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Alleviation activity of *Ziziphus talanai* (Blanco) Merr. ethanolic leaf extract on acetaminophen-induced hepatic aberrations in male Sprague-Dawley (S.D.) rats

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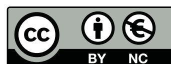

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ABSTRACT

Liver disease remains a global health concern, prompting interest in natural plant derivatives such as *Ziziphus talanai* as potential treatments. Although recent studies documented therapeutic uses, other medicinal properties remained unexplored. This study evaluates the alleviation activity of *Ziziphus talanai* ethanolic leaf extract (ZTELE). Phytochemicals were identified through Thin-layer chromatography (TLC) analysis. Quantification of total flavonoid content (TFC) and total phenolic content (TPC) was determined using alu-

minium chloride and Folin-Ciocalteu colourimetric assay. Twelve healthy male Sprague-Dawley (S.D.) rats were induced with acetaminophen (APAP) and divided into four treatment groups: T+ (Silymarin); T- (Distilled water); T1 (250 mg/kg of ZTELE); T2 (750 mg/kg of ZTELE). Hepatotoxicity in APAP-induced mice was confirmed by elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). TLC analysis revealed the presence of alkaloids, anthraquinones, anthrones, higher alcohols, essential oils, glycosidic flavonoids, steroids, phenols, tannins, and flavonoids in the extract. High contents of flavonoid and phenolic compounds were recorded with 42.98 ± 2.44 mg CG/g and 720.04 ± 31.20 mg GAE/g, respectively. A decline in ALT and AST levels has been observed in ZTELE-treated (T1 and T2) and silymarin-treated mice. On macroscopic examination, the liver was distinctly pale in the negative treatment. Despite no significant difference from liver index, histopathological examination showed hepatoprotection activity of ZTELE and silymarin. Remarkably, T2 showed a significant difference in silymarin-treated samples, indicating its greater hepatoprotective efficacy. In conclusion, ZTELE has various phytochemicals that can be associated with its alleviation of hepatoxicity effects of APAP-induced liver toxicity.

Introduction

The liver plays a crucial role in metabolism, detoxification, and the maintenance of homeostasis. However, it is particularly susceptible to injury caused by alcohol consumption, pharmaceutical agents, environmental toxins, and infectious diseases. Although conventional therapies are available for managing liver disorders, their effectiveness may be limited and can sometimes contribute to further hepatic burden. Consequently, there is increasing interest in identifying safer, more effective alternative therapeutic approaches [1]. The liver is also susceptible to hepatotoxicity induced by drugs such as acetaminophen, also known as *N*-acetyl-para-aminophenol (APAP), an extensively used over-the-counter medication to relieve pain and fever, which accounts for most cases of liver failure [2]. The notable rise in the cytoplasmic enzyme, Alanine Aminotransferase (ALT) and mitochondrial enzyme called Aspartate Aminotransferase (AST) indicates liver toxicity and damage [3]. This damage leads to the prevalence of liver diseases worldwide, which requires immediate action. Liver diseases remain a significant global public health concern, with non-alcoholic fatty liver disease (NAFLD) emerging as one of the most prevalent chronic liver disorders worldwide. Recent estimates indicate that NAFLD affected approximately 1.27 billion individuals globally in 2021, accounting for 48.31 million incident cases, 97,403 deaths, and 2.67 million disability-adjusted life years (DALYs) among adults aged 20–49 years. The burden of NAFLD continues to increase across all regions, driven by rising obesity rates, metabolic disorders, population growth, and socioeconomic disparities. These trends highlight the urgent need for effective preventive and therapeutic strategies to address the growing impact of liver diseases on global health [4].

Medicinal plants have been widely investigated as sources of bioactive phytochemicals with significant therapeutic potential. These compounds, including flavonoids, alkaloids, terpenoids, and phenolic acids, possess diverse pharmacological activities and serve as valuable lead compounds in drug discovery and development. Their contribution to the development of modern medicines highlights the importance of plant-derived compounds in the search for safer and more effective therapeutic agents [5]. Pampanga is home to endemic plants, including the *Ziziphus talanai*. Several studies have confirmed that it exhibits antimicrobial and anti-necrotic potential [6–8], attributed to the presence of various phytochemicals, including tannins, alkaloids, flavonoids, and saponins. Despite recent discoveries, hepatoprotective drugs derived from this plant remain underexplored and may show promise in treating liver diseases. Thus, this study has a main objective in assessing the phytochemicals present in *Z. talanai* and evaluating its hepatoprotective effect in alleviating APAP-induced liver damage in rats.

Material and methods

Animal model

A total of twelve (12) healthy male S.D. rats were distributed into four (4) treatment groups, with four (4) replications, weighing 130-210 g, with

ages ranging from twelve to fourteen weeks old, obtained from Esteleydes Animal Laboratory Research Facility (EALRF), City of Dasmariñas, Cavite, Philippines, to determine the hepatoprotective potential of *Z. talanai*. The experimental protocol used in the animal study was reviewed and approved by the Bureau of Animal Industry, Office of the EALRF, City of Dasmariñas, Cavite, Philippines. All rats were kept in a controlled environment for one (1) week to acclimatise before the administration of chemicals. All rats were fed using commercial rodent pellets and water *ad libitum* [10].

Plant material

The *Z. talanai* collection was obtained from Xevera, Tabun, Mabalacat City, Pampanga, Philippines. Two (2) kg of plant leaf samples were extracted and further concentrated at the Department of Science and Technology (DOST), City of San Fernando, Pampanga, Philippines. A plant specimen was sent to the Bureau of Plant Industry (BPI) at the Social Action Centre of Pampanga (SACOP), Maimpis, City of San Fernando, Pampanga, for plant authentication. Mr Ronnie M. Manuel labelled the specimen as *Ziziphus talanai.*

Plant extraction and phytochemical screening

Collected leaves of *Z. talanai* were washed with running tap water to remove debris, then with distilled water. The leaves were then air-dried for seven days. It was ensured that the plant leaves were not exposed to sunlight and were kept in a well-ventilated area to preserve their bioactive compounds. After air-drying, the leaves were chopped and ground into a powder using a blender. Powdered plant leaves were sieved through a 120-mesh screen. Extraction was performed by cold maceration in 95% ethanol at a 1:2 (w/v) ratio (100 g of powdered leaves in 200 mL of ethanol) for 72 hours. The extract was filtered through Whatman No. 1 filter paper and collected in amber bottles protected from light. The filtrate was concentrated using a rotary evaporator, and the resulting crude extract was stored under refrigerated conditions (4°C) until further use.

Phytochemical screening of *the* ethanolic leaf extract of Ziziphus talanai (ZTELE) was performed and confirmed by thin-layer chromatography (TLC). A systematic trial-and-error approach was employed to determine the appropriate solvent system ratio of hexane, ethyl acetate, and ethanol [9]. Optimal separation was achieved at a solvent ratio of 5:1:0.6 (v/v/v). Different chromatograms were sprayed with various reagents and observed under UV light at 365 nm to detect the presence of phytochemicals. Retention factor (R_f) values of the positive bands were calculated. Phytochemical screening was performed using thin-layer chromatography (TLC) following standard phytochemical detection procedures. Developed chromatograms were sprayed with specific visualisation reagents to detect secondary metabolites. Dragendorff's reagent was used for alkaloids, Borntrager's reagent for anthraquinones, anthrones, and coumarins, vanillin-sulfuric acid reagent for higher alcohols and essential oils, antimony (III) chloride reagent for glycosidic flavonoids and steroids, and potassium ferricyanide-ferric chloride reagent for phenolic compounds, tannins, and flavonoids. The chromatograms were examined under visible and ultraviolet (365 nm) light, and retention factor (Rf) values were calculated for positive bands [39].

Total flavonoid content (TFC) was quantitatively determined using the aluminium chloride ($AlCl_3$) colourimetric assay. In the process, 0.5 mL of the plant extract or standard quercetin solution is mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride solution, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture is incubated in the dark at room temperature for 30 minutes to allow formation of a flavonoid-aluminium complex. After incubation, absorbance was measured at 415 nm using a UV-Vis spectrophotometer. TFC in plant extracts and standard solutions was calculated from calibration curves generated from quercetin standards at six concentration levels (1.0–70.0 µg/mL). Spike recovery was determined using the formula:

$$Spike\ recovery\ (\%) = \frac{(obtained\ concentration\ x\ 100)}{spiked\ concentration}$$

The calculated TFC values will be expressed as milligrams of Catechin Equivalent (CE) per gram of sample.

Meanwhile, the total phenolic content (TPC) was quantitatively measured using the Folin-Ciocalteu assay. A small amount of ZTELE was mixed with Folin-Ciocalteu reagent. Sodium carbonate was then added after five minutes. The

mixture was then left at room temperature for 30 minutes in the dark. Phenolic compounds were indicated by the mixture turning blue. Using a UV-Vis Spectrophotometer, absorbance is measured at wavelengths ranging from 745 to 785 nm. The computed result was expressed as milligrams of gallic acid equivalent (GAE) per gram of sample.

Hepatotoxicity induction

Initially, all treatment groups received an oral intragastric gavage of 1000 mg/kg of APAP (T-) daily for the first seven days. Subsequently, during the second week, 500 mg/kg of APAP was administered four hours post-treatment on each SD rat on the next seven days to ensure successive liver damage [11]. Concurrently, S.D. rats were administered specific concentrations of chemicals and ZTELE in both the control and treatment groups.

Treatment groups and administration of ZTELE

The study focused on the hepatoprotective effects of Z. *talanai* ethanolic crude leaf extract (ZTELE) against acetaminophen (APAP)-induced hepatic abnormalities, assessed using enzyme concentration, gross morphology, liver weight, and histopathology in male Sprague-Dawley (S.D.) rats. **Table 1** provides a comprehensive description of the treated groups. S.D. rats received 1000 mg/kg APAP alone for the first seven (7) days of the administration period. The administration of treatments was executed every 8 a.m. It was first introduced, followed by a 4-hour post-induction of 500 mg/kg of APAP toxicity on the next seven days of the experiment via oral gavage technique. S.D. rats under the T- control received (1.50 mL/150 g bw) distilled water. The T+ control was treated with 200 mg/kg of silymarin, which served as the standard control. In T1 and T2, rats were administered 1.50 mL/150 g bw of low (250 mg/kg) and high (750 mg/kg) doses of ZTELE, respectively.

Biochemical analysis

Blood samples were collected using the lateral tail vein – tail snipping method for baseline, intermediate and endpoint of blood serum. About 2 ml of blood was collected from each rat every 8:00 AM on the 1st, 8th, and 15th day of the administration period. Eight (8) hours of fasting were observed before the blood collection. The blood serum liver enzyme levels of AST and ALT were measured and analysed by a Veterinary expert at the Esteleydes Animal Laboratory Research Facility, City of Dasmariñas, Cavite, Philippines.

Macroscopic examination of liver

The body weights of S.D. rats were assessed on the 1st, 8th, and 15th days of the experiment to determine the appropriate dose. Sedation was used in rats at the end of the administration period. All rats were euthanised. for histopathological analysis. Each liver organ of the rat was completely removed, trimmed and fixed in 10% neutral buffered formalin [13]. The excised liver was weighed and divided by each rat's body weight to obtain the absolute liver index calculated using the adopted equation below [14]:

$$Liver\ index\ =\ \frac{liver\ weight}{rat\ body\ weight} x\ 100 \left(\frac{g\ body\ weight}{100g}\right)$$

Histopathological examination of liver

Livers were extracted a day after the last administration period. The livers were trimmed to obtain

Table 1. Description of treatment groups of SD rats administered with acetaminophen.

Treatment groups		Description
Treatment symbol	Treatment name	
T+	Positive control	S.D. rats were treated with silymarin (200 mg/kg) and administered with 1000 mg/kg and 500 mg/kg acetaminophen (1.50mL/150 g bw)
T-	Negative control	S.D. rats were treated with distilled water (1.50 ml/150 g bw) and administered with 1000 mg/kg and 500 mg/kg acetaminophen (1.50mL/150 g bw)
T1	(250 mg/kg ZTELE)	S.D. rats received 250 mg/kg dose leaf extract of *Ziziphus talanai* (1.50mL/150 g bw) and administered with 1000 mg/kg and 500 mg/kg acetaminophen (1.50mL/150 g bw)
T2	(750 mg/kg ZTELE)	S.D. rats received 750 mg/kg dose leaf extract of *Ziziphus talanai* (1.50mL/150 g bw) and administered with 1000 mg/kg and 500 mg/kg acetaminophen (1.50mL/150 g bw)

the right lobe of the liver. The lobe samples of the livers were immediately sent to the High-Precision Diagnostic Laboratory at the City of San Fernando, Pampanga, for histopathological preparation. Hematoxylin and eosin dye (H&E) were used to stain the specimens [13]. Tissue sections were observed under digital microscopy using the scanner (40x), low-power (100x), and high-power (400x) objectives. This analysis was conducted at Pampanga State Agricultural University – Department of Veterinary Medicine (PSAU-DVM), Magalang, Pampanga, Philippines. The scores in the table were determined using the lesion scoring system employed [15], as shown in **Table 2**.

Statistical analysis

Significant differences in serum (ALT and AST) levels between time periods of induction (baseline and post-induction) were determined using the paired t-test to assess APAP-induced liver damage in treated rats. Meanwhile, One-way Analysis of Variance (ANOVA) was used to determine significant differences among the four treatments: T+, T-, T1, and T2. Tukey's post hoc test was utilised to identify significant pairwise differences between treatments. A significant difference was considered at $p < 0.05$. Results were presented as the mean ± standard error of the mean of three replicates (n = 3). The IBM Statistical Package for the Social Sciences (SPSS) v.22.0 was used to perform the statistical analyses and was verified by a statistical analyst.

Results and discussion

Phytochemicals present in ZTELE

Various phytochemicals were identified in ZTELE by TLC (see **Table 3**). **Figure 1** shows the phytochemicals observed using different reagents

Table 2. Rubrics for histopathological lesions.

Score	Reading	Remarks
0	No alteration observed	Normal
1	1–25% of tissue section affected	Minimal Injury/Damage
2	26–50% of tissue section affected	Mild Injury/Damage
3	51–75% of tissue section affected	Moderate Injury/Damage
4	76–100% of tissue section affected	Severe Injury/Damage

Histopathological scoring adapted from Meles et al. (2018).

Table 3. Phytochemical results of Balakat tree (*Ziziphus talanai*) crude ethanolic leaf extract via TLC.

Reagent used	Constituents tested/observed	Result	Distance traveled by the solute (mm)	R_f value
Dragendorff's reagent	**Alkaloids** – Brown to orange visible spots immediately upon spraying; colors are not stable	+	A. Brown spot: 5	R_{fa} = 0.125
			B. Orange spot: 33	R_{fb} = 0.825
Borntrager's reagent	**Anthraquinones** – orange coloration	+	A. Orange coloration: 9	R_{fa} = 0.225
	Anthrones – yellow (UV 365) zones	+	B. Yellow coloration: 8	R_{fb} = 0.2
	Coumarins – blue (UV 365) zones	-	None	None
Vanillin-sulfuric acid	**Higher alcohol** – appear mainly as blue-violet spots	+	A. Blue-violet spots: Ranging from 18–37	R_{fa1} = 0.45 R_{fa2} = 0.925 The R_f value is ranging from 0.45–0.925
	Essential oils – form zones with a wide range of colors	+	B. Zones with wide range of colors: Ranging from 1–28	R_{fb1} = 0.025 R_{fb2} = 0.7 The R_f value is ranging from 0.025–0.7
Antimony (III) chloride	**Glycosidic flavonoids** – intense yellow to orange visible zones	+	A. Yellow-orange zones: 9	R_{fa} = 0.225
	Steroids – fluoresce under UV 365	+	B. Fluorescence: 3	R_{fb} = 0.075
Potassium ferricyanide – Ferric chloride	**Phenols, tannins, and flavonoids** – blue spots	+	A. Blue spots: 5	R_f = 0.125

+ = present; - = absent; Rfa = Retention factor value of the first detected chromatographic band; Rfb = Retention factor value of the second detected chromatographic band.

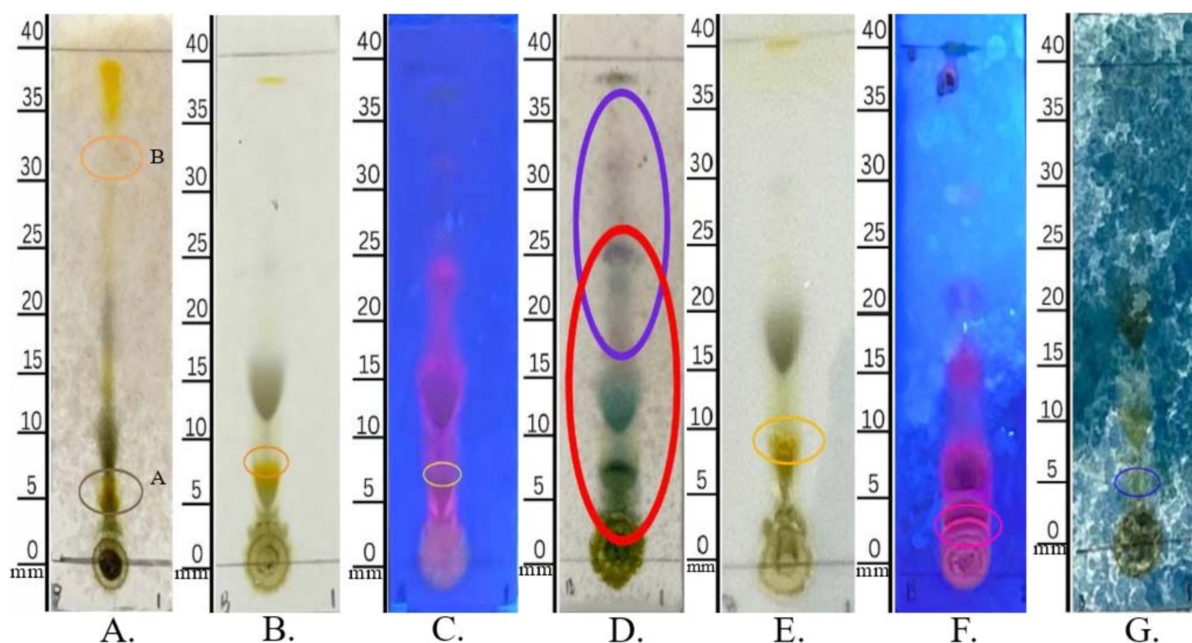

Figure 1. Phytochemical results of Balakat tree (Z. talanai) crude ethanolic leaf. (A) Dragendorff's Reagent; (B) Borntrager's Reagent, (C) Borntrager's Reagent under UV 365; (D) Vanillin Sulfuric Acid; (E) Antimony (III) chloride; (F) Antimony (III) chloride under UV 365; (G) Potassium ferricy anide-Ferric chloride.

under UV light at 365 nm. These include alkaloids, anthraquinones, anthrones, higher alcohols, essential oils, glycosidic flavonoids, steroids, phenols, tannins, and flavonoids. Only the coumarin was not detected in the tests conducted. R_f values also revealed insights about the polarity of detected phytochemicals.

In addition, quantitative colourimetric assays proved that ZTELE has 720.04 ± 31.20 mg GAE/g (Gallic Acid Equivalents per gram of extract) of phenolic content and 42.98 ± 2.44 mg CE/g (Catechin Equivalents per gram) of flavonoids, as summarised in **Table 4**.

Phytochemicals are known for their various bioactivities. The prominent phytochemical constituents with hepatoprotective effects are phenolics and flavonoids. Studies suggest that these compounds have antioxidant properties, which can help lower liver enzyme levels, thereby reducing oxidative stress and other inflammatory responses [16–18]. Although the phytochemical profile of ZTELE was established by TLC and corroborated by total phenolic and flavonoid analyses, the specific bioactive constituents responsible for the observed hepatoprotective activity were not identified. Future studies should employ advanced analytical techniques such as HPLC, LC-MS/MS, or GC-MS to characterise and quantify the major active compounds and to facilitate extract standardisation.

Biochemical analysis

Administration of 1000 mg/kg APAP was performed to induce hepatotoxicity before assessing the treatment groups. Notably, both ALT and AST were upregulated after APAP administration. **Figure 2A** and **2B** show a significant rise in ALT and AST levels as opposed to baseline values. Notably, the T-control showed a drastic increase in ALT levels, from 84 U/L to 178 U/L (111.9% increase). This upregulation was comparable to that of the T+ group, which ranged from 106 U/L to 167 U/L (+57.55%). Similarly, trends were observed in the treatment groups: T1 increased from 106 U/L to

Table 4. Quantitative phytochemical analysis of phenolics and flavonoids of ZTELE.

Score	Procedure	Remarks
Total Phenolics Content	Folin-Ciocalteu Method	720.04 ± 31.20 mg GAE/g
Total Flavonoid Content	Aluminum Chloride Colorimetric Assay	42.98 ± 2.44 mg CE/g

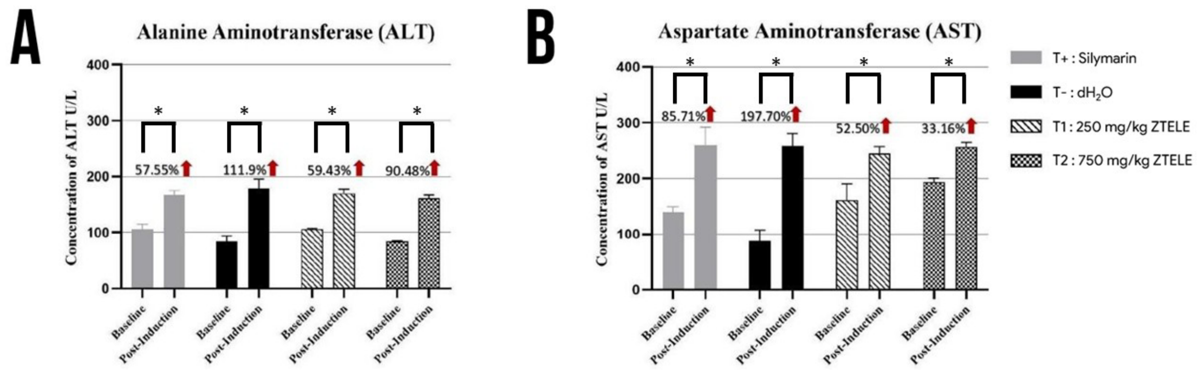

Figure 2. Concentration levels of serum levels (A) Alanine Aminotransferase (ALT) (B) Aspartate Aminotransferase (AST) (U/L) between baseline and post-induction across treatment groups.

169 U/L (+59.43%), and T2 increased from 84 U/L to 160 U/L (+90.48%). Interestingly, a significant increase in AST regulation after APAP administration. The T- control exhibited a more pronounced increase than the T+ control. T- control had a massive upregulation of AST from 87 U/L to 259 U/L (+197.70%), surpassing the increase of T+, which rose from 140 U/L to 260 U/L (+85.71%). While the AST of rats treated with 250 mg/kg and 750 mg/kg displayed congruent trends, with an increase from 160 U/L to 244 U/L (+52.50%) and from 193 U/L to 257 U/L (+33.16%) for T2.

Figure 3A demonstrates significant reductions in ALT levels following treatment introduction in positive and ZTELE-treated APAP-induced rats. Notably, the distilled water + APAP negative control group showed a counterintuitive increase from 178 to 301 (a 69.1% increase), in contrast to the treatment groups. The T+ control exhibited the greatest decrease, dropping from 167 to 89 (a 46.71% decrease). Consistent with the ALT observations, **Figure 3B** also reveals significant decreases in AST levels across treatment groups. T- (Distilled Water + APAP) remained the only group to exhibit an increase, rising from 259 to 292 (12.74%). Silymarin again displayed a substantial decrease from 260 to 73 (-71.92%). The dose-dependent treatment groups, T1 and T2, demonstrated significant reductions from 244 to 152 (-37.70%) and from 257 to 93 (-63.82%), respectively.

Similar results were observed, in which APAP contributes to the production of reactive oxygen species (ROS) in the liver [19]. This accumulation of ROS in hepatocytes promotes apoptosis or liver necrosis [20], thereby compromising liver function and upregulating liver enzymes [11]. Nevertheless, rats treated with ZTELE at 250 mg/kg (T1) and 750 mg/kg (T2) had reduced levels of both ALT and AST. This occurred due to the presence of essential secondary metabolites, including flavonoids, saponins, and related compounds.

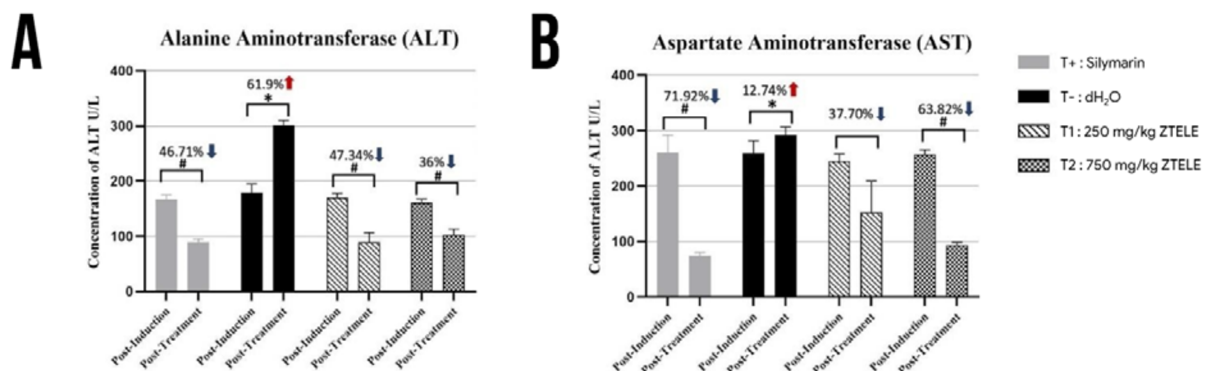

Figure 3. Concentration levels of serum levels (A) Alanine Aminotransferase (ALT) (B) Aspartate Aminotransferase (AST) (U/L) between post-induction and post-treatment across treatment groups.

In addition, studies have reported the potency of these metabolites in downregulating ALT and AST in rodent models [12–14].

The present study focused on conventional biochemical and histopathological markers of liver injury. Additional biomarkers associated with hepatic fibrosis and extracellular matrix remodelling, such as hyaluronidase activity and hyaluronic acid levels, may provide further insight into the hepatoprotective mechanisms of ZTELE and warrant exploration in future investigations.

Macroscopic examination of liver

Figure 4 illustrates the macroscopic features of mice treated with different substances: Silymarin + APAP (T+), Distilled water + APAP (T-), ZTELE 250 mg/kg + APAP (T1), and ZTELE 750 mg/kg + APAP (T2). Figure 4A shows the liver of mice treated with Silymarin, which appears red. This colouration suggests the effectiveness of Silymarin in counteracting APAP-induced hepatotoxicity, consistent with other findings [15,16] on the macroscopic characteristics of healthy rat livers.

Interestingly, the mice treated with distilled water + APAP (see **Figure 4B**) exhibited a pale liver compared to the other treatment groups. This observation aligns with other studies [17,18], in which a pale liver colour indicates histopathological abnormalities and damage. In contrast to the T-group, the livers of rats treated with ZTELE at 250 mg/kg (T1) and 750 mg/kg (T2) showed notable improvements in morphology. This hepatoprotective effect is attributed to the phytochemical constituents of ZTELE, which act against APAP-induced damage [19].

Moreover, various secondary metabolites in the ZTELE extract, such as alkaloids [30], anthraquinones, essential oils, flavonoids [31,32], phenols [33], and tannins, play critical roles in the liver's recovery and protection from abnormalities.

Additionally, **Figure 5** shows the mean liver index across the treatment groups, indicating no significant difference. A similar study [34] aligns with this result, reporting no correlation between liver weight and body weight in the control and

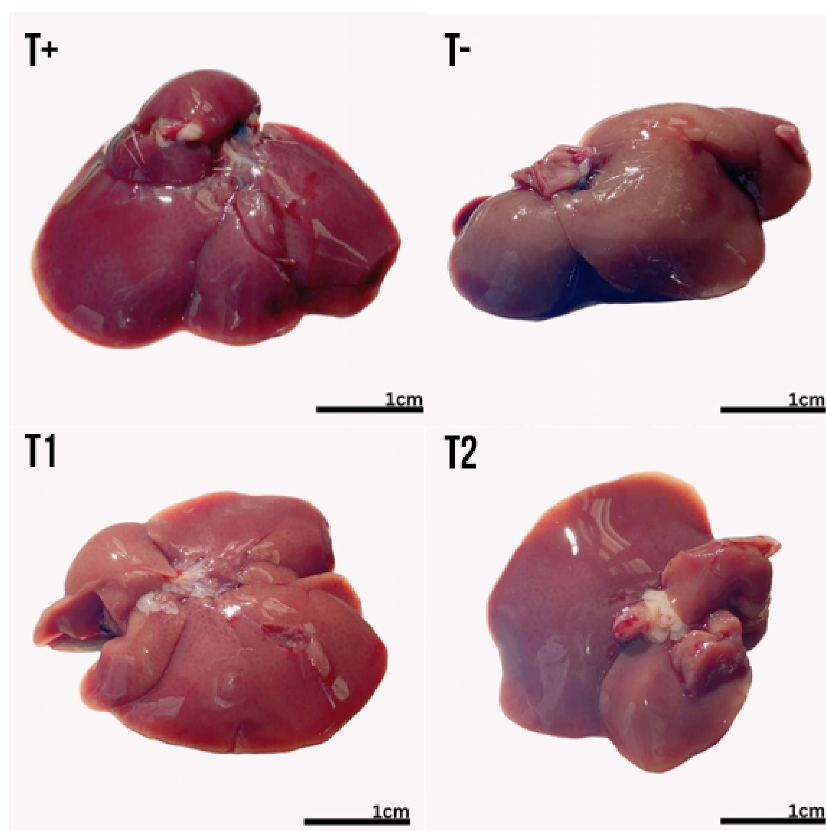

Figure 4. Gross morphology of the liver in a representative group of S.D. rats with APAP-induced liver toxicity. T+ (Silymarin + APAP); T- (Distilled water + APAP); T1 (250 mg/kg ZTELE + APAP); T2 (750 mg/kg ZTELE + APAP).

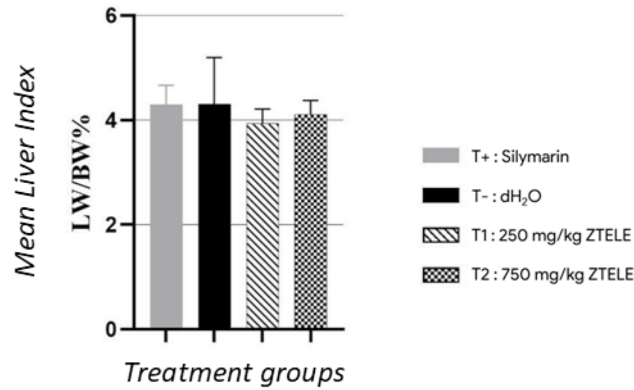

Figure 5. Liver index across treatment groups.

treatment groups. It may be implied that body weight is considered to monitor liver health, but it is not always directly related, as they reflect different physiological aspects.

Histopathological examination of liver

The effect of ZTELE on rats treated with APAP is shown in **Figure 6** and **Table 5**. **Figure 6A** illustrates the healthy liver histology of rats treated with Silymarin + APAP, revealing a normal distribution of hepatocytes and resident macrophages. In contrast, rats treated with water + APAP displayed significant liver abnormalities caused by oxidative stress. **Figure 6B** shows prominent degeneration of hepatocytes, including necrotic cells and leukocyte infiltration near a large vessel. These cascading effects are typical of hepatic injury accompanied by inflammation. As liver cells degenerate, inflammatory cells such as leukocytes play a critical role in combating oxidative stress, thereby explaining the extensive leukocyte infiltration observed in **Figure 6B** [35,36].

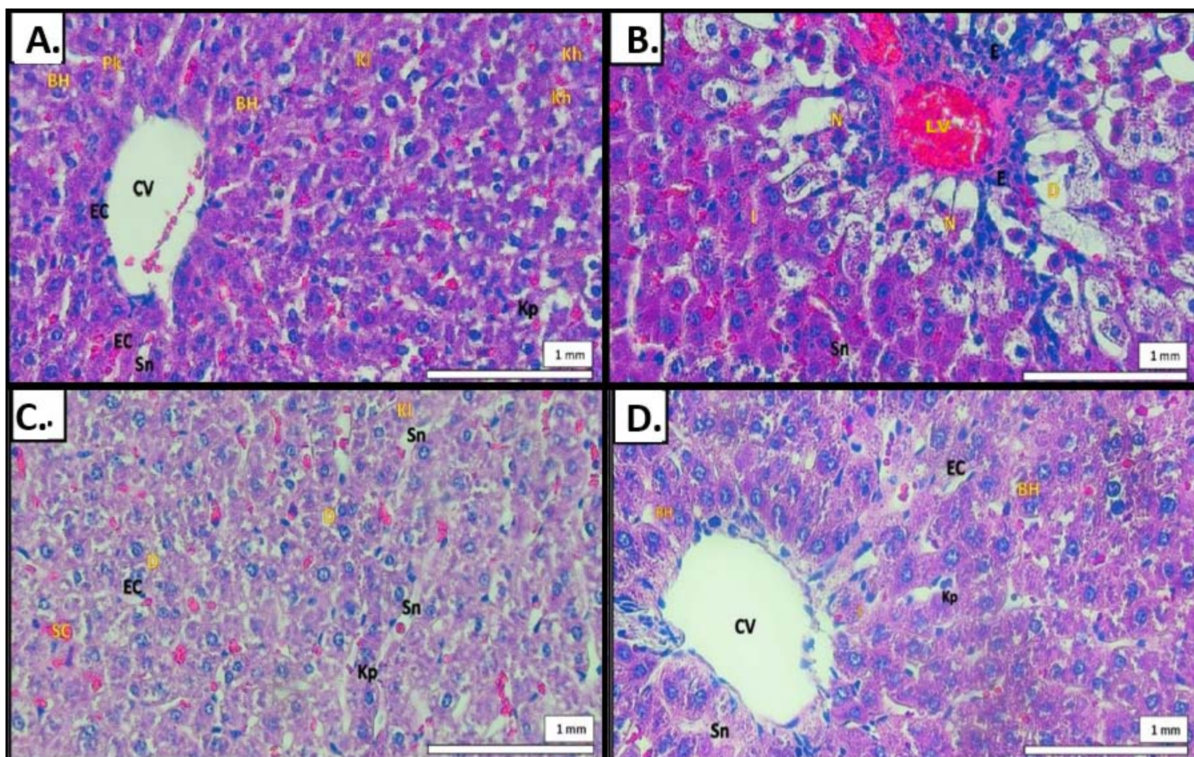

Figure 6. Histopathological examination of the right lobe section of rat liver treated with A) Silymarin + APAP; B) Distilled Water + APAP; C) 250 mg/kg ZTELE + APAP; D) 750 mg/kg ZTELE + APAP at magnification of HPO 400x.

Table 5. Histopathological lesion scores and general description of right-lobe liver.

Treatment	Sample No.	Lesion Severity Grading	Reading	Description
T+	T+R1	2	26–50%	Minimal to mild signs of hepatocyte degeneration
	T+R2	3	51–75%	Minimal to mild signs of hepatocyte degeneration
	T+R3	3	51–75%	Minimal to mild signs of hepatocyte degeneration
T-	T-R1	4	76–100%	Extensive signs of hepatocyte degeneration
	T-R2	4	76–100%	Extensive signs of hepatocyte degeneration
	T-R3	2	26–50%	Minimal to mild signs of hepatocyte degeneration
T1	T1R1	2	26–50%	Minimal to mild signs of hepatocyte degeneration
	T1R2	2	26–50%	Minimal to mild signs of hepatocyte degeneration
	T1R3	1	1–25%	Minimal signs of hepatocyte degeneration
T2	T2R1	1	1–25%	Minimal signs of hepatocyte degeneration
	T2R2	1	1–25%	Minimal signs of hepatocyte degeneration
	T2R3	1	1–25%	Minimal signs of hepatocyte degeneration

Interestingly, rats treated with ZTELE at 250 mg/kg and 750 mg/kg showed near-normal liver histology with minimal aberrations, including slight degeneration in the T1 group. The presence of normal Kupffer cells, endothelial cells, hepatocytes, and central veins was evident in both the T1 and T2 groups, as shown in **Figures 6C** and **6D**, respectively. This recovery strongly indicates that the potent secondary metabolites in ZTELE contributed to its hepatoprotective activity [37,38].

Conclusions

TLC analysis confirmed the presence of several phytochemical constituents in Ziziphus talanai ethanolic leaf extract, including alkaloids, anthraquinones, anthrones, higher alcohols, essential oils, glycosidic flavonoids, steroids, phenols, tannins, and flavonoids. Quantitative assays further revealed high levels of total phenolic and flavonoid contents, suggesting that the extract is a rich source of bioactive compounds.

Administration of ZTELE significantly reduced serum ALT and AST levels and improved liver histoarchitecture in acetaminophen-induced Sprague-Dawley rats. Histopathological findings demonstrated hepatoprotective activity comparable to, and in some parameters exceeding, that of silymarin, particularly at the 750 mg/kg dose.

The findings suggest that ZTELE possesses promising hepatoprotective potential and may serve as a candidate source of plant-derived therapeutic agents for liver disorders. However, the study was limited by the absence of detailed compound identification and investigations of molecular mechanisms. Future studies should focus on bioassay-guided isolation of active compounds, standardisation of extracts, advanced phytochemical characterisation using chromatographic techniques, and evaluation of additional biomarkers associated with liver injury and fibrosis. Such investigations may facilitate the future development of Z. talanai-based phytopharmaceutical and nutraceutical products.

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Author contribution
The contributions of each author to this work are as follows: Kyla Sison oversaw the overall project and was responsible for reading of AST and ALT levels. Charnes Arsent Regala conducted the macroscopic examination. Joshua Lopez weighed the liver and calculated the liver index. Kyle John Malabanan managed the acclimation of the S.D. rats. Marie Stephanie Sanchez was responsible for inducing APAP. John Dave Dicuangco performed the phytochemical screening and statistical analysis. Glen Nolasco conducted the reading of histopathological samples. All authors contributed to the writing and editing of the manuscript.

Ethical Consideration
This study involved animal experimentation and was conducted in accordance with the ethical guidelines and standards for the care and use of laboratory animals. Before the commencement of the study, ethical approval was obtained from the Institutional Animal Care and Use Committee (IACUC) through an Animal Research Clearance (Approval/Reference No. AR-2024-0060). The study was reviewed and approved from December 2023 to January 2024. All procedures involving male Sprague-Dawley rats were performed

with consideration for animal welfare, minimising discomfort and ensuring appropriate handling throughout the experimental period.

Conflict of interest statement

The authors declare no conflict of interest.

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