure is the extraction of bioactive chemicals from plant sources. The goal of the extraction process is to separate the plant's insoluble cellular marc (residue) from its soluble compounds. Tanning agents, flavonoids, alkaloids, glycosides, phenolics, and terpenoids are only some of the bioactive components found in crude extracts. The concentrations of bioactive metabolites in natural medicines are relatively low. The extraction of crude plant extracts necessitates the development of a selective and effective method. In general, two categories of extraction procedures are frequently employed to extract the bioactive metabolites of plants.

1. Hot extraction
2. Cold extraction

The extract is subsequently concentrated, and the constituents are isolated using a variety of methods, including chromatography. As a standard precautionary measure against material loss, concentrated extracts should be stored in the refrigerator.

Method of Plant Extraction

Medicinal extraction is the separation of the biologically active constituents of plant and animal tissues using certain solvents and recognized methods. Products derived from plants are composed of intricate mixtures of metabolites. They can be administered orally or topically and can be in the form of liquid, semisolid, or desiccated particles (after solvent removal).

Extracting the active components from a medicinal plant is the first step of purification.

Plant collection

The leaves of C. barbata were collected from Chittagong, Bangladesh, in June and July 2022 for the purposes of this investigation. During the collection process, any form of adulteration was rigorously prohibited. A Taxonomist from the Bangladesh National Herbarium in Mirpur Dhaka, identified the sample. (DACB Accession No.: 78801). The fundamental principle is to cut the leaves into small pieces, which increases the surface area for extraction and, in turn, the rate of extraction. The optimal solvent-to-dry weight ratio has been reported in previous investigations to be 10:1 (v/w).

Preparation of Crude Extract:

Drying and grinding

The collected plant was separated from any unwanted components or other elements. They spent two weeks in a shelter, dehydrated and without any sunlight. Using the suitable grinder, the plant components were turned into coarse flour. Until the study was carried out, the material was kept in a secure container in a dry, cold, dark surroundings.

Cold extraction (ethanol extraction)

A pristine, flat-bottomed glass vessel was utilized to submerge 250 g of powdered substance in 1250 ml of ethanol. The contents of the container were securely stored for a duration of 14 days, with periodic agitation and mixing. A pristine, white cotton cloth was utilized to do a rough filtering of the entire mixture. Subsequently, it underwent filtration using Whatman filter paper.

Evaporation of the solvent

Rotary evaporation was employed to evaporate the filtrates (ethanol extracts) that were obtained. Then, concentrated extracts were transferred to a beaker. The aperture of the beaker was covered with a sheet of aluminum foil, and perforation was performed to facilitate the evaporation of ethanol. The beaker was then stored in a cold and dry location for a few days.

Finally, the beaker was subjected to evaporation under a table fan until it was completely dry. It produced a concentrate of the deep crimson paste variety. Crude ethanolic extract was the designation of the concentrate.

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Figure 4.1: Evaporation of solvent by Rotary Evaporator

Preparation of Crude Extract

Plant collection

↓

Dry powder of the plant

↓

Macerated with 95% ethanol for 14 days

↓

Filtration with cloth and cotton plug

↓

Filtration using filter paper

↓

Evaporate

↓

Obtain Crude ethanol extract

Yield Determination

Total weight of the dried extract of C. barbata leaves was 24 gm from 350 gm powder that was macerated.

So, Yield = (24 gm / 350 gm) ×100 = 6.85%

Phytochemical Screening

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids, phenolic compounds, steroids, quinones, saponins, glycosides, flavonoids. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities. Alkaloids are used as anaesthetic agents and are found in medicinal plants.

MATERIALS & METHODS

Test Material

Ethanolic extract of Trema Orientalis leaves.

Reagents

1. Benedict's Reagent
2. Fehling's Solution A
3. Fehling's Solution B
4. Ferric chloride solution
5. Potassium dichromate solution
6. Concentrated H_2SO_4
7. Dilute Sodium hydroxide solution
8. Molish Reagent
9. Dragendroff's Reagent
10. NaOH solution
11. Nitric acid
12. Chloroform
13. Sodium bicarbonate solution
14. Ethanol
15. Distilled water

Composition of Reagents used for the different chemical group test Benedicts Reagent

1.73 gm cupric sulphate, 1.73 gm sodium citrate and 10 gm anhydrous sodium carbonate were dissolved in water and the volume was made up to 100 ml with water.

Fehling's solution A

34.64 gm copper sulphate was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml.

Fehling's solution B

17.6 gm of sodium potassium tartarate and 7.7 gm of sodium hydroxide were dissolved in sufficient water to produce 100 ml.

5% Ferric chloride solution

500 mg ferric chloride was dissolved in 10 ml water.

10% Potassium dichromate solution

1 gm Potassium dichromate was dissolved in 10 ml water.

Molish Reagent

2.5 gm of pure α-naphthol was dissolved in 25 ml of ethanol.

Dragendroff's Reagent

1.7 gm basic bismuth nitrate and 20 gm tartaric acid ware dissolved in 80 ml water. This solution was mixed with a solution contains 16 gm potassium iodide and 40 ml water.

Test procedure for identifying different chemical groups

Test procedure for identifying different chemical groups were done. Testing of different chemical groups present in extract represents the preliminary phytochemical studies. In each test 5% (w/v) solution of extract in ethanol was taken unless otherwise mentioned in individual test. The chemical group test, which are performed as follows-

Tests for reducing sugar

Benedict's Test

0.5 ml of aqueous extract of the plant material is taken in a test tube. 5ml of benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously. The formation of a red color precipitate of cuprous oxide indicates the presence of a reducing sugar.

Fehling's Test

2ml of an aqueous extract of the plant material is added 1ml of a mixture of equal volumes of Fehling's solutions A and B and boiled for few minutes. The formation of a red or brick red color precipitate was formed which indicates the presence of a reducing sugar.

Tests for tannins

Ferric Chloride Test

5 ml solution of the extract is taken in a test tube. Then 1 ml of 5%Ferric chloride solution is added. Greenish black precipitate and indicated the presence of tannins.

Potassium dichromate test

5 ml solution of the extract was taken in a test tube. Then 1 ml of 10% Potassium dichromate solution was added. The formation of a yellow precipitate indicates the presence of tannins.

Test for flavonoids

Test for flavonoids was done as previously described by Agora 1981. 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H_2S0_4. A yellow coloration observed in each extract indicates the presence of flavonoids. The yellow color disappears on standing.

Test for saponins

1 ml solution of the extract is diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Formation of one centimeter layer of foam indicates the presence of saponins.

Test for carbohydrates or Gums

5 ml solution of the extract is taken and then molish reagent and sulphuric acid are added. Formation of red violet ring at the junction of two liquids indicates the presence of gums and carbohydrate.

Test for Steroids

Sulphuric acid test

1 ml solution of chloroform extract is taken and then added1ml Sulphuric acid. Red color indicates the presence of steroid.

Test for alkaloids

Dragendroff's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid are taken in a test tube. Then 1 ml of Dragendroff's reagent is added. Formation of orange brown precipitate indicates the presence of alkaloids.

Test for glycosides

General test

Test for glycosides was done as previously described by Shanthip *et al.* 2010. A small amount of alcoholic extract of sample was dissolved in 1.0 ml of water and then aqueous solution of sodium hydroxide was added. Formation of a yellow color indicates the presence of glycosides.

Test for proteins-xanthoprotein

Xanthoproteic Test

Test for proteins-xanthoprotein was done as previously described by Tiwari *et al.* 2011. The extracts were treated with few drops of concentrated nitric acid. Formation of yellow color indicates the presence of proteins.

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Test for Terpenoids

Salkowski test

Test for terpenoids was done as previously described by Kumar *et al*. 5 ml of extract was mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated (H2SO4). A layer of the reddish brown colouration was formed at the interface thus indicating a positive result for the presence of terpenoids.

Test for Acidic Compounds

Test for acidic compounds was done as previously described by Dixit *et al*. 2014. To the 2ml of alcoholic extract, 1ml sodium bicarbonate solution was added. The effervescence produced indicated the presence of acidic compounds.

Observation

Tests for standard

Table 5.1: Different chemical group tests (for standard) are performed and are mentioned below:

Test	Reagent	Standard	Observation	Inference
Reducing sugar test	Fehling's Solution	Dextrose	Brick red colored precipitate was formed	Presence of reducing sugar
	Benedict's reagent	Dextrose	Brick red colored precipitate was formed	Presence of reducing sugar
Tannin test	Ferric Chloride solution	Rose petal	Greenish black precipitate was formed	Presence of tannin
	Potassium dichromate solution	Rose petal	A yellow precipitate was formed	Presence of tannin
Flavonoid test	Dilute ammonia solution, H_2SO_4	Rose petal	A yellow color was formed	Presence of flavonoid
Saponin test	Distilled water	Detergent	Formation of foam	Presence of saponin
Gum test	Molish's reagent	Liquid glue	Reddish ring	Presence of gum
Steroids test	Sulphuric acid	Ethinyl estradiol 0.03 mg	Red color was observed	Presence of steroids
Alkaloids test	Dragendroff's reagent	Nicotine	Orange brown precipitate was observed	Presence of alkaloids
Glycosides test	NaOH solution	Aloe vera	Yellow color was found	Presence of glycosides
Protein test	Concentrated nitric acid	Egg albumin	Yellow color was found	Presence of protein

Tests for extract

Table 5.2: Different chemical group tests (for extract) are performed and are mentioned below:

Test	Reagent	Observation	Inference
Reducing sugar test	Fehling's Solution	Brick red colored precipitate was not formed	Absence of reducing sugar

	Benedict's reagent	Brick red colored precipitate was not formed	Absence of reducing sugar
Tannin test	Ferric Chloride solution	Greenish black precipitate was formed	Presence of tannin
	Potassium dichromate solution	A yellow precipitate was formed	Presence of tannin
Flavonoid test	Dilute ammonia solution, H_2SO_4	A yellow color was formed	Presence of flavonoid
Saponin test	Distilled water	Layer of foam was not produced	Absence of saponin
Gum test	Molish's reagent	Reddish ring was formed at the junction of two liquids	Presence of gum
Steroids test	Sulphuric acid	Red color was observed	Presence of steroids
Alkaloids test	Dragendroff's reagent	Orange brown precipitate was observed	Presence of alkaloids
Glycosides test	NaOH solution	Yellow color was found	Presence of glycosides
Protein test	Concentrated nitric acid	Yellow color was not found	Absence of protein
Terpenoid test	Chloroform, concentrated H_2SO_4	Reddish brown coloration was formed at the junction of two liquids	Presence of terpenoids
Acidic compound test	Sodium bicarbonate solution	Effervescence was not produced	Absence of acidic compounds

RESULT

This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Phytochemical screening of ethanolic extract of *Trema Orientalis* leaves was performed in order to identify various bioactive compounds. The results of the phytochemical screening of leaf of *Trema Orientalis* are presented in Table 7.3. The leaves extract showed positive results for alkaloids, carbohydrates, glycosides, flavonoids, steroids, tannins and terpenoids.

Table 5.3: Result of phytochemical screening of ethanolic extract of *Trema Orientalis* leaves

• **Reducing sugar**	-
• **Tannins**	+
• **Flavonoids**	+

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• **Saponins**	-
• **Carbohydrates / Gums**	+
• **Steroids**	+
• **Alkaloids**	+
• **Glycosides**	+
• **Proteins**	-
• **Terpenoids**	+
• **Acidic compounds**	-

'+' indicates presence and

'-' indicates absence

Toxicological Investigation

Acute Toxicity Test

Human beings have exclusively increased the use of pharmacological chemicals via the intake of food, medications, drinks, as well as other industrial and home items. These compounds may exhibit chronic or acute toxicity, which can range from moderate to severe depending on their nature. Acute toxicity refers to the occurrence of immediate or short-term undesirable consequences when chemicals are provided in a single or multiple dosage during a 24-hour period.

Acute toxicity studies offer data regarding:

- The possibility of immediate harm to humans due to toxicity
- A calculation of the safe amount of the medication that can be taken at once by humans
- The organs in the body that may be affected by the drug's toxicity
- The timeline of observable clinical effects caused by the drug
- The correct dosage for research on the drug's toxicity when taken numerous times
- Differences in the drug's toxicity between different species.

It is imperative to have access to data on the acute toxicity of medications in order to anticipate the potential effects of human overdose. In phase III trials, these data can be used. Outpatient clinical trials must investigate acute toxicity for therapeutic reasons that include patient categories at risk of overdose, such as sadness, pain, and dementia

Objectives

Acute toxicity tests assess whether a single or multiple doses may kill or cause serious toxicological effects. They provide dose-related information for subsequent studies and create another opportunity for determining compound-induced effects that are observed morphologically, clinically or other evaluations. They also serve to provide an early indication of possible target organ(s).

MATERIALS AND METHODS

Test Material

Ethanolic extract of *Trema Orientalis* leaves.

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Experimental animal

The research used 23 to 25 gm Swiss-albino mice aged 4 to 5 weeks. Khulna University's pharmacology lab provided the animals. Animals were acclimated at Khulna University's Pharmacy Discipline animal facility for a week.

Experiment design

The assessment was conducted following the methodology outlined in the OECD GUIDELINE FOR TESTING OF CHEMICALS, with minor adjustments. After randomly selecting 40 experimental animals, they were separated into 8 groups labeled as group-I, group-II, group- III, group-IV, group-V. Each group included five mice. Ethanolic extract of *Cyclea barbata* leaves was administered to the groups I, II, III, IV and V at doses of 250mg/kg, 500mg/kg, 1000mg/kg, 2000mg/kg, and 3000mg/kg body weight, respectively. In this study, Group VI was designated as the control group and was administered an oral 2% tween-80 water solution.

Preparation of sample suspension

By adding a little amount of tween-80, the extract was lyophilized unidirectionally to generate the suspension of the test samples at dosages of 250mg/kg, 500mg/kg, 1000mg/kg, 2000mg/kg, and 3000mg/kg body weight. Then, distilled water was progressively added. The final suspension volume was 5 ml. Stabilizing the suspension required intense vortex mixing. Each group of mice got a 0.01 microliter solution depending on their gram weight.

Preparation of control solution

To prepare 2% Tween-80 in water, 1ml Tween-80 is mixed with 25 ml distilled water and then volume is adjusted to 50 ml by adding adequate amount of distilled water.

Table 6.1: Effects of ethanolic extract of *Trema Orientalis* leaves (mortality rate) in mice after 24 hours.

Group	No. of treated mice	No. of dead mice after 24 hours	Mortality rate
Group-I (250mg/kg)	5	0	0
Group-II (500mg/kg)	5	0	0
Group-III (1g/kg)	5	0	0
Group-IV (2g/kg)	5	0	0
Group-V (3g/kg)	5	0	0
Group-VI (Control group)	5	0	0

Experimental procedure

The method of Lorke was used to administrate 100, 200, 400, 800, 1000, 2000, and 3000 mg/kg body weight aqueous extract orally to the groups I, II, III, IV, V, VI and VII. The vehicle was provided exclusively to the control group. For 24 hours and 14 days, the animals were evaluated for changes, mortality, and body weight.

Group	Avg. Weight on 1st day	Avg. Weight on 14th day
Group-I (250mg/kg)	23.5±1.19	28.2±0.99
Group-II (500mg/kg)	24.8±1.32	31.4±1.12
Group-III (1g/kg)	22.7±0.99	26.1±1.03

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Group-IV (2g/kg)	24.6±1.73	29.5±1.91
Group-V (3g/kg)	24.3±1.73	30.1±1.91
Group-VI (Control group)	23.1±1.35	29.4±1.36

Table 6.2: Effects of ethanolic extract of *Trema Orientalis* leaves on body weight in mice

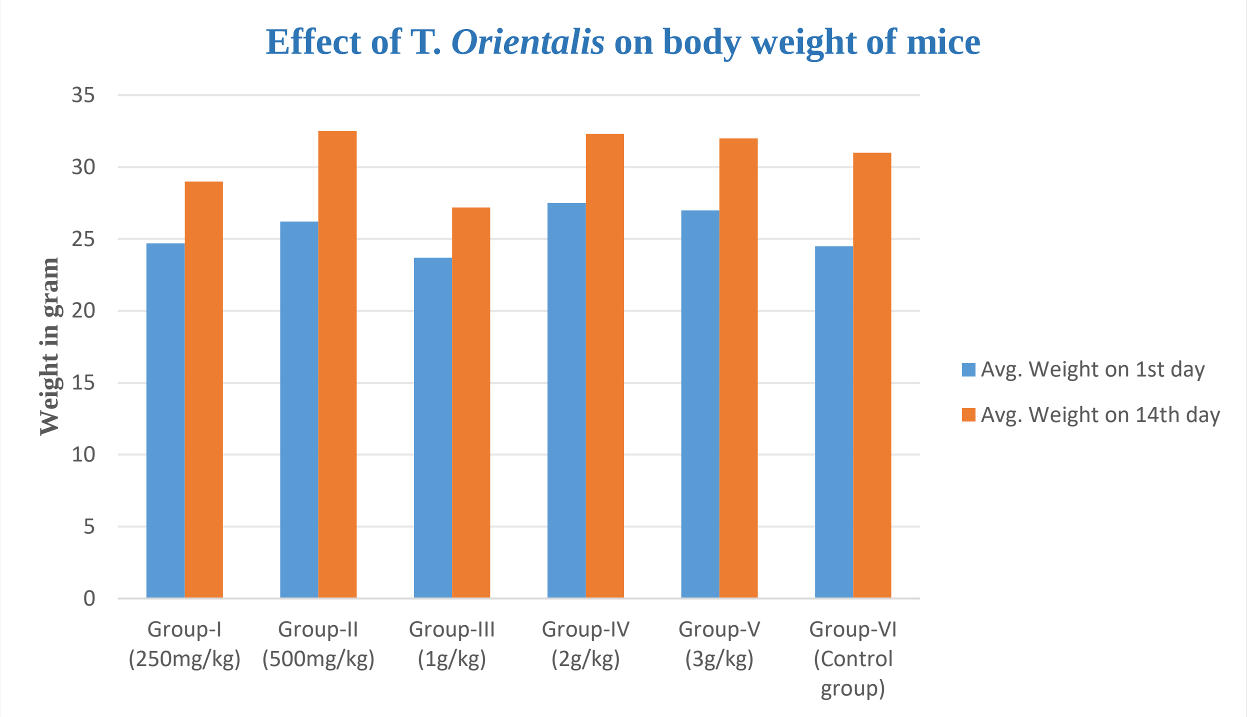

Fig 6.1: Effects of ethanolic extract of *Trema Orientalis* leaves on body weight of mice.

RESULTS AND DISCUSSION

Mice administered orally with an ethanolic extract of *Trema Orientalis* leaves up to a dosage of 3000 mg/kg body weight showed no evidence of toxicity, behavioral changes, or death, proving that the extract is safe.

Conclusion

According to the present study, none of the subjects died at a dose of 3g/kg, suggesting that the ethanolic extract of *Trema Orientalis* leaves may be safe for acute administration. There is no assurance that this plant will have negative consequences based on this investigation.

Sub-Acute Toxicity Test

The purpose of a subacute toxicity study is to evaluate a new medicine's safety during a two- to four-week treatment gap. The purpose of these research is to assess the development and recovery from drug-induced damage, as well as to establish the dosage levels that will be most effective in future investigations of sub-chronic and chronic toxicity. Furthermore, subacute toxicity studies may provide supplementary evidence for initial clinical trials, which may involve a treatment period of up to four weeks The pharmacological significance of medicinal plants has received a lot of attention recently, thanks to the growing interest of academics throughout the globe. Research into these plants' traditional usage has been extensive. But the detrimental impacts of only a few of plants have been the subject of much research. In order to guarantee effective and transparent communication of data about the short and long-term toxicity of herbal treatments and phytochemicals, it is imperative to conduct more inquiry.

Assessing the toxicological impact of therapeutic plant extracts, whether for clinical or pre- clinical usage, is an essential aspect of evaluating their possible harmful consequences. Applying the sub-acute toxicity test allows one to evaluate a substance's toxicity over a long duration of a subject's life. This test is also aligned with the Globally Harmonized System (GHS). This study aims to gather data on the significant toxicity of major organs, identify any systemic toxic effects, and monitor various parameters to estimate the levels of exposure that do not cause observable adverse effects (NOAEL). These findings will be used to determine appropriate dosage levels for long-term studies and establish safety guidelines for human exposure.

MATERIALS AND METHODS

Test Material

Ethanolic extract of *Trema Orientalis* leaves.

Experimental animal

The research used 23–25-gram Swiss-albino mice aged 4–5 weeks. Khulna University's pharmacology lab provided the animals. Animals were acclimated at Khulna University's Pharmacy Discipline animal facility for a week.

Experiment design

The sub-acute toxicity test of the ethanolic extract of *Trema Orientalis* leaves was conducted in accordance with the procedure previously described by McConnell, E., et al and Anandan, S., et al., with minor modifications. Ten experimental animals were randomly selected and divided into two groups: Negative Control and Test-I, each of which contained five mice. The Test-I group was orally administered 500mg/kg of the test extract for a period of 14 days. The vehicle (2% tween 80 in water) was administered orally to the negative control group.

Preparation of sample suspension

Dose of extract - 500 mg/kg

For a 500 mg/kg body weight suspension of test samples, 500 mg of extract was quantified. Add a little tween-80 to grind the extract in one direction. Before adding distilled water, extract and tween-80 were combined properly. Adjusting suspension volume to 5 ml. Ultrasonic agitation stabilized the suspension. To adhere to the 500 mg/kg dose, mice in this group received a 0.01 × body weight in grams milliliter solution.

Preparation of control solution

To prepare 2% Tween 80 in water, 1ml Tween 80 is mixed with 25 ml distilled water and then volume is adjusted to 50 ml by adding adequate amount of distilled water.

Experimental procedure

After 14 days of taking 500 mg/kg orally of the test extract daily, the Test-I group outperformed the control group, which only got a vehicle. The experimental animals were observed every day for any abnormal symptoms or deaths in the 14 days after the extraction operations. After 14 days of observation, blood samples were taken from the animals' jugular vein and middle abdominal vein without the application of an anticoagulant (EDTA). Painkillers were administered to the animals. Blood samples were spun at 1500 rpm for 20 minutes to ensure contamination-free biochemical analysis. After that, several biochemical parameters were calculated by separating the serum.

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Results

Group	Average body weight(1^{st} day)	Average body weight (After 14 days)
Negative control Test-I (500mg/kg)	24 ± 1.2	26.2 ± 1.51
	25 ± 2.5	27.8 ± 1.21

Table 5.2.1 shows the results of the short-term safety tests of an ethanolic extract of *Trema Orientalis* leaves on mice. During the study, the dose of treatment did not change the body weight of mice compared to the control group.

Table 6.3: Effect of *Trema Orientalis* leaves on average body weight of mice

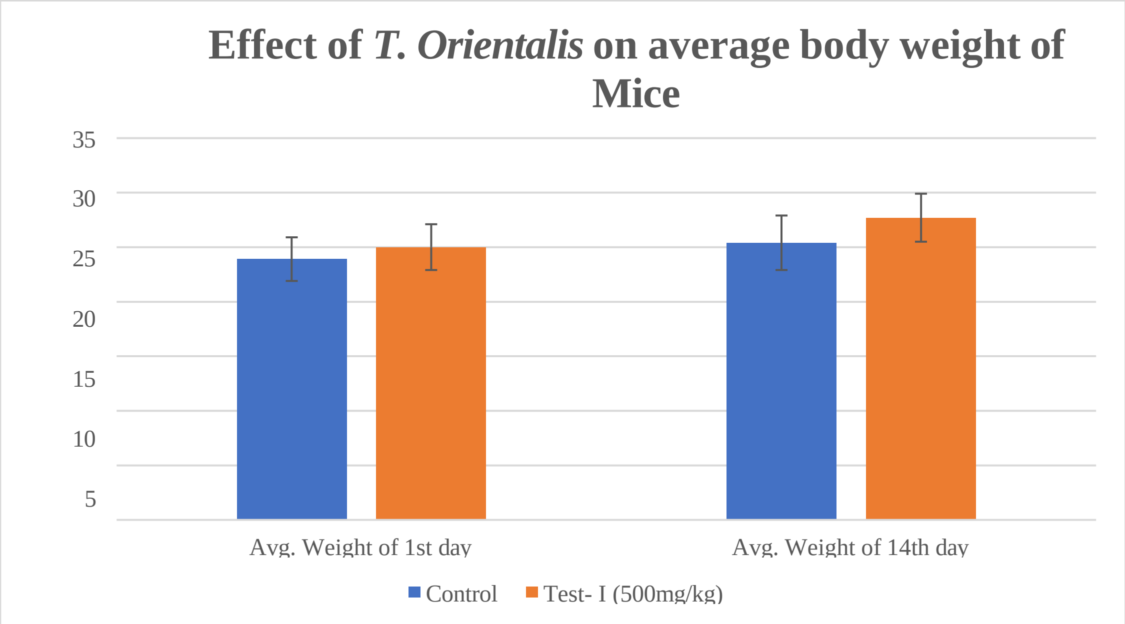
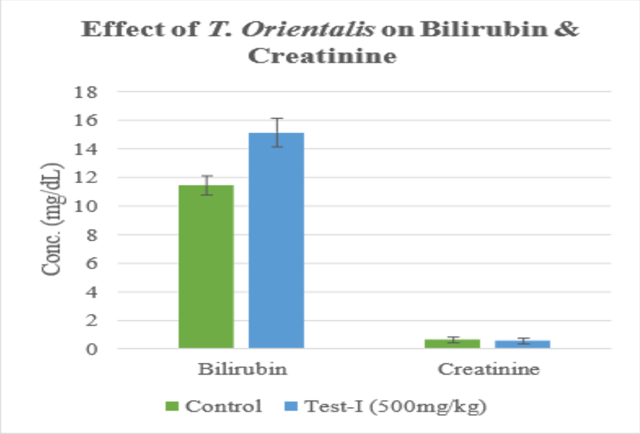


Fig 6.2: Effect of *Trema Orientalis* leaves on average body weight of mice.

Parameter	Control	Test extract (500mg/kg)
SGPT	43.6±4.0 (U/L)	48.9±3.5 (U/L)
SGOT	32.3±5.0 (U/L)	39.9±3.0 (U/L)
ALP	129.1±4.5 (U/L)	123±4.0U/L)
Bilirubin	10.9±0.67 (mg/dl)	13.1±1 (mg/dl)
Creatinine	0.59±0.2 (mg/dl)	0.51±0.2 (mg/dl)
Cholesterol	113.4±5.39(mg/dl)	121.1±4.59
Triglyceride	87.8±4.59(mg/dl)	90.9±5.82
HDL	41.5±5.1(mg/dl)	42.5±4.85

Table 6.4: Effect of ethanolic extract of *Trema Orientalis* leaves on biochemical parameters



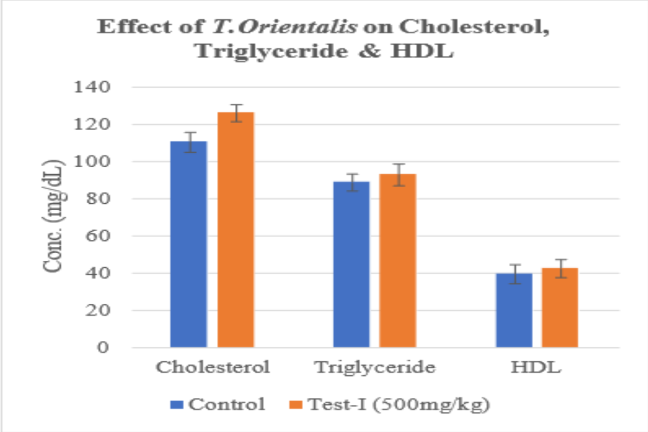


Fig 6.3: Effect of ethanolic extract of *Trema Orientalis* leaves on biochemical parameters

DISCUSSION

Test group mice were exposed to sub-acute toxicity for 14 days after receiving 500 mg/kg of extract. This research primarily aimed to monitor the effects of the extract on the biochemical markers and body weight of the mice. No side effects were noted for any of the assessed parameters after the group was given a dose of 500 mg/kg. This group does not vary significantly in terms of body weight from the control group.

In contrast to the control group, the experimental group showed no statistically significant changes in any of the blood parameters measured in the aforementioned research. So, It can be said that this plant is harmle

CONCLUSION

The present investigation demonstrates that the ethanolic extract of Trema orientalis leaves contains a wide range of bioactive phytochemicals, including alkaloids, flavonoids, tannins, glycosides, steroids, and terpenoids, which are known to contribute to various pharmacological activities. The absence of reducing sugars, saponins, proteins, and acidic compounds indicates a specific phytochemical profile that may be associated with its traditional medicinal uses.

The acute toxicity study showed that the extract is safe up to a dose of 3000 mg/kg body weight, as no mortality or visible signs of toxicity were observed in the treated mice. Furthermore, the sub-acute toxicity study conducted at 500 mg/kg for 14 days revealed no significant changes in body weight or key biochemical parameters related to liver and kidney function, including SGPT, SGOT, ALP, bilirubin, and creatinine. The lipid profile also remained within normal limits, indicating no adverse metabolic effects.

Overall, these findings confirm that the ethanolic extract of Trema orientalis leaves is non-toxic under both acute and short-term repeated administration in mice. The presence of multiple phytochemicals combined with a favorable safety profile supports the traditional use of this plant and suggests its potential for further pharmacological and therapeutic development. However, long-term toxicity and detailed mechanistic studies are recommended to establish its complete safety and efficacy.

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