Acute Oral Toxicity Study of Hydroalcoholic Extract of *Acalypha malabarica* in Albino Wistar Rats

Dhanya P[1]*, Rajasekaran S[2]

[1]Department of Pharmacology, PhD Scholar, Faculty of Pharmacy, Bhagwant University, Ajmer, Rajasthan, India

[2]Department of Pharmacology, Al-Azhar College of Pharmacy, Thodupuzha, Idukki, Kerala, India

*Correspondence:dhanyanidheeshp@gmail.com

Abstract

Background:*Acalypha malabarica* Müll. Arg. is a medicinal plant traditionally used in Indian systems of medicine for the treatment of various ailments, including inflammation and spasmodic disorders. Despite its therapeutic potential, scientific evidence regarding its safety profile is limited. Acute oral toxicity studies are essential for establishing the safety of herbal extracts and determining suitable dose ranges for future pharmacological investigations.**Aim:** To evaluate the acute oral toxicity of the hydroalcoholic extract of *Acalypha malabarica* in female Albino Wistar rats according to OECD Guideline 423.**Methodology:** A hydroalcoholic extract of *Acalypha malabarica* was administered orally as a single dose of 2000 mg/kg body weight to six female Albino Wistar rats. Animals were observed for 14 days for mortality, clinical signs of toxicity, behavioral and neurological changes, and body weight variations. Hematological parameters, including red blood cell (RBC) and white blood cell (WBC) counts, were assessed on Day 14 to determine any toxicological effects.**Results:** No mortality or treatment-related adverse effects were observed throughout the study period. All animals maintained normal respiratory patterns, locomotor activity, reflexes, grooming behavior, and gastrointestinal function. Hematological evaluation showed RBC and WBC counts of $7.5 \times 10^6/mm^3$ and $7.0 \times 10^3/mm^3$, respectively, which were within normal physiological ranges. Body weight increased steadily in all animals from Day 0 to Day 14, indicating normal growth and the absence of systemic toxicity or growth suppression.

Conclusion: The hydroalcoholic extract of *Acalypha malabarica* was found to be safe at an acute oral dose of 2000 mg/kg in female Albino Wistar rats. The absence of mortality, clinical

abnormalities, hematological alterations, and adverse effects on body weight suggests a low toxicity profile. Based on OECD Guideline 423, the extract may be considered relatively non-toxic, with an estimated oral LD_{50} greater than 2000 mg/kg. These findings support further sub-acute toxicity and pharmacological studies to validate its therapeutic potential.

Keywords:*Acalypha malabarica*, acute oral toxicity, hydroalcoholic extract, OECD 423, Wistar rats, safety evaluation.

1. Introduction

Medicinal plants continue to play a significant role in healthcare systems worldwide, particularly in developing countries where traditional remedies remain an important source of primary healthcare. The World Health Organization (WHO) estimates that nearly 80% of the global population relies on herbal medicines for at least part of their healthcare needs. Despite their widespread use, scientific validation of the safety and efficacy of medicinal plants remains essential before their incorporation into modern therapeutic practices (1,2).*Acalypha malabarica* Müll. Arg., belonging to the family Euphorbiaceae, is an ethnomedicinal herb distributed throughout the Western Ghats and other tropical regions of India. The plant has been traditionally used for the management of inflammatory disorders, gastrointestinal disturbances, skin diseases, fever, and urinary ailments (3,4). Phytochemical investigations of the genus *Acalypha* have revealed the presence of flavonoids, phenolic compounds, tannins, alkaloids, saponins, and glycosides, which are known to possess diverse biological activities including antioxidant, anti-inflammatory, antimicrobial, and wound-healing effects (5,6).Previous pharmacological studies on *Acalypha* species have demonstrated significant therapeutic potential. Extracts of *Acalypha indica* and related species have shown anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, and antidiabetic activities in experimental models (7–9). Preliminary phytochemical studies on *A. malabarica* have reported the presence of bioactive secondary metabolites that may contribute to its traditional medicinal uses (10). However, despite the increasing interest in the pharmacological properties of *A. malabarica*, limited information is available regarding its toxicological profile, particularly following oral administration. The absence of adequate safety data represents a major limitation for its future development as a therapeutic agent.Acute oral toxicity studies constitute an important component of preclinical safety evaluation. These studies provide information regarding the potential toxic effects of a substance following a single exposure and help establish dose ranges for subsequent

pharmacological and repeated-dose toxicity studies (11). The Organisation for Economic Co-operation and Development (OECD) Guideline 423 recommends the Acute Toxic Class Method as a reliable and internationally accepted approach for assessing acute oral toxicity while minimizing animal use (12).The present investigation was based on the hypothesis that the hydroalcoholic extract of *Acalypha malabarica* possesses a favorable safety profile and does not produce significant toxic effects when administered orally at the limit dose recommended by OECD Guideline 423. Demonstration of its safety would support the traditional use of the plant and facilitate future pharmacological and therapeutic investigations.Therefore, the objective of the present study was to evaluate the acute oral toxicity of the hydroalcoholic extract of *Acalypha malabarica* in female Albino Wistar rats following OECD Guideline 423. The study aimed to assess mortality, clinical signs of toxicity, behavioral and neurological responses, hematological parameters, and body weight changes after administration of a single oral dose of 2000 mg/kg body weight. The findings are expected to provide preliminary safety data and establish a scientific basis for future sub-acute toxicity and pharmacological efficacy studies involving this medicinal plant.

2. Materials and Methods

2.1. Preparation of Hydroalcoholic Extract

The aerial parts of *Acalypha malabarica* Müll. Arg. were collected, authenticated, shade-dried, and pulverized into coarse powder. The powdered material was extracted using a hydroalcoholic solvent system consisting of 70% ethanol and 30% water. The extract was filtered and concentrated under reduced pressure using a rotary evaporator, followed by drying to obtain a semisolid mass. The dried extract was stored in airtight containers at 4°C until further use. Prior to administration, the extract was reconstituted in distilled water to obtain the required concentration for oral dosing (13,14).

2.2. Experimental Animals

Healthy female Albino Wistar rats weighing 20–25 g were procured from the Animal House Facility of Devaki Amma Memorial College of Pharmacy, Malappuram, Kerala, India. The animals were acclimatized for seven days under standard laboratory conditions, maintained at a temperature of 22 ± 2°C, relative humidity of 50–60%, and a 12 h light/dark cycle. Standard pellet diet and drinking water were provided ad libitum except during the fasting period before

dosing (15,16). All experimental procedures were conducted in accordance with OECD Guideline 423 and institutional ethical requirements (17).

2.3. Acute Oral Toxicity Study Design

The acute oral toxicity study was performed according to the Organisation for Economic Co-operation and Development (OECD) Guideline 423, Acute Toxic Class Method (17). A total of six female Wistar rats were used in a stepwise dosing procedure consisting of two sequential groups of three animals each. The hydroalcoholic extract of *A. malabarica* was administered orally at a limit dose of 2000 mg/kg body weight. No separate control group was included, as recommended by the OECD acute toxic class method (17).

2.4. Dose Administration

Animals were fasted overnight (12–16 h) before administration of the test substance, while water was supplied ad libitum. The extract was administered as a single oral dose of 2000 mg/kg body weight by gastric gavage at a dosing volume of 10 mL/kg body weight. Following treatment, food was withheld for an additional 3–4 h and subsequently restored (17,18).

2.5. Clinical and Behavioral Observations

Following administration of the test extract, animals were observed individually during the first 30 min, periodically during the first 4 h, and thereafter at regular intervals during the first 24 h. Daily observations were continued for 14 consecutive days as recommended by OECD Guideline 423 (17). Parameters evaluated included mortality, changes in skin and fur, eyes and mucous membranes, respiratory pattern, salivation, tremors, convulsions, locomotor activity, posture, autonomic responses, behavioral changes, and neurological reflexes. Body weights were recorded on Day 0 and subsequently monitored throughout the 14-day observation period (17,19).

2.6. Hematological Evaluation

At the end of the observation period (Day 14), blood samples were collected under mild anesthesia through retro-orbital plexus puncture. Hematological parameters including red blood cell (RBC) count and white blood cell (WBC) count were determined using an automated hematology analyzer. The obtained values were compared with normal physiological ranges reported for Wistar rats to assess any treatment-related hematological alterations (20,21).

2.7. Statistical Analysis

Body weight and hematological data were expressed as mean ± standard deviation (SD). Descriptive statistical analysis was performed using Microsoft Excel software. Clinical observations and mortality data were recorded and reported qualitatively according to OECD Guideline 423 recommendations (17).

3. Results

3.1. Clinical Signs and Behavioral Observations

The acute oral administration of the hydroalcoholic extract of *Acalypha malabarica* at a dose of 2000 mg/kg body weight did not produce any mortality during the 14-day observation period. All animals survived until the scheduled termination of the study. No treatment-related clinical signs of toxicity were observed at any time point following administration. The treated rats exhibited normal respiratory patterns, locomotor activity, grooming behavior, posture, and alertness throughout the study period.

Furthermore, no abnormalities were detected in neurological and reflex responses, including corneal and righting reflexes. Ocular appearance, skin condition, fur texture, and mucous membranes remained normal. No gastrointestinal disturbances such as diarrhea, salivation, or reduced feed and water intake were observed. The clinical and behavioral observations are summarized in Table 1.

Table 1. Clinical and behavioral observations in female Albino Wistar rats following acute oral administration of hydroalcoholic extract of *Acalypha malabarica* (2000 mg/kg).

Parameter	Observation
Mortality	0/6[1]
Respiratory pattern	Normal[1]
Locomotion	Normal[1]
Reflexes (corneal, righting)	Normal[1]
Ocular signs	Normal[1]
Gastrointestinal signs	Normal[1]
Mortality	0/6[1]
Respiratory pattern	Normal[1]

3.2. Hematological Parameters

The hematological parameters evaluated on Day 14 are presented in Table 2. The mean red blood cell (RBC) count and white blood cell (WBC) count were found to be $7.5 \times 10^6/mm^3$ and $7.0 \times$

$10^3/mm^3$, respectively. These values were within the normal physiological ranges reported for healthy Wistar rats. No evidence of hematological abnormalities or treatment-related alterations was observed following administration of the extract.

Table 2. Hematological parameters of female Albino Wistar rats treated with hydroalcoholic extract of *Acalypha malabarica* (2000 mg/kg).

Parameter	Observation (Mean)
RBC count	$7.5 \times 10^6/mm^{31}$
WBC count	$7 \times 10^3/mm^{31}$

3.3. Body Weight Changes

The body weight data of female Albino Wistar rats treated with the hydroalcoholic extract of *Acalypha malabarica* (2000 mg/kg) are presented in **Table 3**. A gradual and continuous increase in body weight was observed in all animals throughout the 14-day observation period. The initial body weights ranged from 22.13 to 23.35 g on Day 0, while the final body weights ranged from 23.57 to 24.93 g on Day 14.All treated animals exhibited normal growth patterns without any reduction in body weight or abnormal fluctuations during the study period. The percentage increase in body weight ranged from approximately 6.5% to 7.9% among the animals. No evidence of growth suppression, wasting, or treatment-related adverse effects on body weight was observed.

The progressive increase in body weight throughout the study indicates that the hydroalcoholic extract of *Acalypha malabarica* was well tolerated at the administered dose of 2000 mg/kg and did not adversely affect the general health status of the animals (Table 3).

Table 3. Body weight (g) of female Albino Wistar rats following acute oral administration of hydroalcoholic extract of *Acalypha malabarica* (2000 mg/kg).

Day	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Animal 6
0	23.12	22.13	23.35	23.16	23.33	23.11
7	23.89	22.92	23.99	23.81	24.02	24.15
14	24.56	23.57	24.68	24.59	24.78	24.93

Overall, no mortality, clinical abnormalities, behavioral changes, hematological alterations, or adverse effects on body weight were observed in rats treated with the hydroalcoholic extract of *Acalypha malabarica* at the limit dose of 2000 mg/kg body weight.

4. Discussion: Acute toxicity studies are an essential component of preclinical safety assessment and provide preliminary information regarding the toxic potential of medicinal plant extracts following single-dose exposure. In the present study, the hydroalcoholic extract of *Acalypha malabarica* was evaluated for acute oral toxicity in female Albino Wistar rats according to OECD Guideline 423. The absence of mortality at the limit dose of 2000 mg/kg suggests that the extract possesses a relatively low level of acute toxicity and may be categorized as having an oral LD_{50} greater than 2000 mg/kg (22,23).Clinical and behavioral observations are considered sensitive indicators of systemic toxicity. Toxic substances frequently induce alterations in autonomic, neurological, respiratory, and gastrointestinal functions, which may manifest as changes in locomotion, posture, grooming behavior, reflex activity, salivation, or respiratory distress (24). In the present investigation, no treatment-related abnormalities were observed in any of these parameters throughout the 14-day observation period. The maintenance of normal alertness, locomotor activity, corneal and righting reflexes, respiratory pattern, and gastrointestinal function indicates that the extract did not exert adverse effects on the central nervous system, autonomic nervous system, or other vital physiological functions (25).Hematological parameters serve as important biomarkers for evaluating the toxic effects of xenobiotics because the hematopoietic system is highly sensitive to toxic insults (26). Alterations in red blood cell (RBC) and white blood cell (WBC) counts may indicate anemia, inflammatory responses, immunotoxicity, or bone marrow suppression (27). In the present study, the RBC count ($7.5 \times 10^6/mm^3$) and WBC count ($7.0 \times 10^3/mm^3$) remained within the normal physiological ranges reported for healthy Wistar rats. These findings suggest that the hydroalcoholic extract did not induce hematotoxicity or inflammatory stress following acute exposure (28).Body weight is a widely accepted indicator of general health status and systemic toxicity in experimental animals. Significant reductions in body weight or impaired weight gain are commonly associated with toxic effects, altered metabolism, or reduced feed consumption (29). The treated animals exhibited a progressive increase in body weight throughout the study period, with percentage gains ranging from approximately 6.5% to 7.9%. The absence of weight loss or growth suppression indicates that the extract did not adversely affect nutrient utilization, metabolic processes, or overall physiological well-being (30).The favorable safety profile observed in the present study may be attributed to the presence of naturally occurring phytoconstituents such as flavonoids, phenolic compounds, tannins, and glycosides, which are

generally regarded as possessing low toxicity when administered within therapeutic limits (31). Similar findings have been reported for several medicinal plants belonging to the family Euphorbiaceae, where hydroalcoholic extracts demonstrated minimal acute toxicity and good tolerability in experimental animals (32).Overall, the absence of mortality, clinical signs of toxicity, hematological abnormalities, and adverse effects on body weight demonstrates that the hydroalcoholic extract of *Acalypha malabarica* is well tolerated following acute oral administration at 2000 mg/kg body weight. These findings support its traditional use and provide a scientific basis for future sub-acute, chronic toxicity, and pharmacological efficacy studies.

5. Conclusion

The present study demonstrated that the hydroalcoholic extract of *Acalypha malabarica* was well tolerated following acute oral administration at a dose of 2000 mg/kg body weight in female Albino Wistar rats. No mortality, treatment-related clinical signs of toxicity, behavioral abnormalities, hematological alterations, or adverse effects on body weight were observed during the 14-day observation period. The maintenance of normal physiological and hematological parameters indicates the absence of acute systemic toxicity and supports the safety of the extract under the conditions of the study.According to OECD Guideline 423, the absence of mortality at the limit dose of 2000 mg/kg suggests that the median lethal dose (LD_{50}) of the hydroalcoholic extract is likely to be greater than 2000 mg/kg, indicating a low level of acute oral toxicity. These findings provide scientific support for the traditional use of *Acalypha malabarica* and establish a preliminary safety profile for its future therapeutic development. However, further investigations involving sub-acute, sub-chronic, chronic toxicity, histopathological evaluation, and pharmacological efficacy studies are necessary to comprehensively establish its safety and therapeutic potential.

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