

Evaluation of Hepatoprotective Activity of *Erycibe paniculata* Roxb. Leaf in Wistar Rats: Research Protocol for a Comparative Experimental Study

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ABSTRACT

Introduction: Liver disease is the cause of two million deaths annually. Herbal medicine gained popularity due to its effectiveness and fewer side-effects. About majority of the world's population relies on traditional medicine predominantly based on plant material. The species of genus *Erycibe* possesses hepato-protective activity, and *E. paniculata* has been used by ethnic groups for ailments of the liver. This could be adjuvant or alternative therapy for the management and avoidance of liver-related illnesses.

Need of the study: Given the high global and national burden of liver disease, the ethnomedicinal use and reported hepatoprotective potential of *Erycibe paniculata* necessitate its scientific validation as a safe, effective, and affordable herbal alternative.

Aim: To evaluate the hepatoprotective activity of *E. paniculata* Roxb. leaf in Wistar rats.

Materials and Methods: The experimental study will be conducted at Central Preclinical Research Facility, Datta Meghe College of Pharmacy, Sawangi (Meghe), Wardha, Maharashtra, India. The duration of the study will be three years, which will be from January 2025 and it will be completed on or before December 2028. The present study is an experimental

study in which an acute and sub-acute toxicity study as per (Organisation for Economic Co-operation and Development Test Guidelines) (OECD) guideline 423 and 407, respectively will be performed, followed by a hepatoprotective study. As mentioned below total number of animals will be 82, which will be further divided into acute toxicity study having two groups with 3 animals in each (6), sub-acute toxicity of four groups consisting of 10 animal each (40), 36 for hepatoprotective study consisting of six groups. Groups 1 to 6 will receive water, acetaminophen, silymarin, silymarin with acetaminophen, *Erycibe paniculata* leaf powder, *Erycibe paniculata* leaf powder with acetaminophen, respectively. All the groups will receive the drug orally, only acetaminophen will be given intraperitoneally. The assessment of hepatic damage will be evaluated based on the liver function markers like Aspartateaminotransferases (AST), Aminotransferases (ALT), Alkaline Phosphatase (ALP), Lactate dehydrogenase (LDH), Metabolic byproducts such as creatinine, bilirubin, cholesterol, triglycerides, urea, proteins and glucose and histopathological study. The comparison of biochemical parameters among different treatment groups by One-way Analysis of Variance (ANOVA) Tukey's Honest Significant Difference (HSD) as post-hoc and $p < 0.05$ will be considered significant.

Keywords: Acute toxicity, Drug-induced liver injury, Ethnopharmacology, Liver-protective effect, Scientific validation

INTRODUCTION

Hepatic disease is the cause of death approximately to million deaths every year due to cirrhosis, viral hepatitis and cancer. Worldwide death distribution is 4% of which is one out of every 25 deaths [1]. In females one out of three liver related deaths are observed. Liver illness comes in at number eleven most common cause of mortality and still it is being underestimated [2,3]. In this regard India alone contributed 18.3% of the global death due to liver diseases [4]. The herbal medication have demonstrated the capacity to uphold the typical normal state of the liver experiencing either less severe side-effects or not. Approximately 80% of the global people depends on classical medicine, primarily derived from plant sources [5,6].

Plants are the major component of traditional or folk and ethnomedicinal practice for healing in developing countries and also its plays an important part of history and culture. Medicinal plants are having opportunities to provide tremendous alternative remedies for various disorders. Traditional medicinal plant and their different parts are being used due to their medicinal values in rural areas which can be used to cure and prevent many human diseases. To provide safe and affordable primary healthcare to people the world is now recognising the importance of indigenous medical

practices. Therefore, World Health Organisation (WHO) has passed many resolutions in reaction to this resurgence of interest in the use, recognition and importance of medicinal plants to healthcare of people in any developing countries [7,8].

Liver diseases are now a concern in entire world as a matter of health concern despite tremendous breakthroughs in contemporary medicine therefore the need of search of novel drug is still underway. Presence of different phytoconstituents in medicinal plants is the reason for different pharmacological actions. Plant used in treating diseases is as old as civilisation and traditional medicine are still plays a major role in different maladies. In developing countries folk medicine is playing an important role where the healthcare services are limited. The lack of scientific validation and their use causes serious adverse effect [9].

Erycibe paniculata belong to convolvulace family is a large woody climber known as *Katapergu* in hindi is found in India and Andaman Islands in forest and waste lands [10]. Different etho-pharmacological activities of various part of *E. Paniculata* have been reported. Bark is used for cholera, root for post-delivery complications, leaves for night blindness, whole plant for diuretic and hypotensive, gum infection, bark decoction for malaria, diarrhoea and fever [11,12]. *Erycibe paniculata* is commonly known as *Khoil Khamar* in western

part of Odisha and the leaf is used in liver and Gastrointestinal (GI) disorder by some folk practitioners.

Vernacular Names of *E. paniculata*

Hindi: *Katapergu*, **Kannada:** *Ankola balli*, *Ankole* **Malayalam:** *Nakkuvalli*, *Irimpiyathali* **Tamil:** *Unamkodi*, *Unankodi* **Telugu:** *Putta palatige*, *Putta paalathige* **Odia:** *Khoil khamar*, *Chain Katho*, *Kari* etc.

Morphology: *Erycibe paniculata* is a large woody climber having dense young branches, leaves and inflorescence. Leaves size around 4 to 8 centimetres in long and 2 to 5 centimetres in wide. The shape is generally elliptic or elliptic ovate, with a round base, tip broadly tapering, leathery texture, lateral veins 5-6 pairs, stalk up to 1 cm long. Flowers are panicle cymes in leaf axils found at end part of branch. The fragrant flowers measures 6-8 mm in size, creamy-white. Flowers are bell-shaped, flat-faced, tube 1 to 1.5 millimetre in length, hairy outside, the petals are 5, measuring 2.5 to 3 mm in length. The ovary is one celled with four ovules, and there is no style present. The stigma is spherical. The berry measures 6-8×3-3.5 mm and either ovoid or ellipsoid in shape. It contains a single seed. One-celled ovary with four ovules; no style; spherical stigma. Berry: ovoid or ellipsoid, 6-8×3-3.5 mm, seed 1. The flowering and fruiting times is April to June.

Habitat: it is quite prevalent in an Odisha forest in Sri Lanka and neighbouring regions [13].

Chemical constituents: Alkaloids, Flavonoids, Steroids, Triterpenoids, Glycosides, Cardiac Glycosides, Tannins, Saponins, Anthraquinones, Proteins.

Medicinal uses: Stem bark paste is applied topically to the affected area in case of rickets [14]. The indigenous population of Purulia district in west Bengal utilises the plant's leaf and stem syrup orally to cure sprains in cattle [15]. The people residing in the Nawarangpur district of Odisha, India, have long relied on the traditional use of bark decoction to alleviate symptoms of fever and headache [16]. Indian state of Chhattisgarh folk people uses the extracts from young leaves to treat night blindness [17]. The administration of root extract is effective in treating fever. Consuming ripe fruits can help alleviate constipation, whereas the plant's aerial portion provided the extract exhibits diuretic properties [18]. The Indigenous population of Odisha utilises bark as an ethnomedicine to effectively treat cases of diarrhea [19]. *Erycibe paniculate* is used for hepatic disorders by folklore practitioner in Malkangiri area of Odisha, but till now it has not been documented. The other species i.e., *Erycibe expansa* is documented for its hepatoprotective activity, therefore *E. paniculata* may also have effect in liver disorders [20,21]. Hence, the objective of the study is scientifically validation of hepatoprotective activity in-vivo. This could be adjuvant or alternative therapy for the management and avoidance of liver-related illnesses.

MATERIALS AND METHODS

Aim: Evaluation of phytochemicals of *Erycibe paniculata* Roxb. Leaf and its hepatoprotective activity in-vivo.

Primary objective:

- To evaluate hepato-protective effect of *Erycibe paniculata* Roxb. Leaf powder in the selected animal model.

Secondary objective:

- Evaluate acute and sub-acute toxicity study as per OECD guideline.
- Collect *E. paniculata* Roxb. from its natural habitat and authentication from (Foundation for Revitalisation of Local Health Traditions) (FRLHT).
- Study the phytochemical profile of *Erycibe paniculata* Roxb. leaf.

- Study the pharmacognostic characteristics of the leaf like macroscopic, microscopic features, physicochemical constants and High-performance Thin-layer Chromatography (HPTLC).

Hypothesis

Research (H₁): *E. paniculata* have hepatoprotective effect.

Null (H₀): *E. paniculata* does not have hepatoprotective effect.

The experimental study is designed as a comparative controlled parallel group pre-clinical study on Wistar Rats. The study will be conducted at Central Preclinical Research Facility, Datta Meghe College of Pharmacy, Sawangi (Meghe), Wardha, Maharashtra, India Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Reg. Number-571/PO/ReRcBiBt/S/02/CPCSEA. The same study has been permitted by Institutional Animal Ethical Committee registration no: DMIHER/IAEC/2024-25/38. The committee is in charge of choosing the endpoint and keeping track of the study's development. The duration of the study will be three years, which will be from January 2025 and it will be completed on or before December 2028. In present, total 82 numbers of Wistar rats will be used.

Inclusion and Exclusion criteria: The inclusion criteria for the study will be healthy rats of both sexes from the Wistar strain with an average weight of 200 to 250 grams, while the exclusion criteria will be pregnant Wistar strain rats exhibiting indications of infection all along the study and those participating in another trial.

Study Procedure

Dose fixation: According to the formula developed by Paget and Barnes in 1964 [22], the human dose will be transformed into animal dosage by considering the body surface area ratio.

Suggested human dose of leaf powder (*e. paniculata*)=12 grams per day

Rat dose= 12×0.018 (conversion factor for a rat of 200 gram)
=0.216 gram or 1.08 gram/kg

The dose of individual rats will be derived based on the above calculation

For 1 gram body weight rat test drug dose is=0.183 gram

The present study proposed to take rats with a body weight ranging from 200-250 grams.

Selection of acetaminophen as liver toxicant: Acetaminophen is selected to induced hepatotoxicity, because it is one of the most widely accepted and reproducible models for studying liver injury in preclinical research. It reliably causes centrilobular necrosis in rodents, mimicking human liver damage.

Clinical relevance: Acetaminophen overdose is a leading cause of acute liver failure globally. Using it in experimental models ensures translational relevance to human health scenarios. Selection of silymarin as standard hepatoprotective drug due to comparability with standard drugs. It allows direct comparison with known hepatoprotective agents like silymarin, which are often tested against acetaminophen-induced damage

Dose control and safety: The hepatotoxic dose range in Wistar rats is well-established, enabling controlled induction of liver injury without excessive mortality, which is crucial for ethical and scientific integrity.

The animals will be anaesthetised and all required organs will be washed with normal saline and kept in 10% of formalin store the target organs.

The study group will be divided into acute [Table/Fig-1], sub-acute toxicity [Table/Fig-2], and hepatoprotective activity [Table/Fig-3]. The table represents the groups, sample size, dose, routes of drug administration, vehicle and duration.

Groups	No. of animals	Dose (<i>Erycibe paniculata</i> leaf powder)	Exposure (Day)	Duration of experiment
1 (Test)	3	Water as vehicle	Daily	14 days
2 (Confirmatory)	3	2000 mg/kg bw orally with water	Once, on the first day of the experiment	14 days

[Table/Fig-1]: Experimental design for acute-toxicity oral administration of *Erycibe paniculata* leaf powder.

Groups	No. of animals	Dose/kg Body weight (<i>Erycibe paniculata</i> leaf powder)	Exposure	Duration of experiment
1 (Control)	10 (5 males, 5 females)	Water as vehicle	Daily	28 days
2 (Low dose)	10 (5 males, 5 females)	600 mg/kg bw orally with water	Daily, once a day	28 days
3 (Medium dose)	10 (5 males, 5 females)	800 mg/kg bw orally with water	Daily, once a day	28 days
4 (High dose)	10 (5 males, 5 females)	1000 mg/kg bw orally with water	Daily, once a day	28 days

[Table/Fig-2]: Experimental design for subacute-toxicity oral administration of *Erycibe paniculata* leaf powder.

Groups	Normal control	Acetaminophen control group	Silymarin, control group	Silymarin+Acetaminophen treated group	<i>Erycibe paniculata</i> treated group	<i>Erycibe paniculata</i> leaf powder+Acetaminophen
No. of animals	6	6	6	6	6	6
Intervention	NA	Acetaminophen	Silymarin	Silymarin+Acetaminophen	Leaf powder	Leaf powder and acetaminophen
Vehicle	Distilled water	Normal saline water	Distilled water	Distilled water and normal saline water	Distilled water	Distilled water and normal saline water
Dose of group-specific drugs	1 mL/kg bw	600 mg/kg bw	50 mg/kg bw	50 mg/kg silymarin+Acetaminophen 600 mg/kg bw	1.08 gram/kg	1.08 gram/kg+Acetaminophen 600 mg/kg bw
Route	Oral	Intraperitoneal	Oral	Oral and intraperitoneal	Oral	Oral and Intraperitoneal
Duration	14 days	Single IP injection on 14 th day	14 days	Silymarin administered daily for 14 consecutive days+single IP injection on 14 th day	14 days	<i>E. paniculata</i> leaf powder administered daily for 14 consecutive days+Single IP injection of acetaminophen on 14 th day

[Table/Fig-3]: Experimental grouping and treatment protocol for evaluating the hepatoprotective potential of *Erycibe paniculata* leaf powder against acetaminophen-induced hepatotoxicity in Wistar rats.

The study will be conducted in different phases. The first phase will involve collecting, authenticating, and analysing the trial drug. Then, the toxicity and hepatoprotective study will be conducted. Primarily, the field sample collection of *Erycibe paniculata* leaf will be collected from the Odisha region. The authentication of the raw material will be done by the Foundation for Revitalisation of Local Health Traditions (FRLHT), Bengaluru. The preparation will begin with the collection of leaves, which will be washed with tap water and then shade-dried. After completion of the drying process, it will be subjected to fine powder and stored until use. In Dattatray Rasashala's analytical laboratory at Mahatma Gandhi Ayurveda College and Research Centre (MGACH and RC), Salod, Wardha, the preparation will be standardised in accordance with quality standards.

The raw and powdered leaves of *E. paniculata* will be standardised as part of the analytical analysis. A thorough pharmacognostic study (including Panchbhutik pariksha, organoleptic characters, macroscopy, and microscopy), Preliminary phytochemical analysis at a reputable pharmacognosy laboratory, physico-chemical parameter analysis for *E. paniculata* leaf powder, and an organoleptic character assessment (touch, sight, taste, and smell) will all be part of this.

Loss on Drying (LOD) at 105°C, Ash value, acid soluble/insoluble ash, water soluble/insoluble ash, and High-performance Thin Layer Chromatography (HPTLC) are among the physico-chemical parameters. Methanol-formic acid buffer (20:80 v/v and 30:70 v/v) will be the reagents and mobile phases utilised for HPTLC [23].

The experimental investigation will use 46 and 36 Wistar rats of either sex, weighing between 200 and 250 grams, for the toxicity and hepatoprotective studies, respectively, in accordance with OECD recommendations.

Using a conversion factor, the clinical human dose of the powder made from *E. paniculata* leaves will be extrapolated to an animal dose based on the body surface area ratio for the experimental investigation. Prior to the trial, the animals will be acclimated for 14 days and given unlimited access to food and distilled water.

Primary Outcomes

- Hepatoprotective activity:** It is assumed that acetaminophen administration will induce hepatotoxicity, evidenced by elevated serum levels of ALP, ALT, LDH, AST, and bilirubin. Subsequent treatment with the test drug is expected to significantly reduce these elevations toward baseline. Meanwhile, metabolic parameters- creatinine, cholesterol, triglycerides, urea, total proteins, and glucose- will be assessed and interpretation will be done as per the report.
- Histopathological analysis:** Normal cytoarchitecture is expected as it will show the effect of test drug against the induction of liver toxicant.

Secondary Outcomes

- Preliminary phytochemical profile of *Erycibe paniculata* Roxb. by chemicals tests.
- Different pharmacognostic characteristics of the leaf.
- Evaluation of any toxicity, both acute and subacute of *Erycibe paniculata* Roxb. will be evaluated.

STATISTICAL ANALYSIS

Results will be analysed by statistical method One-way ANOVA test with Tukey's HSD as post-hoc $p < 0.05$ will be considered as significant.

Timeline of the study shown in [Table/Fig-4].

Activity	Q 1	Q 2	Q 3	Q 4	Q 5	Q 6	Q 7	Q 8	Q 9	Q 10	Q 11	Q 12
Registration/approval from IAEC												
Literature review												
Collection, preparation and validation of study material and												

Experimental study												
Toxicity study												
Hepatoprotective activity												
Result, discussion, and thesis writing												
Submission of thesis												
Presentation of thesis												

[Table/Fig-4]: Gantt diagram of the research protocol.
IAEC: Institutional animal ethics committee

Dissemination: To disseminate the hepatoprotective research, this protocol will be published as a thesis. The study design, methods, analysis plan, and ethical issues are all thoroughly outlined in the study protocol. The authors hope that by sharing their research, they will help to expand understanding of this area.

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