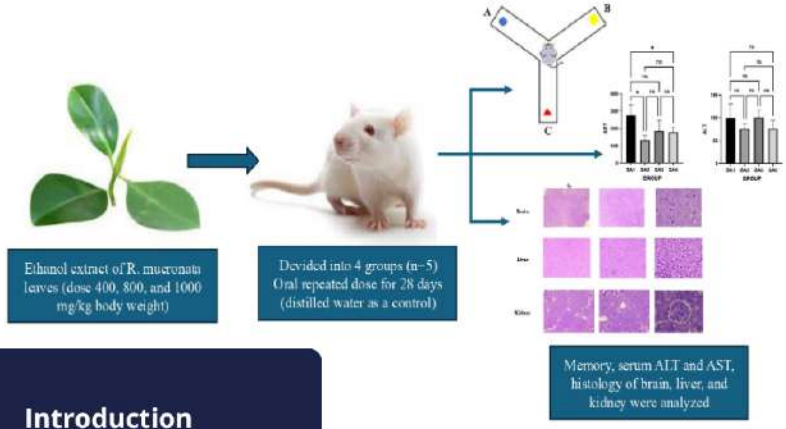
Toxicological and Neurobehavioral Assessment of Rhizophora mucronata Leaf Extract in Rats: Implications for Safe Herbal Use



Introduction

- *Rhizophora mucronata* is the most abundant mangrove species in West Nusa Tenggara, Indonesia
- Rich source of bioactive compounds with significant potential for drug development, offering 10% higher pharmacological activity compared to terrestrial sources
- Historically used in traditional medicine, its leaf extract has been employed to manage diarrhoea and reduce blood sugar levels
- Recent findings highlight its potential in addressing challenging diseases like Alzheimer's (including antioxidant, anti-apoptotic, and anti-Aβ oligomer properties)
- Research on its toxicity remains limited, specifically in terms of its impact on various organ systems
- This gap is important for developing countries, where traditional medicine forms the cornerstone of healthcare, serving nearly 80% of the population

Research Objectives

- To examine potential toxicity of *R.mucronata* leaf extract on various organ systems as recommended by OECD guideline number 407 for oral toxicity test
- To investigate the impact of *R.mucronata* leaf extract on memory function in male Sprague Dawley rats, to provides data for its further exploration as potential anti-Alzheimer's therapy

Method

Animal Care and Ethics

20 Sprague-Dawley male rats (160–190 grams, 8 weeks old), healthy, without physical defects were divided into 4 groups (n=5). The rats were maintained on a standard diet with unrestricted access of water. Ethical clearance number 087/2024.

Extract Preparation

Sonication-assisted maceration at 35°C for 30 minutes with ethanol 96% solvent 1:5 powder-to-solvent ratio was used to extract the leaf fine powders. The thick ethanol extract (hereinafter referred to as EERML) was obtained at 45°C with a rotary evaporator.

Dosage and Administration

Each group were administered daily via an oral probe for 28 days for different treatment; control group with saline solution 1mL/day (SA1) and EERML group with doses 400,800,and 1000 mg/KgBW (SA2, SA3, SA4 respectively).

Toxicological Evaluation

Potential toxicity of the extract was evaluated through serum aminotransferase (ALT and AST) levels and histological assesment of liver, kidney, and brain

Neurobehavioural Assesment

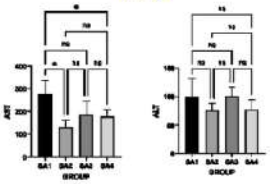
The Y-maze spontaneous alternation method was used to evaluate spatial working memory. the whole procedures was adapted from the Stanford Behavioral and Functional Neuroscience Laboratory and modified by Kraeuter et al. (2018).

Data Analysis

Statistical analysis was conducted using Prism Software (GraphPad 10.4.1, San Diego, CA) and SPSS 29

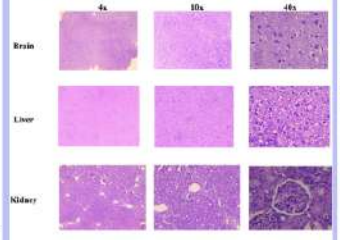
Result & Discussion

Comparison of Serum AST & ALT levels

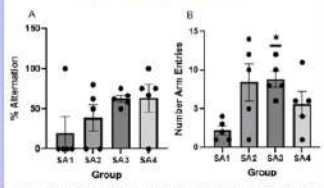


Data represent the mean ±SD of each group (n=5). Significance is indicated by *p<0,05, ns indicated non-significant. SA1, Negative control; SA2, SA3, SA4, EERML dose 400, 800, and 1000 mg/kg body weight, respectively.

Histopathological Imaging of Several Organs After Treatment



Spatial Memory and Exploratory Behavior



Data comparing the percentage alternation (A) and the average number of arm entries (B). The data represent the mean±SEM of each group (n=5). Significance is indicated by *p<0.05 versus SA1 (Negative control). SA2, SA3, SA4, EERML dose 400, 800, and 1000 mg/kg body weight, respectively.

AST and ALT Levels Indicate No Hepatic Injury

- SA2 (p = 0.0113) and SA4 (p = 0.0102) showed a significantly lower AST level compared to control group
- There is no significant differences of ALT level between groups, but SA2 and SA4 posses the lowest level

Microscopic Evidence of Organ Safety

Tissue	Cell Type	Nuclei	Chromatin	Cytoplasm	Mitosis	Architecture	Inflammation	Other
Liver	Hepatocytes	Round-oval	Fine	Extensive	(–)	Normal hepatic sinusoids	Minimal lymphocytes + histiocytes	N/C ratio normal
Kidney	Tubules + Glomeruli	Round-oval	Fine	Sufficient	(–)	No atrophy, no sclerosis	Minimal lymphocytes + histiocytes	Normal stroma
Brain	Astrocytes	Round-oval	Fine	Sufficient	(–)	No necrosis, no hemorrhage	Minimal lymphocytes + histiocytes	Non-edematous stroma

Conclusion

- The ethanol extract of *R.mucronata* leaf has no potential toxicity at any dose given
- The extract also exhibits a dose-dependent memory-enhancing effect, with the optimum efficacy observed at a dose of 800 mg/KgBW
- Further investigation is needed to confirm its potential anti-Alzheimer's effects

Prospective Anti-Alzheimer's Therapy

- An increase in dose corresponded with a higher percentage of alternation, suggesting dose-dependent enhancement of spatial memory
- SA3 significantly increased arm entries, indicating enhanced exploration, possibly reflecting improved motivation, working memory, or attention via dopaminergic and cholinergic pathways
- A prospective study suggests low plasma aminotransferase, particularly ALT, is linked to higher long-term dementia risk. No significant difference indicates no risk of dementia due to EERML administrations

Legis Ocktaviana Saputri[1], Lina Permatasari[1], Arina Windri Rivarti[1], Lale Maulin Prihatina[1], Rohadi[1], Al Hafiez Ariouso[1], Rafi Abdika 'Afwa[1], Ye Muhamad Aprizal[2], I Made Jawi[3], Herpan Syafii Harahap[1*]

1 Faculty of Medicine and Health Sciences, University of Mataram, 83125, Indonesia
2 Universiti Teknologi MARA, 40450, Malaysia
3 Faculty of Medicine, Udayana University, 80234, Indonesia

References