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Research Paper

Nephroprotective Potentials of Ethanol Leaf Extract of Balakat Tree (Ziziphus Talanai) (Blanco) Merrill. Against Gentamicin- Induced Nephrotoxicity in Male Icr Mice (Mus Musculus L.)

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Abstract

Renal dysfunction can be caused by antibiotic drugs like Gentamicin, which can induce lipid peroxidation, increase free radical synthesis, and decrease antioxidant activity, leading to kidney failure. In the antiques, Philippines, an endemic species of Ziziphus, Ziziphus talanai (Balakat tree), is used as a traditional medicine to treat kidney problems. Phytochemical screening of the ethanol leaf extract of the Balakat tree revealed its potential as a nephroprotective agent. Twelve 6- to 8-week-old male ICR mice were divided into four groups, and treatments were administered for 18 days. Gentamicin was administered intraperitoneally, whereas water, leaf extract, and garlic supplement were administered via oral gavage. The ethanolic leaf extract of the Balakat tree exhibited little potential as a nephroprotective agent at the single dosage used (0.3ml/20g bw), and further studies involving higher doses are recommended. These findings suggest that the ethanolic leaf extract of the balakat tree has potential as a nephroprotective agent. Further studies with higher doses are recommended.

Keywords: *Ziziphus talanai (Blanco) Merr; Nephrotoxicity; Nephroprotective agent and Gentamicin*

INTRODUCTION

The Balakat Tree, scientifically known as Ziziphus talanai, is a native plant in the Philippines that contains diverse pharmaceutical bioactive compounds. These compounds exhibit potential health benefits, including mycobacterial, reproductive, and hepatoprotective properties (Anas et al., 2009; Reyes et al., 2016). Traditional herbal medicine practices in San Remegio, Antique, Philippines, have long employed the Balakat tree to treat kidney issues, such as urinary tract infections (UTI), and various skin ailments, including scabies and ringworm (Lee & Nield, 2007; Anas et al., 2009). Despite these anecdotal claims, scientific studies validating the effectiveness of the Balakat tree in the treatment of kidney problems are notably lacking.

The genus Ziziphus, to which Ziziphus talanai belongs, is recognized for its secondary metabolites and phytochemicals of medical and pharmaceutical significance. These properties include antibacterial, anti-aging, anti-tumor, anti-inflammatory, analgesic, and antioxidant properties (Erenmemisoglu et al., 1995; Nisar et al., 2010). Preliminary phytochemical screening of ethanol leaf extract from Ziziphus talanai revealed varying concentrations of triterpenes, alkaloids,

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glycosides, sterols, flavonoids, tannins, and saponins.

Despite the global relevance of botanical treatments, nephrotoxicity remains a pervasive issue with lifelong implications worldwide, affecting countries across Europe, America, and Asia, including the Philippines (Priyadarsini et al., 2012). Nephrotoxicity, often induced by drugs or toxins, presents a significant challenge, with kidney dysfunction resulting from increased production of reactive oxygen species, leading to lipid peroxidation and depletion of endogenous antioxidants (Porter & Bennett, 1981; Tavafi & Ahmadvand, 2011).

In this broader context of pharmaceutical and medical research, medicinal plants with phenolic compounds, like Ziziphus talanai, show promise as potential and accessible nephroprotective agents. Exogenous antioxidants are crucial for safeguarding kidneys against gentamicin-induced nephrotoxicity, an antibiotic widely used for treating gram-negative bacterial infections. Gentamicin has been associated with kidney damage, which is characterized by elevated levels of creatinine and blood urea nitrogen, as well as distinct histological changes in the kidneys (Silan et al., 2007; Lakshmi et al., 2010; Sodimbaku et al., 2016; Adelman et al., 1981). It has been linked to 13.4% of chronic kidney disease cases worldwide from 2020 to 2022.

The significance of gentamicin-induced nephrotoxicity and the potential nephroprotective properties of Z. talanai highlight a notable gap in understanding the specific impact and mechanisms. This study aimed to fill this knowledge gap by evaluating the theoretical and nephroprotective potential of Z. talanai leaf extract against gentamicin-induced nephrotoxicity using ICR mouse models. By doing so, this research not only addresses a critical health issue in the Philippines but also contributes to the broader field of pharmaceutical and medical research, with implications for developing nephroprotective strategies worldwide.

LITERATURE REVIEW

Nephrotoxicity, defined as the adverse impact of drugs or other substances on renal function, poses a significant challenge in clinical practice (Kellum & Lameire, 2020). Among the various agents associated with nephrotoxicity, gentamicin, an aminoglycoside antibiotic, is well-documented for its potential to induce renal damage, leading to acute kidney injury (Friedman et al., 2020). This finding highlights the urgent need to investigate natural compounds with nephroprotective properties to mitigate gentamicin-induced renal injury. This chapter reviews the existing literature to establish the background and rationale for examining the nephroprotective potential of the ethanol leaf extract of the Balakat Tree (Ziziphus talanai) in male ICR mice.

Gentamicin, a widely used antibiotic, is notorious for its nephrotoxic effects, primarily characterized by tubular damage and acute kidney injury (Faubel & Patel, 2014). The mechanisms underlying gentamicin-induced nephrotoxicity involve oxidative stress, inflammation, and mitochondrial dysfunction (Paller, 2016). These mechanisms have been extensively studied, and biomarkers, such as serum creatinine and blood urea nitrogen (BUN), have been identified to assess kidney damage (Perazella, 2019).

Ziziphus talanai, commonly referred to as the Balakat Tree, is a plant native to the Philippines and other regions in Southeast Asia, including parts of Malaysia, Indonesia, and Vietnam. The ecological and geographical distribution of Ziziphus talanai in these specific regions suggests that the plant has adapted to tropical climates and diverse environmental conditions, contributing to its resilience and the potency of its bioactive compounds (Nawwar et al., 1999). This plant is rich in bioactive compounds, including polyphenols, flavonoids, and alkaloids, which exhibit notable pharmacological properties (Nawwar et al., 1999). Previous research has suggested that extracts of Ziziphus talanai possess antioxidant, anti-inflammatory, and nephroprotective properties (Abdalla et al., 2013). These properties have also been reported to improve cognitive function through the effects of a similar gentamicin structure on brain swelling (Nolasco et al., 2023). Despite these

promising findings, there remains a need to explore less explored aspects of Ziziphus talanai's nephroprotective mechanisms, potentially uncovering novel methodologies for investigating its therapeutic potential.

Recent scientific studies have provided compelling evidence regarding the nephroprotective effects of Ziziphus talanai extracts. These effects are largely attributed to the antioxidant properties of the plant, particularly its rich content of flavonoids—a potential exogenous antioxidant. Flavonoids play a crucial role in counteracting gentamicin-induced oxidative stress, thereby preventing tissue damage (Mahmood et al., 2016). The ability of the extract to reduce inflammation and improve mitochondrial function has also been reported (Liu et al., 2021).

However, despite the progress made in understanding the antioxidant properties of Ziziphus talanai, there remains a significant gap in the literature regarding the specific statistical rate at which flavonoid extracts from Z. talanai prevent tissue damage caused by gentamicin. Additionally, there is limited knowledge regarding the precise mechanisms through which Ziziphus talanai exerts its nephroprotective effects and the potential synergistic interactions of its various bioactive compounds. This research aims to fill these gaps by quantitatively analyzing the nephroprotective effects of the ethanol leaf extract of Ziziphus talanai in male ICR mice, thereby contributing to a deeper understanding of its therapeutic potential and laying the groundwork for future clinical applications.

RESEARCH METHOD

This study employed a quantitative research design with an experimental approach to assess kidney tissue abnormalities and evaluate the histological architecture of male ICR (Institute for Cancer Research) mice. The experimental procedure involved treating male ICR mice with gentamicin and the ethanol leaf extract of Ziziphus talanai. Male ICR mice were selected for their genetic uniformity and consistent response to experimental treatments, making them ideal subjects for this study.

The mice were grouped into four (4) different treatment groups, each comprising three (3) replicates (Table 1). This study specifically focused on two key aspects:

1. Assessment of Kidney Tissue Abnormalities: This involved examining male ICR mice treated solely with gentamicin to determine the extent of nephrotoxicity, which was characterized by the presence of tissue abnormalities in the kidneys.
2. Evaluation of Histological Architecture: This study further evaluated the protective effects of the ethanol leaf extract of Ziziphus talanai on the histological architecture of the kidneys in male ICR mice, assessing any potential reversal or prevention of gentamicin-induced damage.

Table 1. Treatments

TREATMENT GROUPS	TREATMENTS
T0 Normal Control	Mice were treated with distilled water alone 1ml/20g bw.
T- Negative Control	Mice were treated with gentamicin alone 0.1ml/20g bw.
T+ Positive Control	Mice were administered garlic soft gel alone0.2ml/20g bw.

T1	Mice were treated with Ethanol leaf extract of Balakat tree 0.3ml/20g bw after 1 hour gentamicin were administered 0.1ml/20g bw.

Experimental Procedures

Plant Material

We collected adult leaves of Ziziphus talanai from the Park of Mauaque Resettlement in Mabalacat City, Pampanga, Philippines. We sent the collected leaves to the Jose Vera Santos Memorial Herbarium at the Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City, Philippines, for authentication.

Acclimatization of Experimental Models

We used twelve (12) male ICR mice, aged six (6) to eight (8) weeks, as the animal models for this study. We acclimatized the mice for four (4) weeks before administering treatments. Each treatment group had three (3) replicates, and each mouse was housed in an artificial cage measuring 5 inches on each side. We constructed cages with chicken wire doors, plywood bedding, and divisions. We fed the mice commercial pigeon pellets and provided distilled water. We kept them at room temperature with a 12/12 h light/dark cycle. We ensured proper sanitation by removing organic waste from the cages and replacing water and pigeon pellets daily to maintain hygiene and ventilation. This conforms to established practices in animal research, in which the physiological adaptation of animals to their environment is crucial for reliable experimental outcomes (Bañares & Totaan, 2014).

Preparation of Ethanol Leaf Extract

We collected 2 kg of fresh adult leaves of Ziziphus talanai from the Park of Mauaque Resettlement in Mabalacat City, Pampanga. We washed the leaves with tap water to remove unwanted materials and dirt and then air-dried them at room temperature for 7 days without exposing them to sunlight to avoid the loss of volatile bioactive compounds. We cut the dried leaves into small portions, ground them using a blender, and homogenized them. Ground leaves were soaked in 2 L of 70% ethanol at room temperature for 72 h. The solution was filtered using a cotton cloth to separate the solid material from the liquid. The ethanol was evaporated using a rotary evaporator. The resulting dried leaves were diluted with distilled water to the required concentration. For every 10 g of concentrated Balakat extract, we used 2 ml of distilled water to dilute it, yielding a final concentration of 5 g/mL. We stored the diluted leaf extract in an ice chest container containing ice until use (Ngobidi et al., 2016). We used the semi-solid material separated from the ethanol to determine the percentage yield obtained from the extraction (Gakunga et al., 2014; Reyes et al., 2016).

Percentage Yield

The percentage yield formula below was adopted from Gakunga et al., (2014). Formula of Percentage yield = (M2/M1) X 100; wherein M1 is the mass of the powdered leaves prior to extraction M2 is the mass of semi-solid portion of the ethanol extract. M1= 753g and M2= 82.276g. Substituting given values to compute percentage yield; % yield= (82.27g/753g) X 100 = 0.11 X 100. Therefore, the percentage yield of Ziziphus talanai ethanol leaf extract was 11%.

Preparation and Administration of Treatments

We procured gentamicin from Multi-first Pharma Inc., Bambang, Manila City, Philippines. We dissolved 80 mg/kg/day of gentamicin in 1 ml of distilled water and administered it intraperitoneally for eighteen (18) days to induce nephrotoxicity (Qadir et al., 2010). We adapted the protocol for administering leaf extract and gentamicin based on previous studies (Qadir et al., 2010; Reyes et al., 2016) with some modifications. We administered treatments to all animal models daily between 4:00 and 6:00 PM for 18 days. We first administered Ziziphus talanai ethanol leaf extract via intragastric gavage, followed by gentamicin one (1) hour later via intraperitoneal injection.

Monitoring and Assessment

We assessed the morphological changes daily. We weighed the mice every Friday using a weighing scale until the end of the experiment to evaluate changes in weight (Qadir et al., 2010).

Preparation of Biochemical Parameters

After 24 h on the 18th day of treatment, we sacrificed each male ICR mouse through cervical dislocation and collected 2 ml of blood via cardiac puncture using a 5 ml disposable syringe. We allowed the blood samples to stand for one (1) hour and then centrifuged them at 3000 rpm for 10 min (10) to separate the serum. We placed the serum in a test tube with a cover and stored it at 20°C. We sent blood serum samples to ML Soliman Medical Diagnostic Laboratory in Angeles City, Pampanga, Philippines for blood creatinine and blood urea nitrogen analysis (Qadir et al., 2010).

Histopathological Examination of Kidneys

We examined 24 kidney tissue samples in longitudinal sections under multiple magnifications (100X, 400X, and 1000X). We analyzed the pathological results of hyaline casts, glomerular congestion, mononuclear cell infiltration, tubular necrosis and degeneration, and intertubular hemorrhage (Sodimbaku et al., 2016).

Statistical Analysis

We present the collected data as mean ± Standard Deviation. We computed statistical significance using one-way analysis of variance (ANOVA) to compare the means of all samples, followed by Dunnett's (Tukey's) Multiple Comparison test using GraphPad Prism version 7 to compare each treatment group. We considered the level of significance at $P < 0.05$ (Sodimbaku et al., 2016).

FINDINGS AND DISCUSSION

Body Weight Variations During Administration. The experiment revealed variations in mean body weight during the initial period until the first week of treatment (Figure 1). Compared with the initial body weight (T0), T1 had the highest mean body weight (27.307g) due to Balakat leaf extract and gentamicin treatment, while T- had the lowest (24.551g) due to gentamicin alone. T0 increased to 21.3g, and T+ increased to 26.103g. Despite these variations, ANOVA and Tukey's tests indicated non-significance.

During the second week (Figure 1), T1 exhibited the highest mean body weight (29.404g), while T- had the lowest (19.516g). T0 and T+ showed increased mean body weights (23.215g and 27.227g, respectively) compared with the first week. ANOVA indicated significance (P-value = 0.0099), with Tukey's test showing mild significance for T- versus T+ and very significant for T- versus T1.

Similarly, Houghton et al. (1975) and Ali et al. (1997) observed a significant decrease in body weight in mice treated with gentamicin alone due to anorexia and acidosis caused by renal dysfunction, indicating nephrotoxicity.

Weight Gain Observations. During the two weeks of treatment, T0 showed an escalating body weight, as supported by Dubnov-Raz et al. (2011), who found that water intake contributes to weight gain. Weekly body weight assessments for all treatment groups (T0, T-, T+, T1) significantly increased, with T- and T+ showing statistical significance and T- and T1 showing very significant increases during the second week. Gulnaz et al. (2010) reported that garlic soft gel supplements contribute to weight gain, and previous studies on Balakat leaf extract supported its potential for weight gain (Shittu et al., 2007; Shittu et al., 2008; Shittu et al., 2009; Azu et al., 2010). Ziziphus tabanai, a related species of Ziziphus spina-christi, demonstrated significant weight gain, as shown by ANOVA and Tukey's tests.

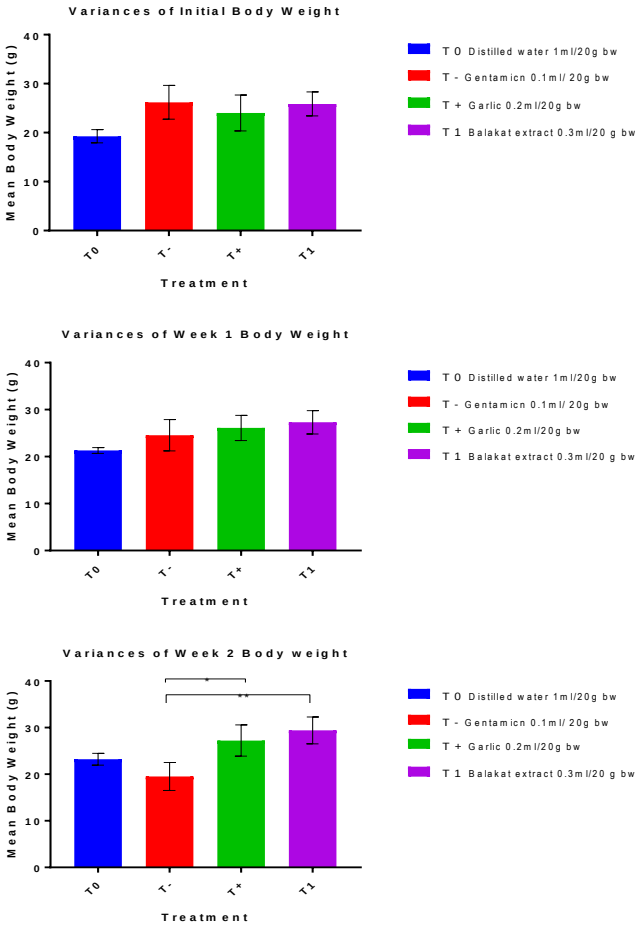

Figure 1. Assessment of the mean body weight from the initial body weight until the 2 weeks of administration of the treatments.

Biochemical Parameters: Creatinine and BUN Levels. Figure 2 depicts the blood serum creatinine (Cr) levels for each treatment. T0 and T+ maintained equilibrium Cr levels, whereas T1 showed a moderate decrease compared to T-, which had the highest creatinine levels. This finding is consistent with Kalayarasan et al. (2009) and Gilbert et al. (1989), who suggested that increased serum creatinine indicates nephrotoxicity. ANOVA revealed a significant decrease in creatinine levels in T1 (P-value = 0.0440), but Tukey's comparisons were not significant.

We examined BUN levels, another parameter of nephrotoxicity (Figure 2). T0 and T+ maintained a consistent BUN level equilibrium, whereas T1 showed a statistically significant

increase compared to T-, which exhibited the highest BUN level. This finding is consistent with Erdem et al. (2000), who noted that elevated BUN levels indicate nephrotoxicity. ANOVA revealed a significant decrease in BUN (P-value = 0.0007), with Tukey's comparisons showing T0 versus T-, T- versus T+, and T+ versus T1 as very significant. T0 versus T1 expressed mild significance. These results suggest that Ziziphus talanai and garlic supplement (Allium sativum) may act as nephroprotective agents.

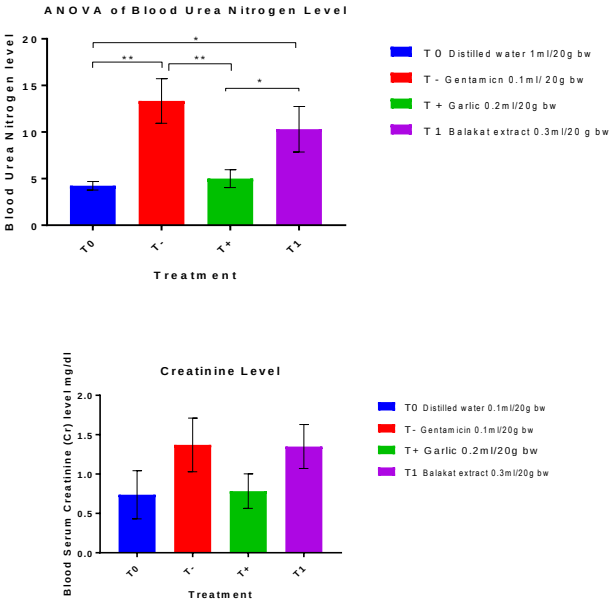

Figure 2. The graph shows the biochemical parameters levels, including creatinine and blood urea nitrogen.

Creatinine exhibited equilibrium levels among treatment groups T0 (normal group) and T+ (positive control), whereas T1 (experimental group) exhibited significantly decreased creatinine level compared to T- (negative control) with the p-value 0.0440.

Histological Observations. Table 2 presents the histological observations of the kidneys. T0, the normal group, exhibited an intact glomerulus and normal renal tubular structure. In contrast, T-, treated with gentamicin, showed an altered glomerulus, desquamated renal tubules, intertubular hemorrhage, and mononuclear cell permeation. These findings are consistent with studies by Shalaby and Hammouda (2014), which linked tissue observations of gentamicin-induced nephrotoxicity to increased Reactive Oxygen Species, leading to lipid peroxidation in the kidneys. Histological evaluation of gentamicin-alone treatment indicated nephrotoxicity, as indicated by the presence of hyaline cast, mononuclear permeation, intertubular hemorrhage, altered glomerulus, and renal tubular desquamation (Sodimbaku et al., 2016).

On the other hand, T+ exhibited normal renal tubular structure and an intact glomerulus. These findings align with Anwar and Meki (2003) and Prasad et al. (1995), who highlighted the potential of garlic supplement (Allium sativum) to reduce oxidative stress through its antioxidant properties, specifically the antioxidant metabolite diallyl disulfide. Observations by Segoviano-Murillo et al. (2008) further emphasized the antioxidant role of garlic in mitigating renal injury induced by ischemia and reperfusion.

T1 exhibited an intact glomerulus, normal renal tubular structure, and moderate intertubular hemorrhage. This histological profile suggests a potential mitigating effect on gentamicin-induced

nephrotoxicity. Ziziphus talanai (Balakat tree) has emerged as a potential nephroprotective agent, supported by its higher concentration of flavonoids, acting as antioxidants. Recent studies have also confirmed the histoprotective and reprotoxic potential of Ziziphus talanai against tetracycline-induced hepatotoxicity and reprotoxicity (Reyes et al., 2016).

In a study by Mierziak et al. (2014), the team highlighted the effectiveness of flavonoids in mitigating the production of reactive oxygen species and thus countering oxidative stress. Flavonoids have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor, and antioxidant properties.

Table 2. Histological observations of all treatments

Treatments	Glomerulus	Renal tubular	Hyaline cast formation	Interstitial hemorrhage	Mononuclear cell permeation
T0 Normal control	intact	normal	**-**	-	-
T- Negative control	Altered	Desquamated	+	+	+
T+ Positive control	Intact	Normal	-	-	-
T1	Intact	Normal	-	Moderate	-

Present = (+)
Absent = (-)

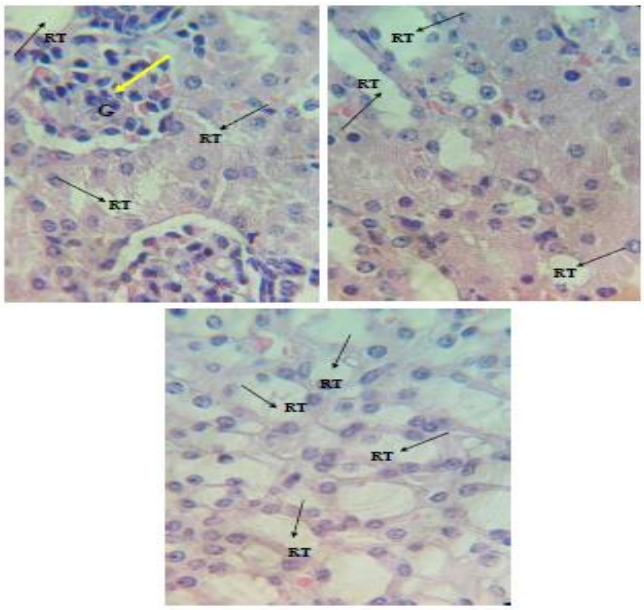

Figure 3. Kidney histopathology of the normal group: A, B- cortex (yellow arrow) exhibited (G) intact glomerulus and (Black arrow) showing (RT) normal renal tubular morphology, C- medulla showing normal morphology of (RT) renal collecting ducts. (H&E X1000)

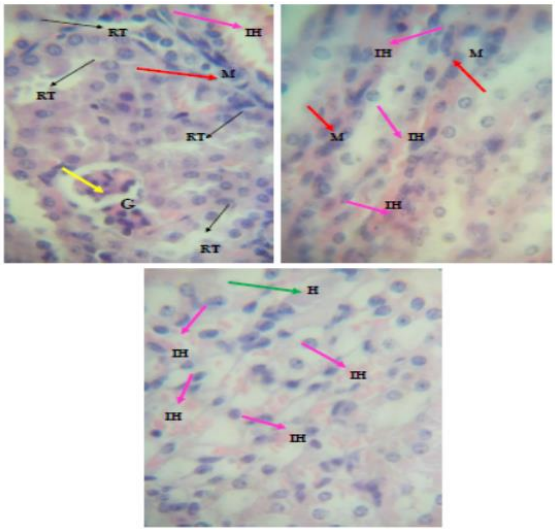

Figure 4. Kidney Histopathology treated with gentamcin- induced- intoxicated group: A, B- cortex (Red arrow) exhibited (M) mononuclear cell permeation (Black arrow) (RT) presented as desquamated renal tubular, (Pitch arrow) displaying (IH) intertubular hemorrhage and (Yellow arrow), exhibited (H) hyaline cast formation (Green arrow), and exhibited (G) alteration of glomerulus. C- Medulla presented intertubular hemorrhage. (H&E X1000)

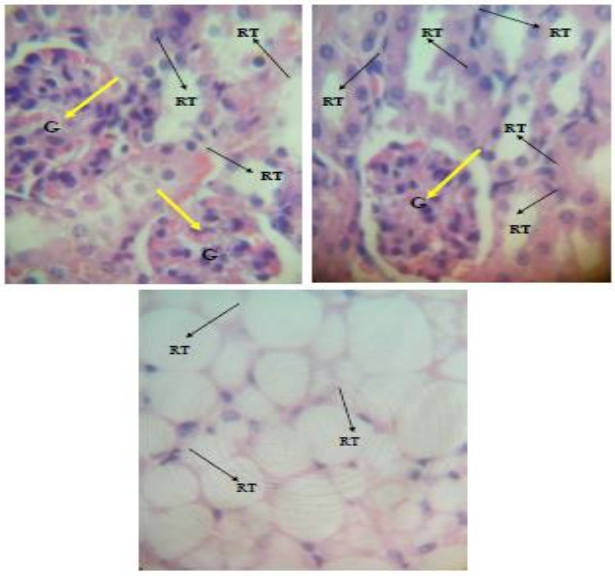

Figure 5. Kidney Histopathology treated with garlic soft gel (Allium sativum); A, B- cortex (Yellow arrow) exhibited (G) intact glomerulus and (Black arrow) displaying (RT) normal renal tubular structures; C- medulla normal morphological features of (RT) renal collecting ducts. (H&E X1000)

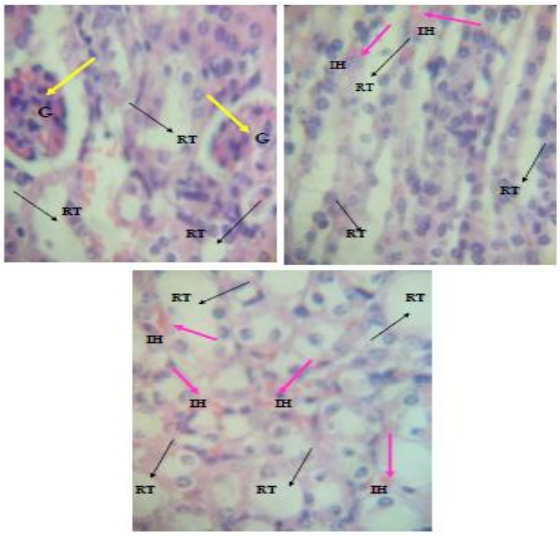

Figure 6. Kidney Histopathology treated with Balakat ethanol leaf extract (Ziziphus talanai); A, B-cortex (Yellow arrow) exhibited intact (G) Glomerulus, (Black arrow) displaying (RT) normal renal tubular and (Pitch arrow) presented (IH) moderate intertubular hemorrhage, C- medulla shows (IH) moderate intertubular hemorrhage. (H&E X1000)

Mice treated solely with gentamicin displayed adverse effects, such as hair thinning, irritable behavior, brownish kidney color, kidney weight loss, decreased mean body weight, and elevated blood serum creatinine (Cr) and Blood Urea Nitrogen (BUN) levels. Histopathological analysis revealed altered glomerulus, intertubular hemorrhage, mononuclear cell permeation, hyaline cast formation, and renal tubular alterations.

Male ICR mice treated with Balakat ethanol leaf extract (Ziziphus talanai) exhibited normal hair, no irritation, and a reddish kidney color. Significant increases in kidney weight, mean body weight, and decreased blood serum levels of Creatinine (Cr) and Blood Urea Nitrogen (BUN) levels were observed. Histopathological examination revealed normal renal tubular structure, intact glomerulus, and moderate intertubular hemorrhage.

Similarly, observations of ICR mice treated with garlic soft gel (Allium sativum) showed natural hair, no irritation, reddish kidney color, significant increases in kidney weight, body weight, and equilibrium in blood serum Creatinine (Cr) and Blood Urea Nitrogen (BUN) levels in the normal group. Histopathology revealed an intact glomerulus and a normal renal tubular structure.

The results indicated that 0.3ml/20g bw of Balakat ethanol leaf extract (Ziziphus talanai) exhibited potential as a nephroprotective agent. Furthermore, based on these findings, it is hypothesized that a higher concentration of crude Balakat tree extract could potentially be used to develop a semi-synthetic drug for nephrotoxicity. Hence, clinical trials are needed to examine the mechanism underlying the development of nephroprotective agents.

CONCLUSIONS

This study examined the nephroprotective potential of Balakat Tree (Ziziphus talanai) ethanol leaf extract against gentamicin-induced nephrotoxicity in male ICR mice. Mice treated with gentamicin alone exhibited renal damage, whereas those treated with Balakat extract showcased significant improvements across various parameters, including kidney weight, body weight, serum creatinine, and BUN levels. Histopathological analysis supported these observations.

In comparison with a standard herbal remedy, garlic soft gel (Allium sativum) and Balakat extract have superior nephroprotective effects, particularly in reducing serum creatinine and BUN

levels. The statistical analysis confirmed the efficacy of 0.3ml/20g bw of Balakat extract as a nephroprotective agent.

These findings suggest that Balakat ethanol leaf extract could serve as a potent natural remedy for gentamicin-induced nephrotoxicity. Furthermore, there is potential for developing semi-synthetic drugs based on higher concentrations of Balakat extract to treat nephrotoxicity. Clinical trials are warranted to explore the underlying mechanisms and translate these promising results into practical therapeutic applications for patients suffering from drug-induced nephrotoxicity.

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LIMITATION & FURTHER RESEARCH

The findings of this research offer valuable insights and warrant several recommendations for future studies and clinical applications:

Further Mechanistic Studies: We conducted in-depth investigations to elucidate the precise mechanisms underlying the nephroprotective effects of Balakat Tree (Ziziphus talanai) ethanol leaf extract. Understanding these mechanisms at the molecular level will enhance our knowledge of their therapeutic potential and guide the development of targeted interventions.

Clinical Trials: Transitioning from preclinical research to clinical trials is crucial. Evaluation of the safety and efficacy of Balakat ethanol leaf extract in humans, particularly those at risk of nephrotoxicity due to medication use or other factors.

Combination Therapies: Investigate the synergistic effects of Balakat extract when used in combination with existing nephroprotective agents. Combination therapies may enhance treatment outcomes and reduce the risk of adverse effects.

Safety and Toxicology: In parallel with clinical trials, conduct comprehensive safety and toxicology studies to ensure that Balakat extract is well-tolerated and does not pose any adverse health risks.

Dosage Optimization: Explore the optimal dosage of Balakat extract should be determined to maximize its therapeutic effects while minimizing potential side effects. This will provide crucial information for developing dosage guidelines for clinical use.

Long-term Studies: Conduct long-term studies to assess the chronic effects of Balakat extract on kidney health and overall physiology. Understanding the long-term impact of treatment is vital for its therapeutic use.

Patient Education: Develop educational materials to inform healthcare professionals and patients about the potential benefits of Balakat extract in preventing and managing drug-induced nephrotoxicity.

Public Health Initiatives: Advocate for public health initiatives that promote the responsible use of nephrotoxic drugs because prevention remains a cornerstone of mitigating nephrotoxicity.

Standardization of Extracts: This step ensures standardization of Balakat leaf extract in terms of its active compounds to provide consistent and reliable results across different studies and clinical applications.

Exploration of Other Benefits: Investigate other potential health benefits of Balakat leaf extract beyond nephroprotection, such as its effects on liver health, cardiovascular health, and its anti-inflammatory properties.

Regulatory Approval: Work towards obtaining regulatory approval for Balakat leaf extract as a nephroprotective supplement or therapeutic agent, ensuring that it meets all safety and efficacy standards required for medical use.

By addressing these recommendations, we can advance the utilization of Balakat Tree ethanol leaf extract as a nephroprotective agent and contribute to the development of safer and more effective treatments for drug-induced nephrotoxicity, ultimately improving the well-being of patients and the quality of healthcare.

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