OPEN ACCESS
Journal of Multidisciplinary Applied Natural Science Vol. 5 No. 1 (2025) https://doi.org/10.47352/jmans.2774-3047.247
Check for updates
Research Article

Acute Toxicity Study of the Leaf and Fruit Extracts of *Avicennia marina* (Forssk.) on Wistar White Male Mice

Duryat Duryat*, Rodiani Rodiani, and Tri Maryono

Received : May 17, 2024	Revised : November 17, 2024	Accepted : November 20, 2024	Online : January 31, 2025

Abstract
Avicennia marina has at least 36 types of flavonoid compounds that are potential anti-fertility agents. The study's objectives were to obtain safe doses and assess the potential risks of using *A. marina* extract. Toxicity testing was conducted on Wistar rats with doses of 0, 250, 500, 1000, 2000, and 4000 mg/kg BW for leaf and fruit extracts. Observations were made to measure the level of mortality and damage to important organs (liver, kidneys, and testes) both macroscopically and microscopically. Macroscopic observation included identifying changes in shape, color, and size. Microscopic observation was done to observe organ damage through histopathological tests. Results of the study show that the administration of *A. marina* extract, both leaf and fruit, resulted in a 100 % survival rate at all doses given and the LD_{50} >8 g/kg BW. Both leaf and fruit extracts of *A. marina* also did not cause a decrease in the size of the kidneys and testes, but at high doses, they potentially reduced liver size. These findings indicate that using *A. marina* extract at recommended doses is safe. Both leaf and fruit extracts of *A. marina*, at a dose of 250 mg/kg BW, did not cause negative effects on the major organs (liver, kidneys, and testes) of mice. This finding suggests that using *A. marina* extract at 250 mg/kg BW is safe for long-term use. Administration of *A. marina* extract at doses up to 500 mg/kg BW did not cause liver damage in mice, but it potentially caused mild kidney damage. This finding indicates that leaf and fruit extracts of *A. marina* still have the potential to be used as drug candidates but with dosage regulation below 500 mg/kg BW. Administration of leaf and fruit extracts at doses of 500, 1000, and 2000 mg/kg BW has been shown to reduce the fertility of mouse sperm cells by up to 30%. This finding indicates that *A. marina* has the potential to be a promising, safe herbal anti-fertility agent.

Keywords: *Avicennia marina*, toxicity test, LD_{50}, anti-fertility, histopathology, herbal medicine

1. INTRODUCTION

As a pioneering mangrove species, *Avicennia marina* thrives in challenging environments, tolerates high salinity, survives in extreme temperatures, prolonges flooding and limited nutrients, which drive essential physiological and biochemical adaptations for survival [1]. These adaptations include the synthesis of secondary metabolites that not only support the plant in enduring these stresses but also exhibit numerous bioactive properties with potential health benefits for humans [2]. *A. marina* contains diverse bioactive compounds, including steroids, naphthalenes, terpenoids, flavonoids, phenylpropanoid glycosides, diterpenoid glucosides, and iridoid glucosides, which have demonstrated therapeutic effects such as anti-microbial, anti-oxidant, anti-aging, anti-inflammatory, anti-tumor, anti-tuberculosis, and anti-atherosclerotic activities [3]-[5]. Particularly noteworthy are the anti-fertility properties of flavonoids, extensively studied as potential male contraceptive agents [6].

Flavonoids have been shown to disrupt spermatogenesis by inducing degeneration in the seminiferous tubules and regression of Leydig cells, leading to sperm production abnormalities [7]. These compounds act as anti-fertility agents through both hormonal and cytotoxic mechanisms, primarily by generating reactive oxygen species (ROS) that damage sperm cell membranes, contributing to male infertility [8]. ROS accumulation also reduces steroid hormone activity, which is crucial for spermatogenesis [9]. Flavonoids stimulate the production of anti-fertility enzymes, such as L-aspartate dehydrogenase and trans-hexaprenyltranstransferase, which inhibit key pathways in steroid biosynthesis, making them potential targets for anti-fertility drugs [10]. Additionally, flavonoids can induce teratogenic effects by causing DNA fragmentation in somatic cells [11].

Rodiani et al. reported that *A. marina* harbors at least 36 distinct flavonoid types across various organs, with 13, 21, 13, 14, and 8 types identified in the roots, fruits, leaves, woods, and barks,

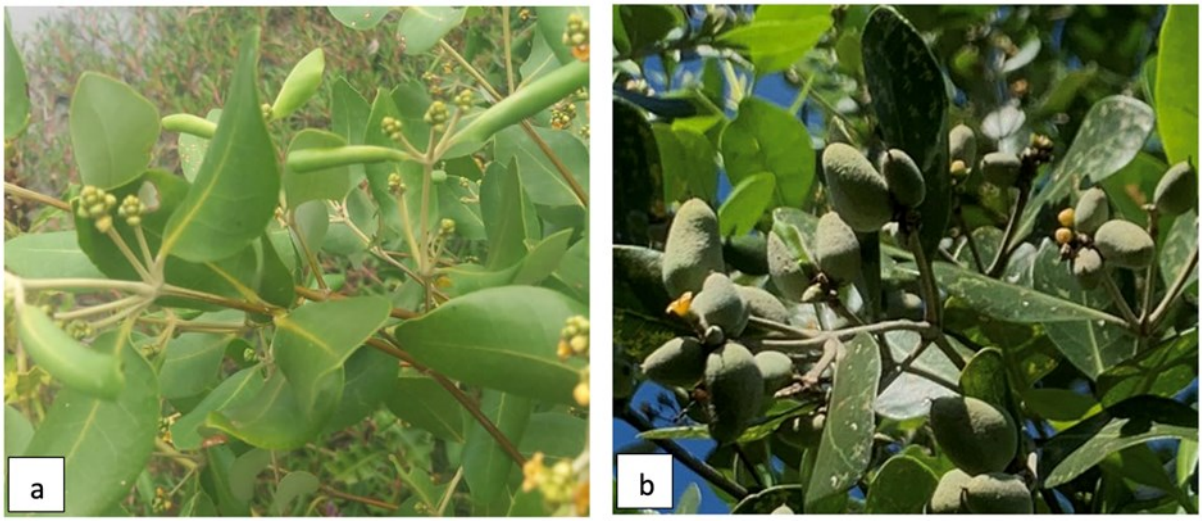


Figure 1. The photograph of *Avicennia marina* (a) leaves and (b) fruits.

respectively [6]. Notably, the fruit exhibits a rich composition of flavonoids and essential vitamins, minerals, and anti-oxidants, highlighting its therapeutic potential. Leaves, though containing fewer flavonoids than fruits and wood, possess unique bioactive compounds and offer practical advantages due to their ease of harvest and year-round availability, making them primary candidates for medicinal applications [12][13]. Furthermore, phytochemical leaf extraction is simpler than other plant organs [14]. The minimal impact of harvesting leaves and fruits on plant survival further supports their utilization. Consequently, this study investigates the medicinal potential of *A. marina* fruit and leaf.

A. marina, with its high flavonoid content, holds significant potential as an affordable, accessible anti-fertility agent for underserved coastal communities with limited access to basic medicines and health facilities [6]. However, toxicity studies are essential to ensure the safety of these herbal preparations before they can be widely used in medicine. Even for remedies with a long history of traditional use, health safety should be confirmed with rigorous scientific evidence. Insights into traditional plants often guide the design of experimental toxicity studies [14]. Ensuring the extract's safety requires confirming the absence of toxic compounds, as toxicity in medicinal plants and herbal products often arises from certain bioactive compounds [15]. Pre-clinical toxicology studies are mandatory in the pharmaceutical industry, providing evidence of a drug's safety profile and identifying potential toxic effects on specific organs [16][17].

Toxicity studies are typically performed on animal models and extrapolated to human health, although some qualitative and quantitative differences may apply [18]. Previous research has examined the toxicity of *A. marina* extracts in various settings—such as freshwater shrimp media, where LC_{50} values for ethyl acetate and methanol extracts were 454 and 740 µg/mL, respectively [19], and acute toxicity tests on rats, which indicated that water extracts of *A. marina* fruit are non-toxic and non-mutagenic, key toxicity data remain unexplored [20]. Notably, the toxicological profile of *A. marina* leaf extract on human gingival fibroblast cells has shown cytotoxic effects, suggesting that it may not be suitable as a mouthwash ingredient [21].

The plant belongs to the Acanthaceae family and is a pioneering mangrove species widely distributed in tropical and subtropical coastal regions [22]. This species thrives in intertidal habitats with high salinity, intense solar radiation, and extreme anaerobic conditions, necessitating specific physiological and biochemical adaptations for survival [23]. Morphologically, *A. marina* features thick, waxy-coated leaves with small stomata and a grayish-white undersurface, which helps minimize water loss through transpiration [24]. Its extensive aerial root system, known as pneumatophores, assists in overcoming oxygen deficiency and stabilizes the plant in the unstable muddy substrate, allowing for its survival in waterlogged soils [23]. Physiologically, *A. marina* possesses salt-excreting

glands on its leaves that help it manage high salinity conditions, alongside the production of various enzymes that facilitate photosynthesis under saline stress. Additionally, it demonstrates a remarkable ability to handle osmotic stress, endure anaerobic conditions, and tolerate drastic fluctuations in tidal movements [25]. Moreover, *A. marina* is known for producing bioactive secondary metabolites, contributing to its medicinal potential and ecological competitiveness [6].

However, no studies have yet assessed the acute toxicity of ethanol 96% extracted from *A. marina* leaf and fruit in Wistar rat models. This study seeks to address this gap by investigating the acute toxicity of these extracts in rats, aiming to determine safe dosage levels and identify any potential risks associated with the medicinal use of *A. marina*. This research is vital for establishing a scientific basis for the safe and effective therapeutic use of *A. marina*, contributing novel insights into its pharmacological potential.

2. MATERIALS AND METHODS

2.1. Chemical and Materials

The chemicals and materials used in this study included 96% ethanol, fresh leaves and fruits of *A. marina*, male *Mus musculus* rats (strain DYY), paraffin embedding equipment, and staining reagents, specifically hematoxylin and eosin (H&E), for histopathological analysis.

2.2. Preparation of Leaf and Fruit Samples of A. marina

The leaves and fruits of *A. marina* were collected from the Ketapang District in South Lampung Regency. The selected samples must meet specific criteria, including being intact, free from pest or disease damage, mature (neither too young nor too old), and vibrant green in color (Figure 1). The samples were thoroughly washed under running water to eliminate surface contaminants. They were then dried in an oven at 80 °C until a constant weight was achieved. Subsequently, the dried samples were ground into a fine powder using a blender.

2.3. Maceration

The leaf and fruit powders of *A. marina*, acquired from the earlier stage, were subjected to 48-h maceration using 96% ethanol as the solvent [26]. The filtrate obtained thereafter underwent filtration, while the residue underwent two further maceration processes employing the same solvent and procedure. Subsequently, the ethanolic extract was concentrated using a rotary evaporator set at 70 °C and a water bath until a consistent weight was attained.

2.4. Preparation of Test Animals and Approval from the Animal Ethics Committee

The test animals used were 165 male mice (*M musculus*) strain DYY, aged 8–12 weeks and weighing approximately 30–50 g. Before treatment,

Table 1. Mortality of test animals in the administration of various doses of *A. marina* extract treatment.

No	Treatment (*A.marina* extract)	Mortality rate (%)
1	Control (Co)	0
2	Leaf extract 250mg/kg BW (D250)	0
3	Leaf extract 500mg/kg BW (D500)	0
4	Leaf extract 1,000 mg/kg BW (D1000)	0
5	Leaf extract 2,000 mg/kg BW (D2000)	0
6	Leaf extract 4,000 mg/kg BW (D4000)	0
7	Fruit extract 250mg/kg BW (D250)	0
8	Fruit extract 500mg/kg BW (D500)	0
9	Fruit extract 1,000 mg/kg BW (D1000)	0
10	Fruit extract 2,000 mg/kg BW (D2000)	0
11	Fruit extract 4,000mg/kg BW (D4000)	0

Table 2. The size of important organs (liver, kidney, and testes) of test mice as the impact of *A. marina* extract treatment.

No	Treatment	Size of organs (cm^3)		
		Liver	Ren	Testis
1.	Control (Co)	11.25	0.75	0.14
2.	Leaf extract 250 mg/kg BW (D250)	11.25	0.75	0.14
3.	Leaf extract 500 mg/kg BW (D500)	6.00	0.75	0.14
4.	Leaf extract 1,000 mg/kg BW (D1000)	3.50	0.75	0.14
5.	Leaf extract 2,000 mg/kg BW (D2000)	6.25	0.75	0.14
6.	Leaf extract 4,000 mg/kg BW (D4000)	11.25	0.75	0.14
7.	Fruit extract 250 mg/kg BW (D250)	7.50	0.75	0.14
8.	Fruit extract 500 mg/kg BW (D500)	11.25	0.75	0.14
9.	Fruit extract 1,000 mg/kg BW (D1000)	7.50	0.75	0.14
10.	Fruit extract 2,000 mg/kg BW (D2000)	13.50	0.75	0.14
11.	Fruit extract 4,000 mg/kg BW (D4000)	7.50	0.75	0.14

the mice were acclimatized as best as possible by being placed at laboratory temperature, and the toxicity testing of *A. marina* extract was conducted *in vivo* for two weeks. Before commencing the acute toxicity test, authorization was obtained from the Animal Ethics Committee at the University of Lampung, with reference number 141/UN.26/KE/X/2024.

2.5. *Acute Toxicity Test*

The method used for acute toxicity tests refers to Organization for Economic Co-Operation and Development (OECD) guidelines for acute oral toxicity testing [27]. The research was conducted at room temperature (25 °C) with alternating dim and bright light periods in 12-h intervals. 165 mice were divided into 11 treatment groups, i.e., control (Co); leaf extract 250 mg/kg BW (D250); leaf extract 500 mg/kg BW (D500); leaf extract 1,000 mg/kg BW (D1000); leaf extract 2,000 mg/kg BW (D2000); leaf extract 4,000 mg/kg BW (D4000); fruit extract 250 mg/kg BW (B250); fruit extract 500 mg/kg BW (B500); fruit extract 1,000 mg/kg BW (B1000); fruit extract 2,000 mg/kg BW (B2000); fruit extract 4,000 mg/kg BW (B4000). Each treatment was replicated three times, resulting in a total of 33 experimental units. Each experimental unit consisted of five mice.

A limit test for single oral administration was conducted, involving test mice that were fasted for 3–4 h before administration but had free access to water. Toxic effects were observed at 30 min, 4 h, and then regularly (every 24 h) for 14 days following administration. Food was provided 1–2 h after administration. Surviving mice were monitored for toxic reactions, and their weights were recorded until the study's conclusion. The LD_{50} was calculated using the Acute Oral Toxicity (AOT) 425 StatPgm software. After the experiment, mice that survived until the end of observation were euthanized for histopathological examination.

2.6. *Observation*

The things that must be observed in the observation period are the number of animals that experience toxic symptoms, such as changes in animal behavior, and the number of animals that died during the test. The observations were also conducted on macroscopic and microscopic organs (liver, kidneys, and testis). Macroscopic observation of organs is conducted by identifying shape, color, and weight changes. Microscopic observation of organs is done through histopathological tests to analyze organ damage from administering extracts from *A. marina* leaves and fruits. Sample organs are paraffinized for observational purposes and then sectioned into observation slides.

Examination of the histopathological structure of the liver includes assessing the presence of cloudy swelling degeneration up to hepatocyte necrosis in five or more large field of view (LFOV) and

observing the histological structure of hepatocytes (liver cells), sinusoids, and liver lobules. Hepatic toxicity is indicated by the presence of stages of cloudy swelling degeneration up to hepatocyte necrosis with the centrilobular liver. Hepatocyte toxicity was categorized as score 0 if central vein and trabeculae, sinusoids are regular/radial; score 1 if cloudy swelling degeneration in 5 or more LFOV; score 2 if cloudy swelling degeneration and necrosis/apoptosis bodies in 5 or more LFOV; and score 3 if cloudy swelling degeneration and bridging necrosis/interlobular.

Examining the histopathological structure of the kidney includes assessing damage to the glomeruli

Table 3. Results of Microscopic Organ Damage Analysis Due to the Administration of *A. marina* Extract.

S No	Treatment	Forms of Organ Damage		
		Liver	Ren	Testis
1.	Control (Co)	None (score 0)	no glomerular damage (score 0)	normal spermatozoa
2.	Leaf extract 250 mg/kg BW (D250)	None (score 0)	no glomerular damage (score 0)	normal spermatozoa
3.	Leaf extract 500 mg/kg BW (D500)	None (score 0)	glomerular damage 25% in 10 LFOV/ glomeruli (mild level)	immature sperm 30%
4.	Leaf extract 1,000 mg/ kg BW (D1000)	degenerative cloudy swelling in 5 LFOV/more (score 1)	glomerular damage 50% in 10 LFOV/ glomeruli (mild level)	immature sperm 30%
5.	Leaf extract 2,000 mg/ kg BW (D2000)	there is damage in the form of degenerative cloudy swelling and the presence of body necrosis in 5 LFOV/more (score 2)	glomerular damage 70% in 10 LFOV/ glomeruli (mild level)	immature sperm 30%
6.	Leaf extract 4,000 mg/ kg BW (D4000)	there is damage in the form of degenerative cloudy swelling and bridging necrosis between lobules in 5 LFOV/more (score 3)	glomerular damage 70% in 10 LFOV/ glomeruli (mild level)	70% immature sperm Leydig cell prolifemiceion
7.	Fruit extract 250 mg/ kg BW (D250)	none (score 0)	no glomerular damage (score 0)	normal spermatozoa
8.	Fruit extract 500 mg/ kg BW (D500)	none (score 0)	glomerular damage 25% in 10 LFOV/ glomeruli (mild level)	immature sperm 30%
9.	Fruit extract 1,000 mg/ kg BW (D1000)	degenerative cloudy swelling in 5 LFOV/more (score 1)	glomerular damage 50% in 10 LFOV/ glomeruli (mild level)	immature sperm 30%
10.	Fruit extract 2,000 mg/ kg BW (D2000)	there is damage in the form of degenerative cloudy swelling and the presence of body necrosis in 5 LFOV/more (score 2)	glomerular damage 70% in 10 LFOV/ glomeruli (mild level)	immature sperm 30%
11.	Fruit extract 4,000 mg/ kg BW (D4000)	there is damage in the form of degenerative cloudy swelling and bridging necrosis between lobules in 5 LFOV/more (score 2)	glomerular damage 70% in 10 large field of view /glomeruli (mild level)	70% immature sperm Leydig cell prolifemiceion

within 10 LFOV per glomerulus and observing the histological structure of glomeruli, tubules, and renal blood vessels. Kidney toxicity is indicated by glomerular damage, namely capsule enlargement, glomerular space narrowing, erythrocyte grains (hemorrhage), cloudy swelling, and hemorrhagic degeneration of tubules. Kidney organ toxicity was categorized into normal if the damage is < 25%; mild if the damage is 25% – 50% within 10 LFOV/ glomeruli; moderate if the damage is 50% – 75% within 10 LFOV/glomeruli; and severe if damage > 75% within 10 LFOV/glomeruli.

The histopathological structure of the testis was examined by observing the histological structure of spermatogenesis (seminiferous tubules), germ cells, Sertoli cells, and Leydig cells. The observation was performed to measure the percentage of immature sperm and the presence of Leydig cell proliferation.

3. RESULTS AND DISCUSSIONS

3.1. Test Animal Mortality

The administration of leaf and fruit extracts of *A. marina* to the test animals did not result in any deaths within the first 24 h, even up to 2 weeks after treatment. The LD_{50} of leaf and fruit *A. marina* ethanol extract is pseudo or not the real LD_{50} >8 g/ kgBW and categorized as practically not toxic (5–15 g/kgBW). However, changes in the behavior of the test animals were observed, characterized by a significant decrease in activity at doses of 2,000 and 4,000 mg/kg BW. These behavioral changes persisted until the 4th day after treatment. From the 5th day onwards, the behavior of the test animals gradually returned to normal, and until the end of the observation period (14 days after treatment), a 100% survival rate was observed. The complete level of mortality of the test animals in administering various doses of *A. marina* extract treatment is presented in Table 1.

The results indicate that up to a dose of 4,000 mg/kg BW of test mice, leaf, and fruit extract of *A. marina* are non-toxic. Several researchers have reported similar results. Ahmed et al., reported that ethanol extracts of *A. Africana* leaves up to a dose of 5,000 mg/kg showed no toxic effects on test animals [28]. Beula et al., reported that extracts of *A. marina* leaves showed no toxic effects on albino mice [29]. Xiu-mei et al., reported that the LD_{50} of water extract from *A. marina* fruit is greater than 10,000 mg/kg BW of test animals [30]. Ali and Bashir reported that extracts from *A. marina* leaves were non-toxic at a dose of 1 g/kg in test mice, but at a dose of 4 g/kg, it caused weight loss and liver size reduction [31].

The absence of mortality in test animals, even at the highest dose (4,000 mg/kg BW), indicates that the extract leaf and fruit of *A. marina* are safe for humans at recommended doses. This finding provides confidence that *A. marina* extract has a sufficiently large safety margin. 49 bioactive compounds based on GC-MS analysis using 96% ethanol solvent, and its high safety margin indicates that *A. marina* has great potential as a source of medicine [6]. However, the mortality rate is just one indicator to measure the safety level of a drug candidate. To comprehensively understand the toxicity effects of *A. marina* extract and to increase confidence in its use, a more in-depth study of vital organs vulnerable to long-term toxicity effects after administration of the herbal extract is required.

3.2. Macroscopic Organ Damage

The observation results of macroscopic damage to vital organs (liver, kidney, and testes) showed inconsistency in liver size with the dose of administration of both leaf and fruit extracts of *A. marina*. Generally, administration of *A. marina* leaf extract decreased mice liver size at doses of 500, 1,000, and 2,000 mg/kg BW; however, the mice liver at the dose of 4,000 mg/kg BW had the same size as the control. Meanwhile, administration of *A. marina* fruit extract led to a decrease in mice liver size at doses of 500, 1,000, and 4,000 mg/kg BW; however, the liver weight of mice at the dose of 2,000 mg/kg BW was higher than the control. Different results were shown regarding kidney and testes size, where administration of *A. marina* extract or fruit did not result in changes in the size of these organs (Table 2).

The administration of *A. marina* extract has been proven to result in hepatic atrophy in mice. This finding contrasts with the report by Ahmed et al. [28] and Beula et al. [29] that the administration of *A. africana* and *A. marina* extracts did not affect the size or weight of the mice's liver organs. Hepatic atrophy in test mice due to *A. marina* extract administration may indicate toxicity; however,

Table 4. Pathological changes and histopathological images of the effects of *A. marina* extract on the liver of test animals.

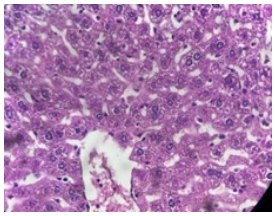
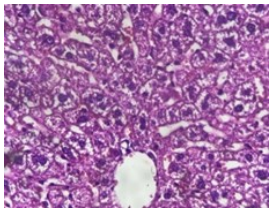
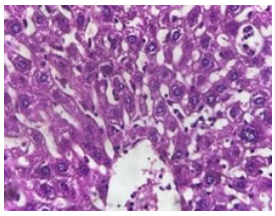
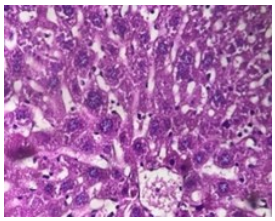
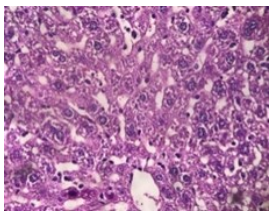
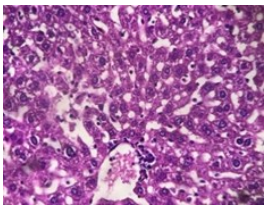
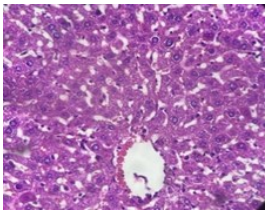
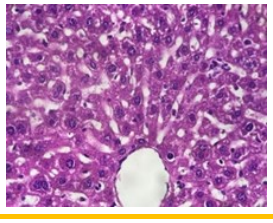
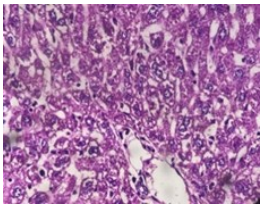
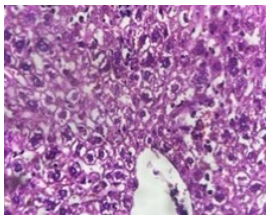
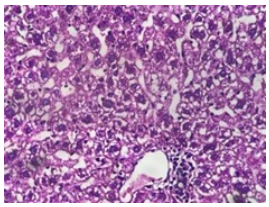
Treatment	Pathological changes	Histopathological images
Control (Co)	• Normal • Presence of central vein and trabecula • Regular/radiating sinusoids	
Leaf extract 250 mg/kg BW (D250)	• Presence of central vein and trabecula • Regular sinusoid/radier • Score 0	
Leaf extract 500 mg/kg BW (D500)	• Presence of central vein and trabecula • Regular sinusoid/radier • Score 0	
Leaf extract 1,000 mg/kg BW (D1000)	• Presence of cloudy swelling degeneration in 5 or more LFOV • Presence of inflammation • Score1	
Leaf extract 2,000 mg/kg BW (D2000)	• Presence of cloudy swelling degeneration in 5 or more LFOV • There is liver cell necrosis in 5 or more LFOV • Score 2	
Leaf extract 4,000 mg/kg BW (D4000)	• Presence of cloudy swelling degeneration in 5 or more LFOV • Bridging interlobular necrosis • Score 3	
Fruit extract 250 mg/kg BW (D250)	• Presence of central vein and trabecula • Sinuoid temiceur/radier • Score 0	
Fruit extract 500 mg/kg BW (D500)	• Presence of central vein and trabecula • Sinuoid temiceur/radier • Score 0	

Table 4. *Cont*.

Treatment	Pathological changes	Histopathological images
Fruit extract 1,000 mg/kg BW (D1000)	• Presence of cloudy swelling degeneration in 5 or more LFOV • Presence of inflammation • Score 1	
Fruit extract 2,000 mg/kg BW (D2000)	• Presence of cloudy swelling degeneration in 5 or more LFOV • There is liver cell necrosis in 5 or more LFOV • Score 2	
Fruit extract 4,000 mg/kg BW (D4000)	• Presence of cloudy swelling degeneration in 5 or more LFOV • Bridging interlobular necrosis • Score 3	

further investigation is required to confirm this suspicion. Hepatic atrophy can be a sign of the toxic effects of *A. Marina* extract, where this occurrence is caused by liver cell damage or other structural changes resulting from exposure to bioactive compounds that are toxic [32]. Hepatic atrophy can also be a sign of a response to inflammation that may occur due to the administration of *A. marina* extract. This inflammation may be caused by the body's immune reaction to bioactive components in the herbal extract or because of the direct effects of these extract components [33]. Furthermore, hepatic atrophy is also possible as part of an organ's adaptive response to chronic exposure to *A. marina* extract. Hepatic atrophy may be the body's attempt to compensate for or overcome the negative effects of the extract [32]. In contrast to various potential negatives, hepatic atrophy can also signify the positive effects of herbal extracts, such as reducing liver fat or improving liver function [34]. It could occur if the herbal extract has hepatoprotective properties or can stimulate liver cell regeneration.

The administration of *A. marina* extract did not result in changes in the kidney size of mice. This finding aligns with Ahmed et al. [28] and Beula et al. [29], who reported that extracts of the mangroves *A. africana* and *A. marina* did not affect the kidney size in mice. This indicates that *A. marina* extract is not highly toxic, as high toxicity from herbal extracts can damage the structure and function of the kidneys, potentially leading to a reduction in organ size. Meneses et al. reported that long-term use of certain herbal extracts significantly causes kidney damage in mice, which is reflected in a reduction in kidney size observed histopathologically [35]. However, kidney size is only one parameter in evaluating kidney health. While changes in size can provide an early indication of issues, a comprehensive assessment of kidney health requires deeper analysis. The histological structure of the kidneys needs to be evaluated to detect microscopic damage to kidney tissue. An herbal extract might not affect kidney size but could still impact kidney function or histological structure [36].

The administration of *A. marina* extract did not result in changes in the testis size of mice. This finding aligns with reports by Ahmed et al. [28] and Beula et al. [29], which indicated that extracts from *A. marina* did not produce significant changes in the testis size of mice. This suggests that the compounds in *A. marina* extract do not have sufficient affinity or toxic potential to affect the testes significantly. Furthermore, the testes are organs with good cell regeneration capabilities, allowing any damage to testicular tissue to be

Table 5. Pathological changes and histopathological images of the effects of *A. marina* extract on the renal of test animals.

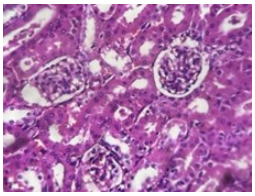
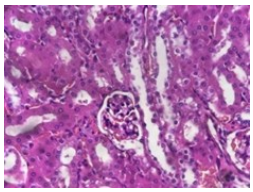
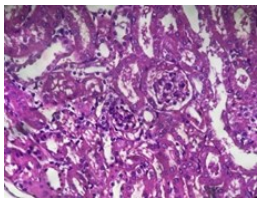
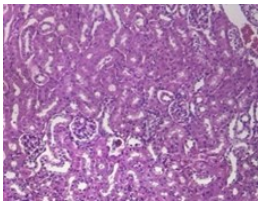
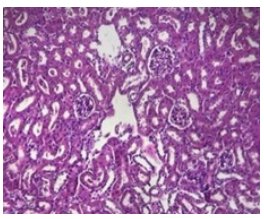
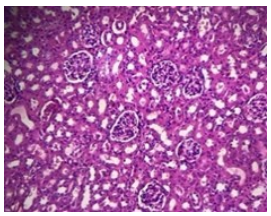
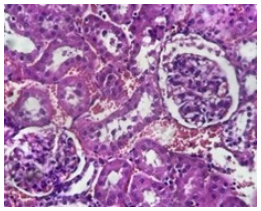
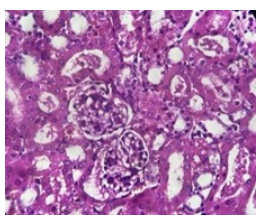
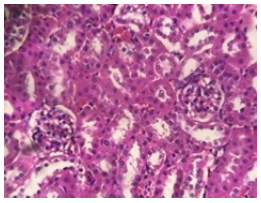
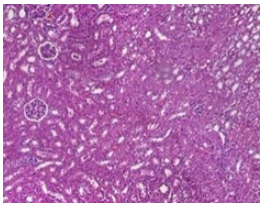
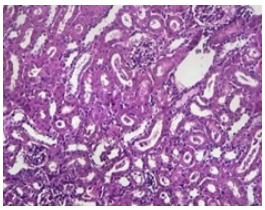
Treatment	Pathological changes	Histopathological images
Control (Co)	• Normal	
Leaf extract 250 mg/kg BW (D250)	• Normal glomeruli	
Leaf extract 500 mg/kg BW (D500)	• Glomerular space narrowed • Tubules exhibit cloudy swelling degeneration, with narrow lumens • 25% damage within 10 LFOV/glomeruli • Mild stage	
Leaf extract 1,000 mg/kg BW (D1000)	• Glomerular space narrowed • tubules exhibit cloudy swelling degeneration, narrow lumen, cell necrosis • 50% damage within 10 LFOV/glomeruli • Modemicee stage	
Leaf extract 2,000 mg/kg BW (D2000)	• Glomerulus shows capsular enlargement • Glomerular space narrows • Tubules exhibit cloudy swelling degeneration, narrow lumen, and cell necrosis • 70% damage within 10 LFOV/glomeruli • Medium stage	
Leaf extract 4,000 mg/kg BW (D4000)	• Glomerulus shows capsular enlargement • Glomerular space narrows • Tubules exhibit cloudy swelling degeneration, narrow lumen, and cell necrosis • 70% damage within 10 LFOV/glomeruli • Medium stage	
Fruit extract 250 mg/kg BW (D250)	• Normal glomeruli	
Fruit extract 500 mg/kg BW (D500)	• Glomerular space narrows • Tubules exhibit cloudy swelling degeneration and narrow lumen • 25% damage within 10 LFOV/glomeruli • Mild stage	

Table 5. *Cont.*

Treatment	Pathological changes	Histopathological images
Fruit extract 1,000 mg/kg BW (D1000)	• Glomerular space narrows • Tubules exhibit cloudy swelling degeneon, narrow lumen • 50% damage within 10 LFOV/glomeruli • Medium stage	
Fruit extract 2,000 mg/kg BW (D2000)	• Glomerulus shows capsular enlargement. • Glomerular space narrows • Tubules exhibit cloudy swelling degeneration narrow lumen, and cell necrosis • 0% damage within 10 LFOV/glomeruli • Medium stage	
Fruit extract 4,000 mg/kg BW (D4000)	• Glomerulus shows capsular enlargement • Glomerular space narrows • Tubules exhibit cloudy swelling degeneration, narrow lumen, and cell necrosis • 70% damage within 10 LFOV/glomeruli • Medium stage	

repaired through cellular regeneration. The testes possess a high regeneration capacity, meaning that even if minor damage occurs, the size and function of the testes can be maintained through internal repair mechanisms [36].

3.3. Microscopic Organ Damage

Generally, administering both leaf and fruit extracts of *A. marina* at the exact dosage will have the same impact on liver, kidney, and testis damage in mice. The results of the microscopic organ damage analysis due to the administration of *A. marina* extract are presented in Table 3.

Table 3 shows that administering *A. marina* extract, both leaf and fruit, at a dose of 250 mg/ kg BW does not cause any negative effects on key organs (liver, kidneys, and testes) in mice. This finding indicates that using *A. marina* leaf and fruit extracts as a beneficial herbal remedy at 250 mg/kg BW can be considered safe for long-term use. As Nalimu et al. noted, herbal extracts that do not cause damage to vital organs such as the liver, kidneys, and testes can be considered safer for long-term use [37]. Based on these findings, *A. marina* leaves and fruits have the potential for the development of herbal products. As stated by Jităreanu et al., herbal products that do not cause side effects have the potential to be developed as potent medicines [38]. This finding can also serve as a scientific basis for developing dosage guidelines for the use of *A. marina* extracts as herbal medicines and important health supplements.

3.3.1. *Liver Damage*

The results of toxicity analysis on the liver showed that both leaf and fruit extracts of *A. marina* at the same dose have approximately similar toxicity levels in the liver. Administration of *A. marina* extract at doses up to 500 mg/kg BW did not cause liver damage in mice. However, administering higher doses resulted in pathological changes such as cell necrosis, inflammation, and fibrosis in the liver (Table 4). Furthermore, administering plant extracts or chemical substances as drugs also increased liver enzyme activity, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as indicators of hepatocyte damage.

Based on the histopathological analysis of the test animals' liver, it is known that the dose of 500 mg/kg BW of leaf and fruit extracts is still categorized as safe, as it does not cause liver damage. This finding indicates that *A. marina* extract is safe to use at relatively high doses. The leaves and fruits of *A. marina* are very rich in bioactive compounds, containing 21 and 13 compounds, respectively [6]. This finding paves the way for utilizing these active compounds in the

Table 6. Pathological changes and Histopathological images of the effects of *A. marina* extract on the testes of test animals.

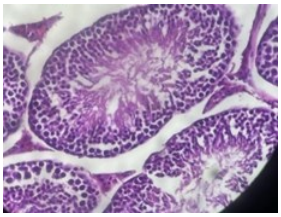
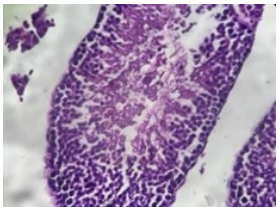
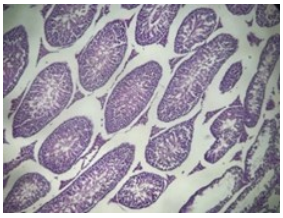
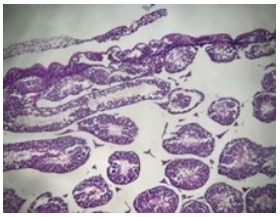
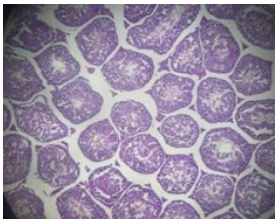
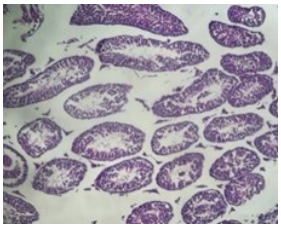
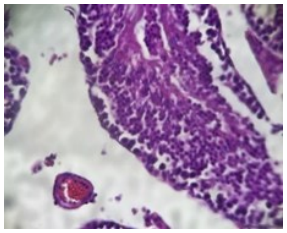
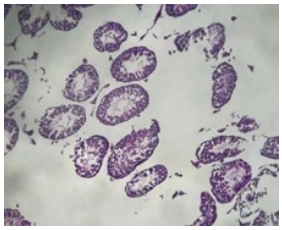
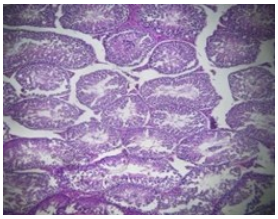
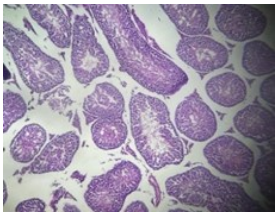
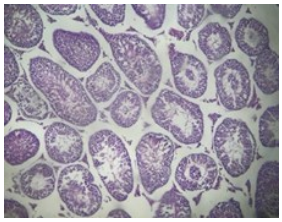
Treatment	Pathological changes	Histopathological images
Control (Co)	• Normal spermatogenesis • Mature sperm	
Leaf extract 250 mg/kg BW (D250)	• Normal spermatogenesis • Mature sperm	
Leaf extract 500 mg/kg BW (D500)	• Immature sperm 30%	
Leaf extract 1,000 mg/kg BW (D1000)	• Immature sperm 30%	
Leaf extract 2,000 mg/kg BW (D2000)	• Immature sperm 30%	
Leaf extract 4,000 mg/kg BW (D4000)	• Immature sperm 70% • Leydig cell proliferation	
Fruit extract 250 mg/kg BW (D250)	• Normal spermatogenesis • Matur sperm	

Table 6. *Cont.*

Treatment	Pathological changes	Histopathological images
Fruit extract 500 mg/kg BW (D500)	• Immature sperm 30%	
Fruit extract 1,000 mg/kg BW (D1000)	• Immature sperm 30%	
Fruit extract 2,000 mg/kg BW (D2000)	• Immature sperm 30%	
Fruit extract 4,000 mg/kg BW (D4000)	• Mature sperm 70% • Leydig cell proliferation	

treatment of various diseases or health supplements with minimal side effects. As stated by Liwa and Jaka, the minimal side effects of herbal ingredients can open the door to using these extracts to treat various diseases [39].

3.3.2. *Renal Damage*

The histopathological analysis of the kidneys revealed that a 500 mg/kg BW of leaf and fruit extracts of *A. marina* could cause mild renal damage. This finding indicates that leaf and fruit extracts of *A. marina* still have the potential to be used as drug candidates but with dosage regulation below 500 mg/kg BW. The complete histopathological depiction of the effects of *A. marina* extract on the kidneys of test animals is presented in Table 5.

Although the *A. marina* extract shows significant therapeutic potential, renal damage at a dose of 500 mg/kg indicates the need for caution in its use. Administering the plant extract above the safe dosage threshold over the long term can lead to acute tubular renal injury, necrosis, or chronic interstitial nephritis in mice [39]. The findings suggest that a dose of 500 mg/kg is at the safety threshold for kidneys, necessitating a lower dosage adjustment to ensure long-term safety [38]. The finding also opens up opportunities for developing alternative formulas or combinations of *A. marina* extract with other compounds that can neutralize or reduce the toxic effects on the kidneys without diminishing the therapeutic benefits of *A. marina* leaf and fruit extracts.

3.3.3. *Testes Damages*

Histopathological analysis of the testes can provide insights into the structural and histological changes that may occur due to exposure to herbal compounds. This includes changes in the number and morphology of spermatogenic cells, the presence of Leydig cells, and signs of inflammation or fibrosis in the testicular tissue. The research showed that both leaf and fruit extracts of *A. marina* at the same dose demonstrated similar effectiveness

regarding sperm damage. The histopathological images depicting the impact of *A. marina* extract on the testes of test animals are presented in Table 6.

Leaf and fruit extracts of *A. marina* have been shown to reduce the fertility of mice sperm cells by up to 30% at concentrations of 500, 1,000, and 2,000 mg /kg BW. The research results also indicate that sperm motility increases with the dose of *A. marina* extract administered. At a dose of 4,000 mg/ kg BW, sperm immaturity even reaches 70%. These findings suggest that *A. marina* leaf and fruit extracts have the potential to be promising herbal anti-fertility agents. As stated, a significant decrease in sperm count, sperm motility, or overall sperm quality below the normal threshold indicates the potential of anti-fertility agents [40].

The leaf and fruit extracts of *A. marina* have been proven to possess properties as anti-fertility agents. This finding scientifically confirms the effectiveness of using *A. marina* as a herbal anti-fertility agent in the ethnopharmacological practices of the Lampung Coastal community [41]. However, administration of extracts at doses exceeding 500 mg/kg BW may cause moderate side effects of liver and kidney damage. Therefore, it is essential to develop alternative formulas or combinations of *A. marina* leaf and fruit extracts with other compounds that can neutralize or reduce the toxic effects on the liver and kidneys without diminishing the anti-fertility benefits. Antioxidants such as vitamin C, E, or selenium combined with herbal extracts have been suggested to counteract oxidative stress and reduce potential toxicity [42]. Additionally, incorporating herbs with known hepatoprotective or nephroprotective properties, such as milk thistle (*Silybum marianum*) or green tea (*Camellia sinensis*), into herbal formulations can help safeguard against liver or kidney damage [43]. By synergizing herbal extracts with complementary compounds, researchers aim to create safer and more effective herbal remedies that offer therapeutic benefits while minimizing the risk of adverse reactions.

4. CONCLUSIONS

The administration of leaf and fruit extracts of *A. marina* to mice at doses of 250–4000 mg/kg BW each resulted in a 100% survival rate. LD_{50} of leaf and fruit A. marina ethanol extract is pseudo or not the real LD_{50} >8 g/kgBW and categorized as practically not toxic (5–15 g/kgBW). Both leaf and fruit extracts of *A. marina* also did not cause a decrease in the size of the kidneys and testes, but at high doses, they potentially reduced liver size. These findings indicate that using *A. marina* extract at recommended doses is safe. Both leaf and fruit extracts of *A. marina*, at a dose of 250 mg/kg BW, did not cause adverse effects on mice's major organs (liver, kidneys, and testes). This finding suggests that using *A. marina* extract at 250 mg/kg BW is safe for long-term use. Administration of *A. marina* extract at doses up to 500 mg/kg BW did not cause liver damage in mice, but it potentially caused mild kidney damage. The finding indicates that leaf and fruit extracts of *A. marina* still have the potential to be used as drug candidates but with dosage regulation below 500 mg/kg BW. Administration of leaf and fruit extracts at doses of 500, 1000, and 2000 mg/kg BW has been shown to reduce the fertility of mouse sperm cells by up to 30%. This finding indicates that *A. marina* has the potential to be a promising herbal anti-fertility agent. The use of *A. marina* extract at recommended doses is safe for humans and can potentially be used as an herbal anti-fertility agent in the long term. Therefore, *in vivo* testing of *A. marina* extract in test animals is needed to determine the effectiveness of this herbal extract as an anti-fertility agent for men.

AUTHOR INFORMATION

Corresponding Author

Duryat Duryat — Department of Forestry, University of Lampung, Bandar Lampung-35145 (Indonesia);

orcid.org/0009-0001-8652-4352

Email: duryat.1978@fp.unila.ac.id

Authors

Rodiani Rodiani — Department of Obstetrics and Gynecology, University of Lampung, Bandar Lampung-35145 (Indonesia);

orcid.org/ 0000-0002-2381-9057

Tri Maryono — Department of Plant Protection, University of Lampung, Bandar Lampung-35145 (Indonesia);

orcid.org/0000-0002-0834-7944

Author Contributions

Conceptualization, R. R. and D.; Methodology, R. R. and T. M.; Software, T. M.; Validation, R. R. and D.; Formal Analysis, D. and T. M.; Investigation, R. R., D., T. M.; Writing – Original Draft Preparation, D. and R. R.; Writing – Review & Editing, T. M.; Visualization, D.; Supervision, R. R.; Project Administration, T. M.; Funding Acquisition, R. R.

Conflicts of Interest

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The author would like to thank the Higher Education for Technology and Innovation (HETI) project at the University of Lampung for the funding support, as well as the The National Population and Family Planning Agency (BKKBN) Regional Lampung for the partnership and collaboration. This research was conducted with the help of students of the Department of Forestry, Faculty of Agriculture, University of Lampung (Hafiz Ansori Dani, Rafli Indra Gozali, M. Andrian Wijaya, Yopi Jordan Saragih, Agilang Ananda Purwayuda, and Kevin Kornelius Kambei).

REFERENCES

[1] D. M. Alongi. (2018). "Impact of Global Change on Nutrient Dynamics in Mangrove Forests". *Forests.* **9** (10). 10.3390/f9100596.

[2] Y. S. Kurniawan, T. Indriani, H. Amrulloh, L. C. Adi, A. C. Imawan, K. T. A. Priyangga, and E. Yudha. (2023). "The Journey of Natural Products: From Isolation Stage to Drug's Approval in Clinical Trials". *Bioactivities.* **1** (2): 43-60. 10.47352/bioactivities.2963-654X.190.

[3] Y. Sun, J. Ouyang, Z. Deng, Q. Li, and W. Lin. (2008). "Structure elucidation of five new iridoid glucosides from the leaves of Avicennia marina". *Magnetic Resonance in Chemistry.* **46** (7): 638-42. 10.1002/mrc.2224.

[4] F. Siharis, N. Ikawati, and N. Lolok. (2019). "The Potency of Antifertility Effect of Stem Bark Extract of Mangrove (Avicennia Marina) on Male White Rats (Rattus Novergicus)". *The Proceedings of the 6th International Conference on Advanced Molecular Bioscience and Biomedical Engineering*. 10.5220/0009587701410146.

[5] F. Cerri, M. Giustra, Y. Anadol, G. Tomaino, P. Galli, M. Labra, L. Campone, and M. Colombo. (2022). "Natural Products from Mangroves: An Overview of the Anticancer Potential of Avicennia marina". *Pharmaceutics.* **14** (12). 10.3390/pharmaceutics14122793.

[6] Rodiani, Duryat, T. Maryono, and D. A. Ramdini. (2023). "Avicennia Marina: A Natural Resource for Male Anti-Fertility in Family Planning". *International Journal of Design & Nature and Ecodynamics.* **18** (5): 1077-1085. 10.18280/ijdne.180508.

[7] M. A. Akbarsha and P. Murugaian. (2000). "Aspects of the male reproductive toxicity/male antifertility property of andrographolide in albino rats: effect on the testis and the cauda epididymidal spermatozoa". *Phytotherapy Research.* **14** (6): 432-5. 10.1002/1099-1573(200009)14:6<432::aid-ptr622>3.0.co;2-i.

[8] M. T. Shaaban, G. M. Fahmy, E. H. Saad, and H. S. Salama. (2024). "Clove and Peppermint Essential Oils Effect on Pathogenic Gut Micro-Biota in Chronic Hepatic Disease Patients". *Bioactivities.* **2** (1): 24-29. 10.47352/bioactivities.2963-654X.213.

[9] M. Maneesh, H. Jayalekshmi, S. Dutta, A. Chakrabarti, and D. M. Vasudevan. (2005). "Role of oxidative stress in ethanol induced germ cell apoptosis - An experimental study in rats". *Indian Journal of Clinical Biochemistry.* **20** (2): 62-7. 10.1007/BF02867402.

[10] K. Sak. (2023). "The Role of Flavonoids as Potential Plant Fungicides in Preventing Human Carcinogenesis: A Short Communication". *Bioactivities.* **1** (2): 39-42. 10.47352/bioactivities.2963-654X.187.

[11] X. Zhang and C. Wu. (2022). "In Silico, In Vitro, and In Vivo Evaluation of the Developmental Toxicity, Estrogenic Activity, and Mutagenicity of Four Natural Phenolic Flavonoids at Low Exposure Levels". *ACS Omega.* **7** (6): 4757-4768. 10.1021/acsomega.1c04239.

[12] K. Kumari, P. Adhikari, A. Pandey, S. S. Samant, M. Lal, and V. Pande. (2024). "Influence of Solvent Polarity on Phytochemicals, Antioxidants, and Antimicrobial Properties of Delphinium denudatum: A Medicinal Herb from Sainj Valley, Himachal Pradesh, India". *Bioactivities.* **2** (1): 30-40. 10.47352/bioactivities.2963-654X.214.

[13] S. Kiran, A. Aslam, A. Parveen, M. Dilshad, and S. Hussain. (2024). "Phytochemistry of Punica granatum Fruit: Its Nutritional and Biological Potential". *Bioactivities.* **2** (1): 57-73. 10.47352/bioactivities.2963-654X.220.

[14] A. Aydın, G. Aktay, and E. Yesilada. (2016). "A Guidance Manual for the Toxicity Assessment of Traditional Herbal Medicines". *Natural Product Communications.* **11** (11). 10.1177/1934578x1601101131.

[15] C. S. J. Woo, J. S. H. Lau, and H. El-Nezami. (2012). In: "Recent Trends in Medicinal Plants Research, (Advances in Botanical Research". 365-384. 10.1016/b978-0-12-394591-4.00009-x.

[16] E. L. Andrade, A. F. Bento, J. Cavalli, S. K. Oliveira, R. C. Schwanke, J. M. Siqueira, C. S. Freitas, R. Marcon, and J. B. Calixto. (2016). "Non-clinical studies in the process of new drug development - Part II: Good laboratory practice, metabolism, pharmacokinetics, safety and dose translation to clinical studies". *Brazilian Journal of Medical and Biological Research.* **49** (12): e5646. 10.1590/1414-431X20165646.

[17] S. Parasuraman, G. S. Thing, and S. A. Dhanaraj. (2014). "Polyherbal formulation: Concept of ayurveda". *Pharmacognosy Reviews.* **8** (16): 73-80. 10.4103/0973-7847.134229.

[18] C. H. C. Leenaars, C. Kouwenaar, F. R. Stafleu, A. Bleich, M. Ritskes-Hoitinga, R. B. M. De Vries, and F. L. B. Meijboom. (2019). "Animal to human translation: a systematic scoping review of reported concordance rates". *Journal of Translational Medicine.* **17** (1): 223. 10.1186/s12967-019-1976-2.

[19] Rozirwan, R. Y. Nugroho, M. Hendri, Fauziyah, W. A. E. Putri, and A. Agussalim. (2022). "Phytochemical profile and toxicity of extracts from the leaf of Avicennia marina (Forssk.) Vierh. collected in mangrove areas affected by port activities". *South African Journal of Botany.* **150** : 903-919. 10.1016/j.sajb.2022.08.037.

[20] M. K. Okla, A. A. Alatar, S. S. Al-Amri, W. H. Soufan, A. Ahmad, and M. A. Abdel-Maksoud. (2021). "Antibacterial and Antifungal Activity of the Extracts of Different Parts of Avicennia marina (Forssk.) Vierh". *Plants (Basel).* **10** (2). 10.3390/plants10020252.

[21] H. A. H. Ibrahim, H. H. Abdel-Latif, and E. H. Zaghloul. (2022). "Phytochemical composition of Avicennia marina leaf extract, its antioxidant, antimicrobial potentials and inhibitory properties on Pseudomonas fluorescens biofilm". *Egyptian Journal of Aquatic Research.* **48** (1): 29-35. 10.1016/j.ejar.2021.10.007.

[22] W. N. Aye, X. Tong, J. Li, and A. W. Tun. (2023). "Assessing the Carbon Storage Potential of a Young Mangrove Plantation in Myanmar". *Forests.* **14** (4). 10.3390/f14040824.

[23] P. J. Hogarth. (2015). "The Biology of Mangroves and Seagrasses". 10.1093/acprof:oso/9780198716549.001.0001.

[24] M. Abu El-Regal and S. Satheesh. (2023). In: "Marine Ecosystems: A Unique Source of Valuable Bioactive Compounds, (Marine Ecology: Current and Future Developments". 1-42. 10.2174/9789815051995123030003.

[25] D. Rančić, I. Pećinar, S. Aćić, and Z. D. Stevanović. (2019). In: "Halophytes and climate change: adaptive mechanisms and potential uses". 152-178. 10.1079/9781786394330.0152.

[26] N. Hasani, H. Rika, and J. Elin. (2023). "Antimicrobial activity test of 96% ethanol

extract of flowers, leaves, and stem bark of tigarun (crateva magna dc.) Against staphylococcus aureus and malassezia furfur". *Medical Sains : Jurnal Ilmiah Kefarmasian.* **8** (3): 1009-1018. 10.37874/ms.v8i3.848.

[27] M. H. Zarei, Z. Lorigooini, H. Amini Khoei, and E. Bijad. (2023). "Acute oral toxicity assessment of galbanic acid in albino rat according to OECD 425 TG". *Toxicology Reports.* **11** : 111-115. 10.1016/j.toxrep.2023.07.001.

[28] M. A. Ahmed, E. O. Ameyaw, F. Ackah-Armah, D. O. Acheampong, P. K. Gathumbi, M. B. Adinortey, G. Ghartey-Kwansah, H. R. Otsyina, and C. K. Adokoh. (2022). "In Vitro and In Vivo Toxicological Evaluation of Avicennia africana P: Beauv. (Avicenniaceae) Leaf Extract in a Rat Model". *Journal of Toxicology.* **2022** : 3434383. 10.1155/2022/3434383.

[29] J. M. Beula, M. Gnanadesigan, P. B. Rajkumar, S. Ravikumar, and M. Anand. (2012). "Antiviral, antioxidant and toxicological evaluation of mangrove plant from South East coast of India". *Asian Pacific Journal of Tropical Biomedicine.* **2** (1): S352-S357. 10.1016/s2221-1691(12)60187-7.

[30] G. Xiu-mei, H. Wei-Dong, and Y. Zen-ji. (2008). "The acute toxicity and bone-merrow micronucleus tests of water extract from Avicennia marina fruits in mice". *Journal of Coastal Development.* **11** (2): 20-74.

[31] B. H. Ali and A. K. Bashir. (1998). "Toxicological studies on the leaves ofAvicennia marina (mangrove) in rats". *Journal of Applied Toxicology.* **18** (2): 111-116. 10.1002/(sici)1099-1263(199803/04)18:2<111::Aid-jat481>3.0.Co;2-9.

[32] B. R. Friesen, R. N. Gibson, T. Speer, J. M. Vincent, D. Stella, and N. A. Collier. (2011). "Lobar and segmental liver atrophy associated with hilar cholangiocarcinoma and the impact of hilar biliary anatomical variants: a pictorial essay". *Insights Imaging.* **2** (5): 525-531. 10.1007/s13244-011-0100-9.

[33] I. M. Petyaev, P. Y. Dovgalevsky, V. A. Klochkov, N. E. Chalyk, D. V. Pristensky, M. P. Chernyshova, R. Udumyan, T. Kocharyan, N. H. Kyle, M. V. Lozbiakova, and Y. K. Bashmakov. (2018). "Effect of lycopene supplementation on cardiovascular parameters and markers of inflammation and oxidation in patients with coronary vascular disease". *Food Science and Nutrition.* **6** (6): 1770-1777. 10.1002/fsn3.734.

[34] B. A. Neuschwander-Tetri and S. H. Caldwell. (2003). "Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference". *Hepatology.* **37** (5): 1202-19. 10.1053/jhep.2003.50193.

[35] M. E. Meneses, D. Martinez-Carrera, L. Gonzalez-Ibanez, N. Torres, M. Sanchez-Tapia, C. C. Marquez-Mota, G. Rendon, V. Mitzi, A. Morales, I. Tello-Salgado, and A. R. Tovar. (2023). "Effects of Mexican Ganoderma lucidum extracts on liver, kidney, and the gut microbiota of Wistar rats: A repeated dose oral toxicity study". *PLoS One.* **18** (4): e0283605. 10.1371/journal.pone.0283605.

[36] X. Xu, R. Zhu, J. Ying, M. Zhao, X. Wu, G. Cao, and K. Wang. (2020). "Nephrotoxicity of Herbal Medicine and Its Prevention". *Frontiers in Pharmacology.* **11** : 569551. 10.3389/fphar.2020.569551.

[37] F. Nalimu, J. Oloro, E. L. Peter, and P. E. Ogwang. (2022). "Acute and sub-acute oral toxicity of aqueous whole leaf and green rind extracts of Aloe vera in Wistar rats". *BMC Complementary Medicine and Therapies.* **22** (1): 16. 10.1186/s12906-021-03470-4.

[38] A. Jităreanu, A. Trifan, M. Vieriu, I.-C. Caba, I. Mârțu, and L. Agoroaei. (2022). "Current Trends in Toxicity Assessment of Herbal Medicines: A Narrative Review". *Processes.* **11** (1). 10.3390/pr11010083.

[39] A. C. Liwa. (2016). "Renal Diseases and Use of Medicinal Herbal Extracts: A Concise Update of Reported Literature in Africa". *Nephrology & Renal Therapy.* **2** (2): 1-5. 10.24966/nrt-7313/100008.

[40] S. W. Choi, K. I. Park, J. T. Yeon, B. J. Ryu, K. J. Kim, and S. H. Kim. (2014). "Anti-osteoclastogenic activity of matairesinol via suppression of p38/ERK-NFATc1 signaling axis". *BMC Complementary Medicine and*

Therapies. **14** : 35. 10.1186/1472-6882-14-35.

[41] Duryat, Rodiani, and T. Maryono. (2023). "Mangroves species diversity and their use as medicinal plants by coastal communities of Pesawaran Lampung". *IOP Conference Series: Earth and Environmental Science.* **1255** (1). 10.1088/1755-1315/1255/1/012027.

[42] T. M. Htay, K. K. Sann, and H. Haini. (2023). "A Comparative Study on Phytochemical Screening and Antioxidant Activity of Aqueous Extract from Various Parts of Bauhinia purpurea". *Bioactivities.* **1** (1): 24-31. 10.47352/bioactivities.2963-654X.183.

[43] L. Abenavoli, A. A. Izzo, N. Milic, C. Cicala, A. Santini, and R. Capasso. (2018). "Milk thistle (Silybum marianum): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases". *Phytotherapy Research.* **32** (11): 2202-2213. 10.1002/ptr.6171.