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Research paper

Histoprotective potentials of ethanol leaf extract of balakat tree (*Ziziphus talanai* (blanco) merr.) against tetracycline-induced hepatotoxicity and reprotoxicity in male mice (*Mus musculus* L.)

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Abstract

Hepatic dysfunction can develop due to ROS and lipid peroxide and may lead to primary liver cancer. Hepatic dysfunction may also develop from tetracycline, an antibiotic with a broad-spectrum of bacteriostatic mechanisms, as it can induce hepatotoxicity and reprotoxicity. In addition, liver tumors secrete hormones that can result to testicular atrophy. Thus, there is a need to address liver and testicular damage. Preliminary phytochemical analysis indicated the presence of bioactive compounds with histoprotective potentials in Balakat tree (*Ziziphus talanai*), which is used in Antique against urinary tract infection. Twelve male mice were divided into four groups of three mice each. Treatments were administered via intragastric gavage technique for 30 days. T0 is the negative control group treated only with water at a dose level of 0.5 ml/20 g body weight (bw). T+ is the positive control group treated only with tetracycline at a dosage level of 0.5ml/20 g bw. T1 and T2 received 0.3 ml/20 g bw and 0.5 ml/20 g bw Z. talanai ethanol leaf extract, respectively.
Histopathological evaluation revealed that T+ mice showed hepatotoxicity based on central vein rupture, fibrosis, vacuolations, cytoplasmic degeneration, lymphocyte infiltration, small amount of glycogen deposits and fibrous septa. Reprotoxicity was also observed as indicated by formation of irregularly shaped tubules, narrowing of tubule diameter, widening of the lumen, germinal epithelium exfoliation, reduction in tubules' cross-sectional areas, absence of germ cell bundles and blood-testis barrier destruction. T1 and T2 mice showed normal liver and seminiferous tubule architectural design as compared to those of T+ mice. Thus, the *Z. talanai* leaf extract mitigated the hepatotoxicity and reprotoxicity effects of tetracycline causing an improvement in the hepatological and testicular parameters. The ethanolic leaf extract of *Z. talanai* (Blanco) Merr. Contains bioactive compounds with histoprotective potentials.

Keywords: *Ziziphus Talanai (Blanco) Merr; Hepatotoxicity; Reprotoxicity; Histoprotective and Tetracycline.*

1. Introduction

The Balakat tree (*Ziziphus talanai*) has been used, in San Remegio, Antique, Philippines, as a traditional medicine for scabies caused by *Sarcoptes scabiei*, a parasitic mite, mycosis such as ringworm and urinary tract infections or UTI (Anas et al., 2009).
In addition, though bioactive compounds in *Ziziphus talanai* exhibit activity against microbes, such effects were not directly observed inside living organisms because majority of the studies on the plant extract were carried out only in vitro (Carvalho et al., 2011).
Studies have shown that the genus *Ziziphus* contains a variety of phytochemicals with potentials to protect the liver and seminiferous tubules against toxicity (Godini, 2009). Specifically, phytochemical analysis of the leaf extract of *Ziziphus talanai* indicated the presence of trace amounts of alkaloids, triterpenes and glycosides; moderate concentrations of sterols; and abundant quantity of flavonoids, saponins, and tannins with histoprotective potentials (Bañares, 2016). Thus, further studies are needed to investigate the effects of *Ziziphus talanai* on selected organs using animal models.
Liver (hepatic) dysfunction can develop due to several causes. It can arise from the disruption of nitrogen metabolism and other signs of hepatocellular damage such as alleviation of serum transaminases level and free radicals (Böcker et al., 1982). It can also develop due to reactive oxygen species (ROS) and lipid peroxide. Hepatic dysfunction may lead to non-alcoholic fatty liver disease and primary liver cancer. In addition, liver tumors secrete hormones that can result to testicular atrophy (American Cancer Society, 2015; Rabey et al., 2014).
Tetracycline, an antibiotic with broad spectrum of bacteriostatic mechanisms, is employed in clinical treatments to cure bacterial infections. Aside from its antibacterial activities, tetracycline is effective in treating pathological ailments such as acute dermatological and periodontal infections or chronic illnesses like rheu-

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matic and neurodegenerative diseases (Bastos et al., 2007). But the medical use of tetracycline has been plagued with many unwanted side effects chiefly hepatic dysfunction and testicular damage among humans and animals (Yin et al., 2006; Wruble and Cummins, 1965).

Tetracycline-induced testicular damage is attributed to the induction of oxidative stress in testicular tissues as a result of reduction in superoxide dismutase, catalase, glucose-6-phosphate dehydrogenase, glutathione-S-transferase activities as well as marked decrease levels of GSH and serum testosterones together with increased action of γ –glutamyltranspeptidase and the development of malondialdehyde (Farombi et al., 2008). Though there are several probable adverse effects resulting from testicular damage, a feared chronic outcome is the persistent shrinkage of the testis or testicular atrophy resulting in relative male infertility (Azu et al., 2010). The impact of male infertility to couples who desire to have their own biological children can be overwhelming. As reported by the Centers for Disease Control and Prevention (2012), male infertility leads to psychological stress, anxiety and depression.

There is a paucity of data regarding the effects and mechanisms of tetracycline on liver and seminiferous tubules as well as histoprotective potentials of *Ziziphus talanai*. Thus, the present study evaluated the hepatoprotective and testiculoprotective potentials of *Z. talanai* leaf extract against tetracycline-induced hepatotoxicity and reprotoxicity using animal models (*Mus musculus* L.).

2. Methodology

2.1. Research design

The study investigated the presence of liver and seminiferous tubule abnormalities among male albino mice treated with tetracycline. The study also evaluated the liver tissue architectural design as well as the stereology of the seminiferous tubules of male albino mice treated with varying concentrations of ethanol leaf extract of *Ziziphus talanai*.

Male albino mice were grouped into four. Each treatment group had three (3) replications (Table 1).

Table 1: Treatment Groups

Treatment Groups	Treatments
T0 (Negative Control)	Male albino mice treated with distilled water alone (0.5 mL/20 g bw)
T+ (Positive Control)	Male albino mice treated with tetracycline alone (0.5 mL/20 g bw)
T1	Male albino mice received leaf extract (0.3 mL/20 g bw) and tetracycline (0.5 mL/20 g bw)
T2	Male albino mice treated with leaf extract (0.5 mL/20 g bw) and tetracycline (0.5 mL/20 g bw)

2.2. Experimental procedures

2.2.1. Plant material

Adult leaves of *Ziziphus talanai* were collected from a piece of land located at Xevera Subdivision, Tabun, Mabalacat City, Pampanga, Philippines. Leaves of *Ziziphus talanai* were sent to Jose Vera Santos Memorial Herbarium, Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City, Philippines for authentication.

2.2.2. Acclimatization of experimental models

A total of twelve (12) six- to twelve-week sexually mature male albino mice were used as animal models in the study. The mice were acclimatized for two (2) weeks prior to administration of the treatments. Each treatment group was housed in an improvised plastic cage which was provided with hardwood pulp as bedding. Furthermore, all mice were provided commercial pigeon pellets and water ad libitum as their source of nutrition. Experimental animals were kept under 12/12 h light/dark cycle, room temperature, proper ventilation and hygienic conditions (Bañares & Totaan, 2014).

2.2.3. Preparation of ethanol leaf extract

A total of 2 kg of fresh *Z. talanai* leaves were collected from the site. Adult leaves of *Ziziphus talanai* were washed with tap water to eliminate dirt and unwanted materials. Leaves were air-dried in a ventilated place at room temperature for 7 days without exposure to sunlight to avoid loss of volatile bio-active compounds. The dried leaf samples were cut into small portions and were homogenized into coarse powder using electric grinder and finely sieved (Gakunga et al., 2014). Powdered leaves were soaked into 2 L of 70% technical grade ethanol for 72 hours at room temperature (26-28°C). The solution then was filtered using a wire strainer to separate solid materials from the liquid portion. The 70% technical grade ethanol was further evaporated using a steam bath. The resulting dried material obtained from the evaporation was diluted with distilled water in order to come up with test solutions of varying concentrations. For every 10 g of dried material, 100 ml of distilled water was utilized to dilute it yielding to a concentration of 0.1 g/mL. The solution was then filtered using a wire strainer to remove solid materials (Oyedeji & Bolarinwa, 2013). Diluted leaf extract was then lyophilized until use (Taati et al., 2011). The semi-solid materials separated from the 70% ethanol were utilized to identify the percentage yield obtained from the extraction (Gakunga et al., 2014).

2.2.4. Percentage yield

The percentage yield formula was adopted from Gakunga et al. (2014). The formula is Percentage yield = (M_2/M_1) X 100; where M_2 is the mass of the semi-solid portion of ethanol extract and M_1 is the mass of leaves prior to extraction. M_2 = 40.79 g and M_1 = 620.13 g. Substituting the given values: Percentage yield = (40.79 g /620.13 g) X 100 = 7%. Therefore, the percentage yield of Z. talanai ethanol leaf extract is 7%.

2.2.5. Preparation and administration of treatments

Commercially available tetracycline was dissolved in distilled water and was administered to male albino mice right after preparation to induce liver toxicity and testicular injury (Shabana et al., 2012; Farombi et al., 2008).

The protocol followed to administer the treatments was adopted from Guevara (2005) with some modifications. Treatments were orally introduced via intragastric gavage technique on a daily basis every 8 o'clock in the morning to 11 o'clock in the morning for a duration of 30 days. The first treatment (tetracycline) was administered 24 hours before the second treatment (*Z. talanai* leaf extract).

Observable physical changes were monitored daily. The weight of the mice was also taken into consideration. Weighing of male albino mice was conducted using digital weighing scale every 7 days (Bañares & Totaan, 2014).

2.2.6. Preparation of liver and testicular tissue samples for histopathology and stereological examination

Six hours after the last treatment (30th day), the mice were sacrificed and excised through cervical dislocation. The livers and testes were collected and were fixed in 10% neutral buffered formalin for fixation purposes (Rao et al., 2012; Bañares & Totaan, 2014; Guevara, 2005). Fixed tissue samples were sent to Angeles University Foundation Medical Diagnostic Center, Pampanga, Philippines for embedding and staining using hematoxylin and eosin.

2.2.7. Histopathological examination of liver

20 liver tissue sections were examined under 100x, 400x and 1000x. Liver tissue sections were examined for the pathological findings of hepatotoxicity based on hepatocyte cytoplasmic degeneration, central vein rupture, fibrosis, lymphocyte infiltration, microvesicular vacuolations and macrovesicular vacuolations. Histological changes in tissues were analyzed as well as glycogen deposits and formation of fibrous septa (Hassan & Abdel-Gawad, 2010; Shabana et al., 2012).

2.2.8. Histopathological and stereological evaluation of seminiferous tubules

A total of 114 tissue sections of testes were subjected to histopathological analysis. Four round or nearly-round seminiferous tubule cross-sections per mouse were randomly selected for stereological analysis using a micrometer scale to measure the following parameters: seminiferous tubule diameter, germinal epithelium height, lumen diameter and cross-sectional area (Gilio et al., 2013; Yama et al., 2011).

2.2.8.1. Diameter of seminiferous tubules

Mean diameter was obtained by getting the average of two diameters situated at 90 degrees, D1 and D2. The purpose of such measurement is to eradicate longitudinal characteristics that might demonstrate varying amounts of tubule damage along the tubule length and display asymmetrical reduction (Yama et al., 2011).

2.2.8.2. Lumen diameter of seminiferous tubules

Average lumen diameter was determined by taking the sum of two lumen diameters at right angles, L1 and L2, and dividing it by 2, which is constant (Yama et al., 2011).

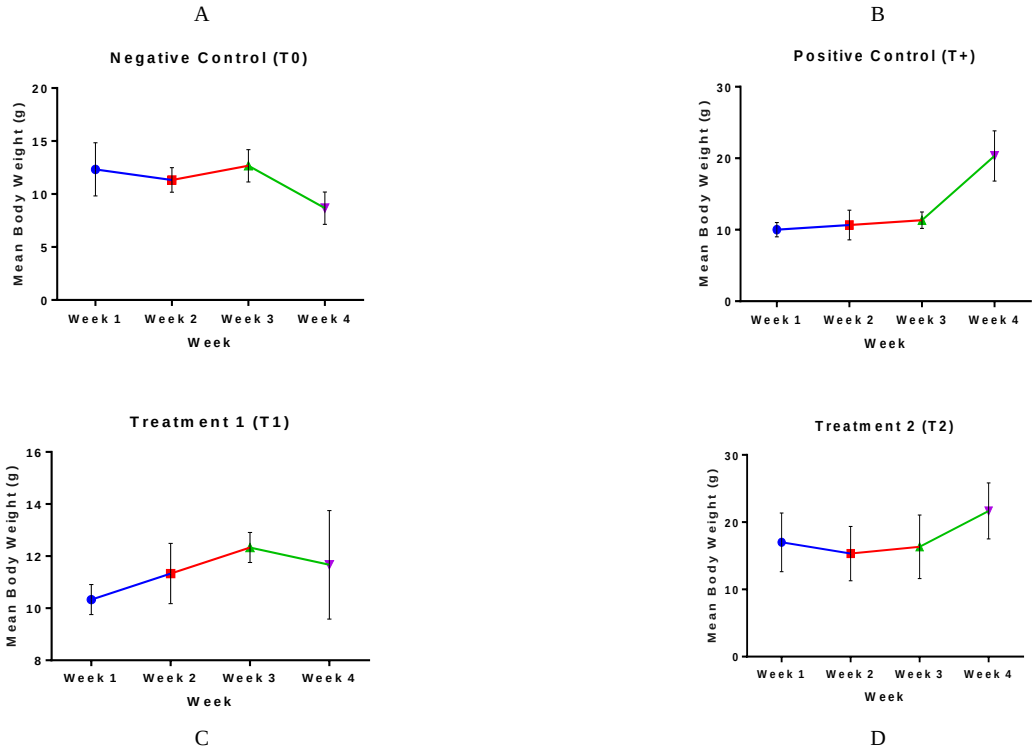


Fig. 1: Line Graphs Displaying the Relative Changes on Mean Body Weight of 4 Treatment Groups Mice for 4 Weeks. (A) T0; (B) T+; (C) T1; (D); T2.

2.2.8.3. Germinal epithelium height

The germinal epithelial height of seminiferous tubules were measured from the spermatogenic cells located at the inner surface of the basement membrane to the innermost advanced cell types surrounding the lumen of the seminiferous tubules (Sakr & Shalaby, 2011).

2.2.8.4. Cross-sectional area of seminiferous tubule

The cross-sectional area for each seminiferous tubule was calculated by applying the formula as prescribed by Yama et al. (2011). The formula is $AC = D^2 \times p/4$; wherein AC is the cross-sectional area; D^2 is the square of the mean diameter; p is a constant equal to 3.142; and 4 is also a constant.

2.2.9. Statistical analysis

Collected data were presented as Mean ± Standard Deviation. Comparisons of between-group were assessed using One Way Analysis of Variance (ANOVA). Post Hoc Test specifically Tukey's Multiple Comparison Test was utilized to identify where the statistical significance lies among compared treatment groups. The level of significance was considered at $p < 0.05$. Statistical analyses were done using GraphPad Prism Version 6 (Bañares & Totaan, 2014).

3. Results

3.1. Observable physical changes of male albino mice

Male albino mice in T+ exhibited localized hair loss and thinning. This is consistent with the findings of Jain et al. (2012) that many medicines including anticancer drugs, hormonal drugs, NSAIDs as well as Beta blockers may cause hair loss. Mice in T0, T1 and T2 did not exhibit hair thinning and hair loss.

The essential oil of a related species of our plant of interest, *Zizyphus jujube*, is used as traditional herb and medicine to treat hair loss. Thus, mice treated with the extract did not exhibit hair loss because the leaf extract may contain phytochemicals necessary for hair rejuvenation (Adhirajan et al., 2003). Male albino mice in T+ exhibited lousy movements and were not active as compared to mice in the T0, T1 and T2. Such results were supported by the findings of Hines (1956) in which animal models developed ephemeral weakness, hyperpnea and anorexia after an intravenous dosage of 50 and 100 mg Chlortetracycline, a derivative of tetra-

cycline, and a dosage of 150 mg/kg body weight caused respiratory distress, general paresis, somnolence and death within several hours.

3.2. Body weight of male albino mice

Figure 1 shows the trend in the body weight of male albino mice in each treatment group for four weeks. As shown in Figure 1A, T0 suffered from weight fluctuation due to the fact that water was not found to consistently induce weight gain or trigger weight loss (Stookey, 2015; Muckelbauer et al., 2009; Hernández-Cordero et al., 2014; Maersk et al., 2012; Stookey et al., 2014). Figure 1B displays the weight gain experienced by T+ after administration of tetracycline. The preceding findings regarding the weight gain of male albino mice in T+ were supported by the findings of Cunningham et al. (1954); Deichmann et al. (1964); and Hines (1956) wherein male mice treated with tetracycline showed weight gain and hastened growth rates. Figure 1C illustrates the weight gain of male mice in T1 on weeks 2 and 3. It means that *Z. talanai* leaf extract has the potential to induce weight gain. Such findings are supported by Shittu et al. (2007); Shittu et al. (2008); Shittu et al. (2009); and Azu et al. (2010). However, weight loss can be recognized on week 4 probably because of tetracycline-induced toxicity (Farombi et al., 2008). Figure 1D displays the weight gain of male mice in T2 on weeks 3 and 4. It is most likely that the *Ziziphus talanai* leaf extract in T2 has the potential to induce weight gain.

As shown in Figure 2A, statistical analysis revealed that there was a significant difference between T+ versus T2 on week 1. This means that *Z. talanai* leaf extract in T2 has the potential to induce weight gain during preliminary consumption. However, Figure 2B and Figure 2C show that there was no significant difference with respect to body weight of male albino mice on weeks 2 and 3. Such findings may be due to the weight loss of male albino mice due to tetracycline-induced toxicity that had an influence on body weight (Farombi et al., 2008). As presented in Figure 2D, statistical analysis showed that there was a significant difference between T0 versus T+, T2 versus T0, T+ versus T1 and T1 versus T2. Such comparisons collectively mean that both tetracycline and *Z. talanai* leaf extracts have the capacity to induce weight gain (Shittu et al., 2007; Shittu et al., 2008; Shittu et al., 2009; Azu et al., 2010; Cunningham et al., 1954; Deichmann et al., 1964; and Hines, 1956).

The results regarding the weight gain and weight loss brought about by tetracycline can be attributed to its rate of absorption in the GI tract. Absorption of tetracycline can be impaired by milk products, sodium bicarbonate, aluminum hydroxide and iron preparations due to chelation and increase in gastric pH (Sande & Mandell, 1990). In a range-finding study of NTP (1989), groups of mice were provided diets containing 470, 950, 1800, 3700 or 7500 mg/kg bw/day tetracycline for 13 weeks. Notice that the study lasted for many weeks and large concentrations of tetracycline were used in the study of NTP (1989) as compared to the normal oral dosage of tetracycline which is 250 or 500 mg every 6 hours as specified by the US National Formulary and British Pharmacopoeia. It is most likely that very large amount of tetracycline absorbed by the body is toxic thus leading to weight loss. Results of the study by NTP (1989) showed that the final mean body weight was slightly decreased in males by 16%.

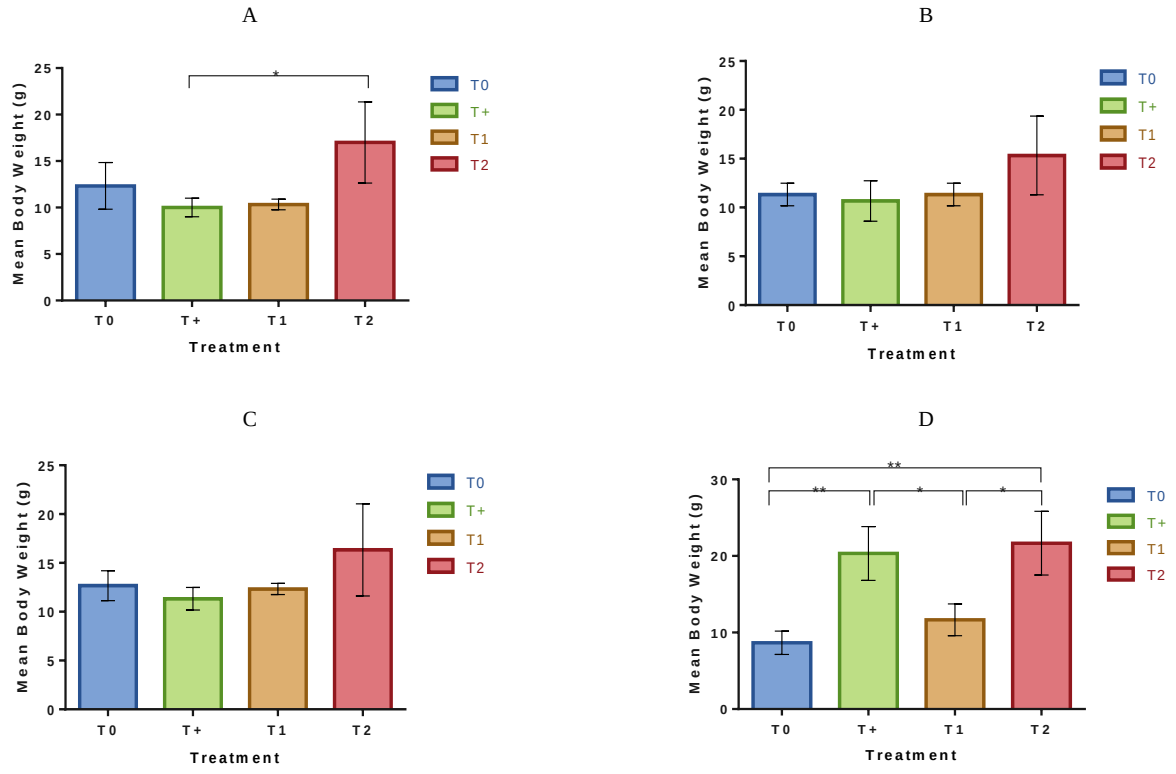


Fig. 2: Bar Graphs Showing the Mean Body Weight and Statistical Significance. The Bracket Indicates the Comparison Among Two Different Treatment Groups. * Signifies That the Comparison Is Statistically Significant. ** Specifies That the Comparison Is Very Statistically Significant. (A) Week 1; (B) Week 2; (C); Week 3; (D) Week 4.

Thus, it can be stated that male albino mice in T+ exhibited weight gain because their body absorbed normal or nearly normal amounts of tetracycline and rate of absorption was impaired by the aforementioned factors that reduce the absorption rate of tetracycline in the GI tract. In contrast, male albino mice in T1 and T2 that exhibited weight loss on some weeks did not suffer from impairment of rate absorption. Thus, weight loss can be a result of toxicity due to absorption of high amounts of tetracycline in the GI tract.

3.3. Histopathological observation in the liver of male albino mice

Figure 3a displays the normal liver architecture of male mice in T0 based on well-conserved central vein, hepatocytes with feathery cytoplasm, glycogen deposits as well as normal spacing between hepatocytes. As shown in 3b, microscopic examination of liver of male mice in T+ showed rupture of the central vein, fibrous septa as well as bridging fibrosis. Moreover, microvesicular and macrovesicular vacuolations and loss of cell membrane and polyhedral structures in some hepatocytes were observed (Figure 4b). In addition, there were few areas of glycogen deposits. Lymphocyte infiltration was relatively observed in some liver microscopic fields thus suggesting the presence of infection. The preceding findings were supported by Shabana et al. (2005); Machado et al. (2013); Tasduq et al. (2005); and Ravi et al. (2010). Figure 3c and 3d present the normal liver architectural design of male albino mice in T1 and T2, respectively. Such findings with respect to the preservation of normal architectural liver design is supported by the findings of Rao et al. (2012) in which a relative species of *Z. talanai*, *Ziziphus oenoplia*, showed hepatoprotective potential of the ethanolic root extract among hepatotoxic rats treated with anti-tubercular drugs.

3.4. Histopathological and stereological observation in the testicular tissue of male albino mice

Figure 5a shows the normal architectural design of seminiferous tubules coming from a representative mouse in T0. As shown in the figure, seminiferous tubules have normal morphology which includes a circular shape with uninjured and intact sex cells, distinguishable lumens as well as clear and noticeable basement membranes. Figure 5b shows histological alterations among seminiferous tubules in T+ which include large numbers of seminiferous tubules having irregular shapes as well as increased in distance between adjacent seminiferous tubules.

Because of the widened lumens, spermatogenic cells lining the distinctive lumens were exfoliated and degenerated; thus, there was noticeable decrease in the number of germ cells and the seminiferous tubules were not composed of germ cell bundles. Furthermore, exfoliation of the blood-testis barrier occurred among these seminiferous tubules. Such exfoliation can lead to the inviability in man because the blood-testis barrier creates an immunologically privileged site for germ cells so that these cells will not be attacked by the body's own immune cells (Carlson, 1999; Scudamore, 2014). Seminiferous tubule atrophy was also observed. In addition, germ cells particularly spermatocytes and spermatozoa were the most affected ones in terms of quantity because of degenerative changes in seminiferous tubules together with lumen widening. Figure 5c displays the seminiferous tubules in T1 that were characterized by less dominant histological alterations. Fig. 5d shows the normal seminiferous tubule stereology. Such preservation of the normal stereology of the seminiferous tubules may be attributed to the *Z. talanai* leaf extract mitigating the toxicity effects of tetracycline causing an improvement in testicular parameters. These findings were supported by Dessau & Sullivan (1958); Dessau & Sullivan (1961); Bañares & Totaan (2014); Gilio et al. (2013); Anuradha & Devi (2011); Gakunga et al. (2014); Shittu et al. (2007); Shittu et al. (2008); and Shittu et al. (2009).

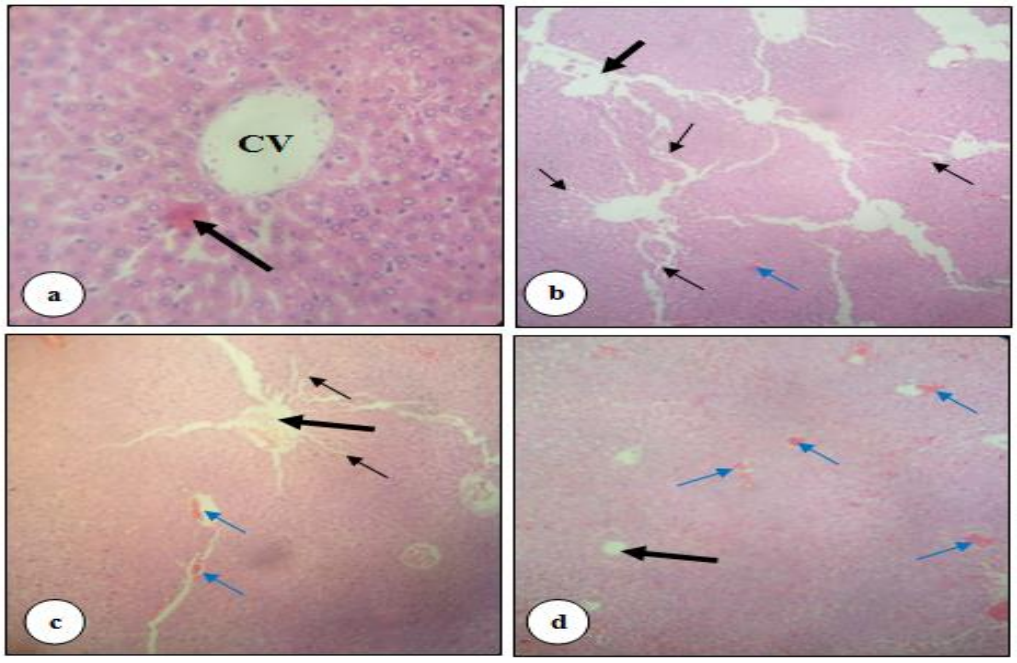


Fig. 3: Histopathological Observations in the Liver of Male Albino Mice (A) Normal Liver Section from T0 Showing A Brought Out Central Vein (CV), Feathery Cytoplasm of Hepatocytes as Well as Glycogen Deposits (Thick Arrow); (B) Liver Tissue Cross-Section from T+ Displaying CV Disruption (Black Thick Arrow), Numerous Fibrous Septa (Black Thin Arrows), Few Glycogen Deposits (Blue Arrow); (C) Liver Tissue From T1 Exhibiting Less Rupture Of CV (Thick Arrow), Few Fibrous Septa (Black Thin Arrows) And Glycogen Deposits (Blue Arrows); (D) Liver Tissue Section Presenting A Normal CV (Black Thick Arrow), Normal Hepatocytes as Well As Numerous Glycogen Deposits (Blue Arrows) (H&E, A- 400x Magnification; B, C, D-100x Magnification).

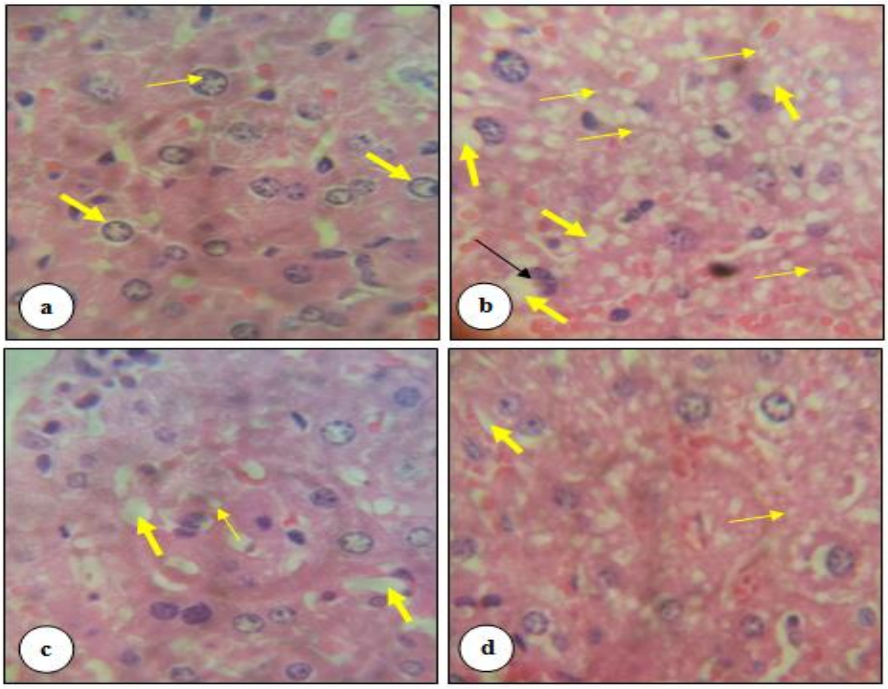


Fig. 4: Histopathological Observations of Macro- and Microvesicular Vacuolations in the Liver of Male Albino Mice (A) Representative Liver Section of Normal Mouse Showing Interstitial Space, Well-Preserved Hepatocytes (Thick Arrows) and Membrane-Bounded Nucleus (Thin Arrow); (B) Representative Liver Tissue Cross-Section Coming from Tetracycline-Treated Mouse Exhibiting Numerous Macrovesicular Vacuolations (Yellow Thick Arrows), Microvesicular Vacuolations (Yellow Thin Arrows) and Cytoplasmic Degeneration (Black Arrow); (C) Representative Section of Liver Coming From Mouse Treated With Tetracycline and 0.3 Ml/20 G Bw Ethanol Extract Showing A Reduction in the Number of Macrovesicular Vacuoles (Thick Arrows) and Microvesicular Vacuoles (Thin Arrow); (D) Representative Micrograph of Liver Tissue of Mouse Treated with Tetracycline and 0.5 Ml/20 G Bw Ethanol Leaf Extract is Characterized by Congestion Among Macro-(Thick Arrow) and Microvesicular Vacuolations (Thin Arrow) (H&E 1000x Magnification).

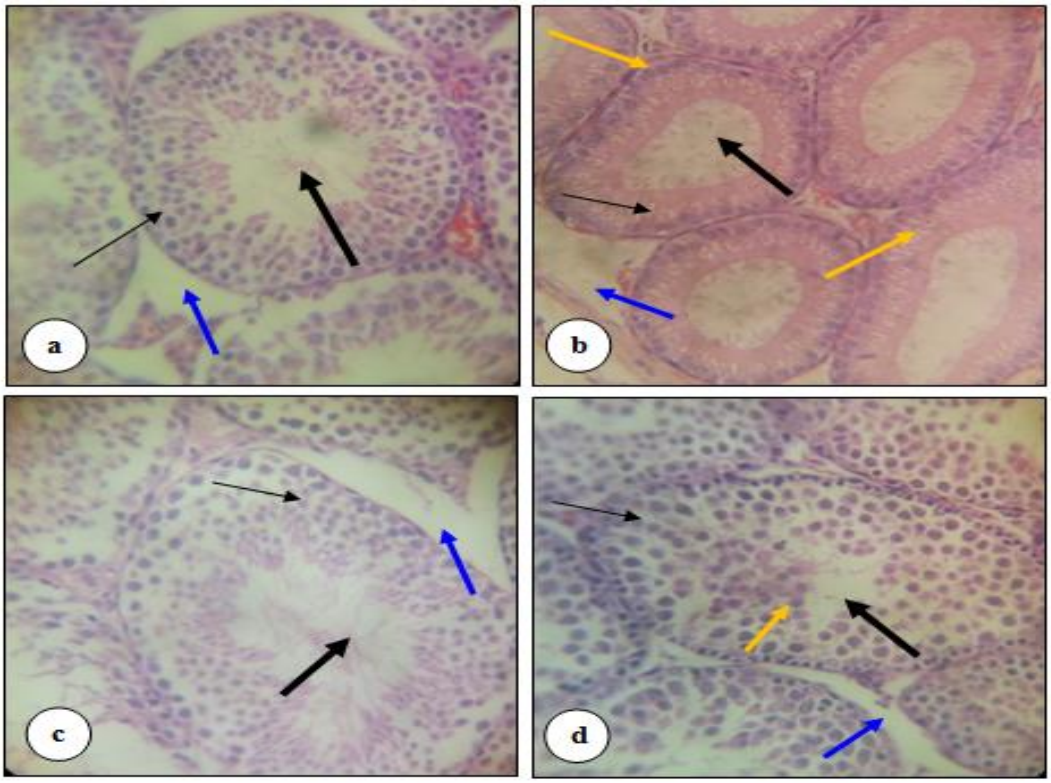


Fig. 5: Histopathological Observations in the Testicular Tissue of Male Albino Mice (A) Representative Seminiferous Tubules Coming From Normal Mouse of T0 Showing Normal Tubule Diameter, Lumen Diameter (Black Thick Arrow), Germinal Epithelium Height Showing the Normal Spermatogenic Cycle (Black Thin Arrow) and Spacing Between Adjacent Tubules (Blue Thick Arrow) ; (B) Representative Seminiferous Tubules of T+ Showing Irregular Shapes of Tubules (Orange Thick Arrows), Reduction In Sizes of Each Seminiferous Tubule Together with Widening of Lumens (Black Thick Arrow), Compacted Germinal Epithelium (Black Thick Arrow), Exfoliated Spermatogenic Cells, Absent Germ Cell Bundles and Abnormal Spacing Between Neighboring Tubules (Blue Thick Arrow); (C) Representative Seminiferous Tubules From Mouse of T1 Showing Vivid Lumens (Black Thick Arrow), Normal Tubule Diameter, Noticeable Germinal Epithelium (Black Thin Arrow) and Normal Spacing Between Adjacent Tubules (Blue Thick Arrow); (D) Representative Seminiferous Tubules Coming From T2 Exhibit Normal Tubule Structure Based on Normal Lumen Diameter (Black Thick Arrow), Germinal Epithelium Height (Black Thin Arrow), Normal Spacing (Blue Thick Arrow), Presence of Germ Cell Bundles (Orange Thick Arrow) and Spermatogenic Layers as Well as Increase in Number of Spermatogenic Cells (H&E 400x Magnification).

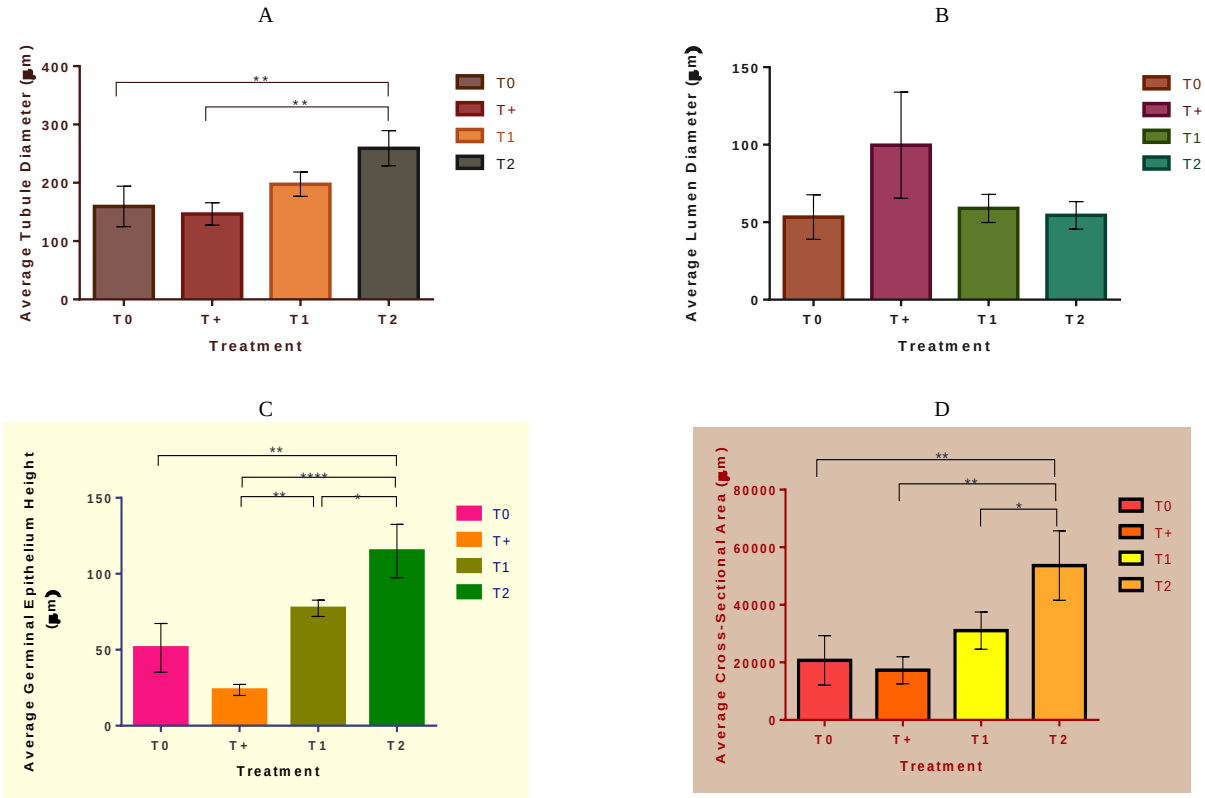


Fig. 6: Bar Graphs Showing the Mean and Statistical Significance in the Following Testicular Parameters: (A) Tubule Diameter; (B) Lumen Diameter; (C) Germinal Epithelium Height; (D) Cross-Sectional Area

Diameter, lumen diameter, germinal epithelium height and cross-sectional area of the seminiferous tubules of male albino mice: Figure 5a displays the normal stereology of the seminiferous tubules in T0. Figure 5b shows evidence of reduction in seminiferous tubule diameter, widening of the lumen, exfoliation of germinal epithelium, disorganization of seminiferous tubule shape and decreased cross-sectional area of seminiferous tubules in T+ due to tetracycline reprotoxicity (Farombi et al., 2008). Figure 5c and Figure 5d illustrate the preservation of the normal architectural design of seminiferous tubules in T1 and T2, respectively.

Statistical analysis on the diameter of seminiferous tubules revealed that T2 versus T0 and T2 versus T+ were statistically significant (Figure 6A). It means that the leaf extract in T2 is more effective than in T1 in widening the diameter of seminiferous tubules as well as mitigating the reprotoxicity of tetracycline. Statistical analysis of lumen diameter showed that there was no statistical significance between treatment groups. However, this does not necessarily mean that the *Z. talanai* leaf extract does not have the potential to mitigate the lumen widening effect of tetracycline because it was found out that there was a decrease in lumen diameter in T1 and T2 when compared to that of T+ (Figure 6B). Analysis of data of germinal epithelium height revealed that T2 versus T0, T1 versus T+, T2 versus T+ and T2 versus T1 were statistically significant. These findings only show that *Z. talanai* leaf extracts have the potential to increase the germinal epithelium height as well as mitigating the germinal epithelium exfoliation due to tetracycline reprotoxicity (Figure 6C). Statistical analysis of the cross-sectional area showed that T2 versus T0, T2 versus T+ and T2 versus T1 were statistically significant. It only means *that Z. talanai* leaf extract has the capacity to increase the cross-sectional area as well as to mitigate the toxicity of tetracycline (Figure 6D).

4. Discussion

The experimental study was conducted to evaluate the observable physical changes, body weight, hepatocyte cytoplasmic degeneration, central vein rupture, fibrosis, lymphocyte infiltration, microvesicular vacuolations, macrovesicular vacuolations, noticeable alterations in the histology of testicular tissue, tubule diameter and lumen diameter of randomly selected seminiferous tubules as well as the cross-sectional areas and germinal epithelium height of randomly chosen seminiferous tubules of male albino mice treated with tetracycline and varying dosages of *Ziziphus talanai* ethanol leaf extract.

The study revealed that male albino mice treated only with tetracycline exhibited lousy movements, localized hair loss, weight gain, hepatotoxicity adverse effects including hepatocyte cytoplasmic degeneration, central vein rupture, fibrosis, lymphocyte infiltration, small amount of glycogen deposits, fibrous septa, microvesicular vacuolations and macrovesicular vacuolations.

In addition, the reprotoxicity of tetracycline extends to the testes inducing damage to seminiferous tubules which includes irregular tubule shapes, exfoliation of the germ cells, absence of germ cell bundles, destruction of the blood-testis barrier, abnormal spacing of the adjacent seminiferous tubules, narrowing of tubule diameter, widening of the lumen diameter, exfoliated germinal epithelium and marked reduction on the cross-sectional areas of randomly selected seminiferous tubules.

Interestingly, male albino mice treated with *Ziziphus talanai* ethanol leaf extract mitigates the toxicity effects of the tetracycline in the liver tissue and seminiferous tubules. Mice treated with the leaf extract were not characterized by lousy movements as well as localized hair loss. Beneficial effects of *Ziziphus talanai* leaf extract on the liver tissue includes the normal liver architectural design based on brought-out central vein, normal integrity of the nucleus and nucleolus of hepatocytes, presence of glycogen deposits and congestion of microvesicular and macrovesicular vacuolations due to tetracycline toxicity.

Furthermore, *Ziziphus talanai* ethanol leaf extract provides favorable effects to the histology and stereology of seminiferous tubules male albino mice. These advantageous effects include the maintenance of the normal tubule shape, abundance of germ cell bundles, preservation of the blood-testis barrier, normal spacing between neighboring seminiferous tubules, increase tubule diameter with vivid lumen diameter as well as increase germinal epithelium height and cross-sectional areas without compromising the normal spermatogenic cycle.

Generally, it was assumed that the beneficial effect of *Ziziphus talanai* leaf extract is dose-dependent. Increasing the concentration of leaf extract may enhance the aforementioned advantageous effects. The preceding findings evidently showed that *Ziziphus talanai* has hepatoprotective and testiculoprotective potentials against adverse tissue alterations. In such cases, in the near future with further related and advanced researches, *Z. talanai* leaf extract can be utilized to treat liver diseases as well as promote wellness in the reproductive health of males.

5. Conclusion

It can be concluded that the ethanolic leaf extract of *Ziziphus talanai* (Blanco) Merr. possesses histoprotective potentials against hepatotoxicity and reprotoxicity. Further studies are hereby recommended to isolate and characterize the bioactive compounds that are responsible for this medical property. Other studies may explore the histoprotective potentials of the other parts of the tree.

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