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REGULAR ARTICLE

TOXICOLOGICAL EVALUATION OF AQUEOUS LEAF EXTRACT OF *SPONDIAS MOMBIN* USING ALBINO RAT

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ABSTRACT

In this study, aqueous leaf extract of *Spondias mombin* was evaluated for toxicity using albino rats was carried out. The test groups were placed on different doses of the studied leaf extract after the lethal dose was determined. The studied extractconsists mainly of flavonoids, saponins, alkaloid, tannins and cyanogenic glycosides, which have considerable therapeutic values, and reduced body weight considerably in test rats against the control. The tested parameters were reduced significantly ($p<0.05$) when compared to the control. However, the observed histopathological changes associated with the kidney and liver may suggest that the toxicity of the extract could be dose dependent. This study has shown the toxicological evaluation of aqueous leaf extract of *S. mombin* using albino rats.

Keywords: Aqueous extract, Bioactive constituents, *Spondias mombin,* Toxicology evaluation

INTRODUCTION

The quest to survive the numerous effects of disease pathogens and to stay alive by man, has led to the trial and establishment of different therapies that were deemed effective against disease causing pathogens [1]. Some of the known therapeutic methods used by man are established and generally accepted [2], some are still at the trial levels and wait for general acceptance, while some have long been rejected due to their obsolete and the questionable effects of their mechanisms in the biological system [3-5]. The therapeutic method that employs the services of plants as raw materials to remedy disease conditions caused by disease pathogens has long been accepted [2], due to its confirmed efficacy and detailed mechanism of action in the biological system [6]. It has been noted that 80% of the world herbal medicine is still the only medicine readily available and accessible to ordinary person [7-12]. Various terminology such as herbalism have been given to this therapeutic method that employs the services of plants to remedy disease conditions [13-19]**.** The plants used as raw materials in such practice are specifically termed medicinal plants [20]. According to Sofowora [17], plants with useful characters can be considered as medicinal. Duru *et al.* [21] noted that medicinal plants are seen as gift of nature against different disease conditions, and are raw materials for medicinal therapy. Plants are embodiment of compounds that are bioactive in nature, with physiological activity when administered to organisms [22-26]. These bioactive compounds are phytochemicals, and phytonutrients [27-30]. Amadi *et al.* [31], Duke [32] and Arukwe *et al.* [33] noted that phytochemicals are found virtually in different part of plants and at different concentrations. Record has it that man for long has taken advantage of the therapeutic value of medicinal plant constituents on production of drugs that are effective against infectious microorganisms and disease conditions. De Silva [34]; Oladunmoye [35]; and Agomuo *et al.* [36] reported that plant based compounds are in use of many modern medical systems.

Spondias mombin L. a tree plant that belongs to the family Anacardiaceae [15], is among the medicinal plants that man has long taken advantage of their medicinal value. It grows to about 12 to 25 m high with trunk covered with thick, rough bark [37]. The leaves are alternate, imparipinnate, and made of 5 to 8 pairs of opposite or alternate leaflets [38]. The fruits are ellipsoid or ovoid drupes, and yellow when matured with astringent flesh [38]. The fruit has sweet taste when ripped and acidic in nature [39]. In industrial worlds, the fruit of *S. mombin* useful in various industries [40]. The leaves are used in phytomedicine for the treatments of diseases like diarrhea, inflammation, prostatitis, herpes labialis, eye diseases, dysentery and painful colic [39, 41]. They are also used as purgative, laxatives and diuretics. It has been noted that the leaves of *S. mombin* can be used in dental problems. The stem bark of *S. mombin* is used against pain, excessive bleeding during menstruation and the hemorrhoid [37].

Despite the therapeutic approach of medicinal plants, studies have exposed their toxicity in the biological system

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[42-44], which could be chronic or acute in nature. This could be the reason why WHO in 2002 called for evaluation of quality, safety and efficacy of herbal medicines [45]. The growing interest in medicinal usage of *S. mombin* calls for it evaluation for precision in terms of administration.

The present study looked into this area and evaluated the toxicity of aqueous leaf extract of *S. mombin* using wistar albino rats.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *S. mombin* used in the present study were obtained from Ihie in Isiala Ngwa North L. G. A of Abia State, Nigeria. The plant was identified and authenticated at the Department of Plant Sciences and Biotechnology, Abia State University, Uturu, Nigeria.

Preparation of the extract

The identified plant leaves were washed with distilled water to remove dirt and contaminants. The leaves were dried under the sun to a fine powder. Two hundred (200g) of the powdered leaf sample was soaked in 1000 ml of distilled water and allowed to stand for 48 h with occasional stirring to allow for proper extraction. One ml of the filtrate was pipetted into a pre-weighed 100 cm^3beaker and evaporated to dryness on a boiling water bath. The beaker was cooled and weighed again and the concentration was determined to be 70 ml.

Determination of acute toxicity

Lethal dose was carried out and median lethal dose was calculated using modified method of Lorke [46].

Experimental animals and designs

Twenty five (25) male albino rats of the same stock were obtained from the animal house of Abia State University, Uturu. The animals were taken to the laboratory where they were housed in plastic cage and placed on commercial feeds bought from the local market as produced by Nigeria Flour Mills, and were allowed food and water *ad libitum*. Ethical principles in animal handling were adhered to strictly. The rats were allowed to acclimatize for the initial period of four days as the same feed and water were given to them within this period. After the acclimatization, the rats were randomly divided into five groups of five animals each. Group serves as the control, while the remaining four groups served as the tests groups. The treatments given to the experimental animals are as follow

Group 1: Feed+potable water(Control). Group 2: 200 mg/kg b. w of *S. mombin* leaf extract+feed+potable water Group 3: 400 mg/kg b. w of *S. mombin* leaf extract+feed+potable water Group 4: 600 mg/kg b. w of *S. mombin* leaf extract+feed+potable water Group 5: 800 mg/kg b. w of *S. mombin* leaf extract+feed+potable water

The body weights of the rats were taken at interval of 7 d from the day of commencement of feeding of the rats as day zero to the 28^{th} day. After the treatment period of twenty-eight (28) days, the rats were sacrificed, and blood was collected by cardiac puncture from the rats for analysis. Blood for liver enzyme studies were placed in anticoagulant free tubes, while the ones for hematological studies were placed in anticoagulant tubes.

Determination of liver integrity

Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were analyzed suing the methods as described by Reitman and Frankel [47].

Evaluation of the haematologicalparameters

Haematological parameters were estimated according to the methods of Dacie and Lewis [48].

Histopathological studies

Histopathological studies were conducted by following the method described previously [71].

Statistical analysis

Results were presented as means and standard deviations of triplicate determinations. Duncan multiple range test of data analysis was used to establish significant difference between groups at $p<0.05$.

Preliminary phytochemical evaluation of aqueous leaf extract of *S. mombin* as presented in table 1, shows the presences of tannins, flavonoids, glycosides, alkaloids, steroid and terpenoids. Both the beneficial [49-50], and lethal [51] effects of the observed constituents have earlier been noted. However, their lethal effects in a biological system depend on dosage or rather concentrations. Tannin is known for astringency [49]. Amadi *et al.* [51] noted that though the usefulness of flavonoids cannot be overstated, but they are toxic at high concentrations in the body system as well as in plants. Onwuka [52] noted that the knowledge of glycoside content of food is vital because its cyanide is an effective cytochrome oxidase inhibitor, and interferes with aerobic respiratory system. Alkaloids are associated with nerve poison [49]. High concentrations of steroid and terpenoids may definitely possess one or two negative effects on some steroids hormones. Observed concentrations of tannins, flavonoids, and terpenoids were in high concentrations, while glycosides, alkaloids, and steroids were in moderate concentrations (table 2).

RESULTS AND DISCUSSION

Table 1: Preliminary phytochemical screening of aqueous leaf extract of *S. mombin*

Phytochemicals	Status
Tannins	+++
Flavonoids	+++
Glycosides	++
Alkaloids	++
Steroids	++
Terpenoids	+++

Key: ++= present in moderate concentration, +++= present in high concentration

Table 2: Acute toxicity test (LD_{50}) of aqueous leaf extract of *S. mombin*

Group of rats/No of rats	Dose (mg/kg)	Number of deaths recorded/Mortality
1	400	Nil
2	800	Nil
3	1600	Nil
4	2500	Nil
5	5000	Nil

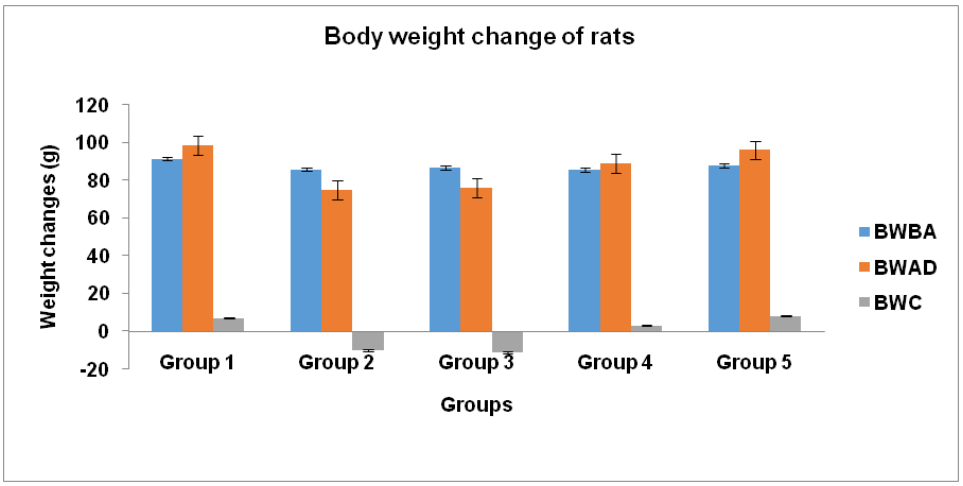

Fig. 1: Body weight change of rats

Key: BWBA: Body weight before administration; BWAD: Body weight after administration; BWC; Body weight change. Bars of the same group with different letters of alphabets are statistically significant ($p<0.05$).

Table 3: Effect of aqueous extracts of *S. mombin*on liver enzymes

Groups	ALT (IU/l)	AST (IU/l)	ALP (IU/l)	Total bilirubin (mg/l)	Conjugate bilirubin (mg/dl)
Group 1	18.87 ± 0.31^{a}	20.12 ± 0.42^{a}	24.25 ± 9.09^{a}	0.540 ± 0.008^{c}	0.143 ± 0.013^{a}
Group 2	18.23 ± 0.14^{b}	20.79 ± 0.65^{b}	31.13 ± 7.34^{b}	0.520 ± 0.008^{c}	0.170 ± 0.04^{a}
Group 3	19.19 ± 0.23^{c}	23.36 ± 0.46^{c}	35.53 ± 1.61^{c}	0.5000 ± 0.014^{b}	0.160 ± 0.00^{a}
Group 4	21.00 ± 0.13^{d}	36.90 ± 0.46^{d}	37.17 ± 0.70^{d}	0.485 ± 0.021^{b}	0.170 ± 0.00^{a}
Group 5	29.00 ± 0.13^{e}	42.49 ± 0.30^{e}	39.34 ± 3.38^{e}	0.468 ± 0.015^{a}	0.173 ± 0.005^{a}

Values are mean and standard deviation of triplicate determinations. Values with letters of alphabets down the column or groups as superscript are statistically significant at $p<0.05$.

Oral administration of *S. mombin* from 400 to 5000 mg/kg (table 2), did not produce any toxicity on the albino rats. Mortality was not recorded throughout the duration of this study. No death was recorded at all doses at the end of the 24 h of study. This indicates that the LD_{50} of the extract is far above 5000 mg/kg body weight.

The observed body weight change (BWC) of the test rats reduced significantly ($p<0.05$), when compared to the control (fig. 1) in the present study. This is not in line with the earlier observations made by Duru *et al.* [28], on rats given "udu, an antimalarial concoction made from combination of herbal leaves, and Mbongue *et al.* [53] on rats treated with *P. guineese* for 55days. Zhou *et al.* [54] attributed body weight increase in rats to androgenic property of the substance given to the rats as a result of anabolic activity, while Agomuo *et al.* [36]; and Yehya (2001) noted that loss in weight could be as result of increase in sympathetic tone.

Nisirm [56]; Mathew [57]; and Duru *et al.* [58] noted an increase in activity of serum aminotransferase. It has been noted that injury in the liver may be reflected by elevation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) along with elevation of alkaline phosphatase (ALP) activity [59]. Different authors [60-64] have shown that ALT is a more specific enzyme of liver damage than AST. Levels of AST and ALP in test rats (Groups 2-5) increased significantly ($p<0.05$) against the control. ALT in group 2 rats significantly ($p<0.05$) reduced against the control (Group 1), while AST levels in groups 3, 4 and 5 increased significantly ($p<0.05$), when compared to AST of group 1 (control). According to Enemor *et al.* [59], small increase in AST and ALT may be as a result of wide range of liver diseases. The observed increase in ALT and AST could be indication of hepatocelluar damage by aqueous extract of *S. mombin*. Duru *et al.* [65] noted that bilirubin is associated to protein breakdown in the system.

Table 4: Effect of *S. mombin* leaf extract on haematological parameters

Treatment	Haemoglobin (g/dl)	Haematocrite (%)	Erythrocyte (10^3/ul)	Leukocytes (10^3/ul)
Group 1	10.61 ± 0.60^a	45.78 ± 2.64^b	7.01 ± 0.32^d	19.58 ± 3.40^d
Group 2	14.82 ± 0.24^b	47.92 ± 0.80^c	8.05 ± 0.16^c	20.24 ± 2.61^b
Group 3	16.12 ± 0.20^c	48.80 ± 0.85^d	8.10 ± 0.10^b	21.24 ± 2.50^b
Group 4	17.00 ± 0.21^c	51.00 ± 0.87^a	9.00 ± 0.15^a	25.02 ± 2.41^a
Group 5	20.01 ± 0.30^d	60.10 ± 0.90^a	9.06 ± 0.20^a	26.32 ± 1.50^a

Values are mean and standard deviation of triplicate determinations. Values with letters of alphabets down the column or groups as superscript are statistically significant at $p<0.05$.

The levels of conjugated bilirubin of test rats increased insignificantly ($p>0.05$), when compared to the control, while total bilirubin reduced significantly ($p<0.05$) against the control. This could be bilirubin retention, which may signify liver damage arising from the administration of the aqueous leaf extract of the studied plant.

According to Duru *et al.* [65, 66], assessment of haematological parameters is very useful. Haematological parameters are of diagnostic significance in the routine clinical evaluation of the state of health [67]. The results in table 4 established the haematological activity of the studied leaf extract. Haemoglobin is known to be a protein used by erythrocytes to distribute oxygen to other tissues and cells in the body [68]. The haemoglobin and haematocrite levels increased ($p<0.05$) in test rats (groups 2-4) against group 1(Control) rats. Defective haematopoiesis is indicated by reduction in erythrocytes number and haemoglobin content, and such was not observed in the present study. Duru *et al.* [65] noted that increase in haemoglobin level is normally followed by corresponding increase in haematocrite level. The increase ($p<0.05$) in erythrocyte of test rats (Groups 2-4) could be indication that the balance between rate of erythrocyte production and destruction. Levels of leukocytes significantly increased ($p<0.05$) in test groups 2-4, against group 1(Control). Adebayo *et al.* [69], and Celik and Suzek [70] noted leukocytosis may be directly proportional to the severity of the causative stress condition.

Histopathology of the kidney from rats (Groups 2-4) placed on the aqueous leaf extractas compared to those of the control (Group 1) revealed normal renal histo-architecture, glomeruli, renal tubules, blood vessels, and Bowman's capsule. Sections of the kidney from group 5 which was administered the highest dose of the extract showed changes consistent with renotoxicity. Normal structures of the glomeruli and Bowman's capsules were observed. However, the renal tubules in both the cortex and outer medulla showed varying degenerative and necrotic changes in the tubular epithelial lining cells. The lesions were randomly observed affecting all the proximal convoluted tubules, pars recta and distal convoluted tubules of the cortex and outer medulla while the collecting ducts appeared normal. The changes varied from cellular swelling and vacuolar degeneration to cellular necrosis with nuclear pyknosis and/or karyorrhexis. The basement membranes of the renal tubules were unaffected.

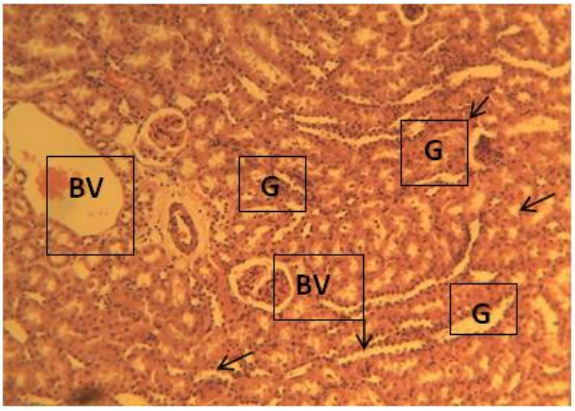

Plate 1: Photomicrograph of the kidney group 1 (Control), showing the normal renal histo-architecture. Glomeruli (G), renal renal tubules (arrow), and blood vessels (BV). H and E x100

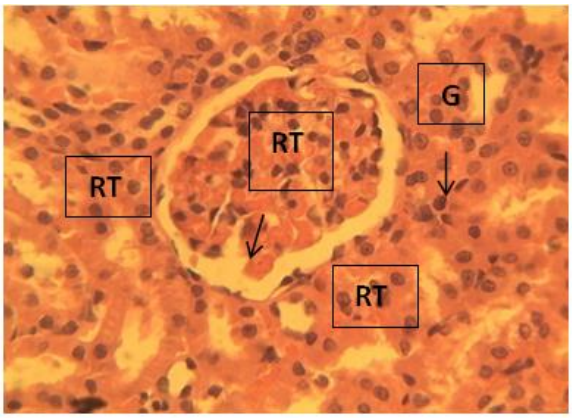

Plate 2: Photomicrograph of the kidney in group 2 showing, the normal renal histo-architecture. Glomeruli (G), tubules (RT), Bowman`s capsule (arrow). HandEx 100

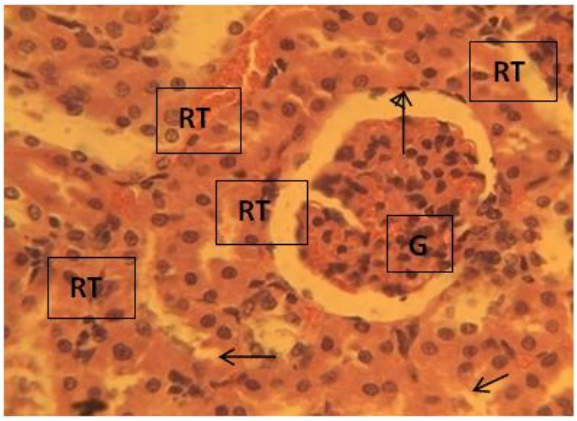

Plate 3: Photomicrograph of the kidney in group 3 showing, the normal renal histo-artitecture. Glomeruli (G), Renal, tubules (RT), Bowman`s capsule (arrow). HandEx100

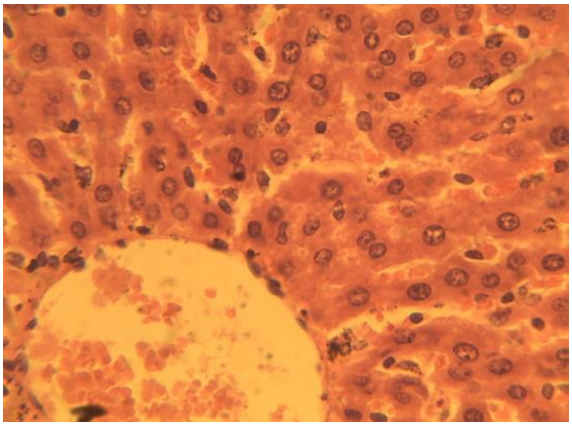

Plate 6: Photomicrograph of the liver collected from group 1showing the arrangement of normal hepatoctes in interconnecting chorts (arrow) around a central view HandEx100

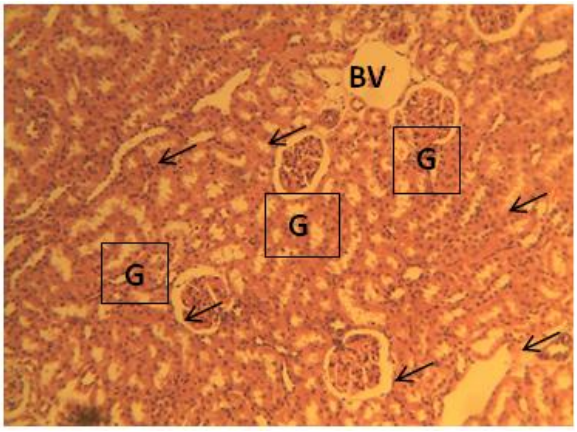

Plate 4: Photomicrograph of the kidney in group 4 showing, the normal renal hist-artitecture. Glomeruli (G), Renal tub-ubules (arrow), Blood vessel (BV). HandEx100

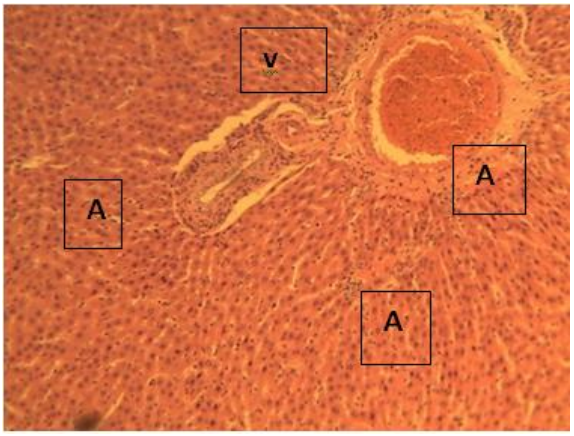

Plate 7: Photomicrograph of the liver collected from group 2 showing the normal hepatic histo-architecture. The hepatocytes can be observed, arranged in chords and converging towards the portal area which contains the hepatic artery (A), hepatic vein (V) and bile duct (B), Central vein(C). H and Ex100

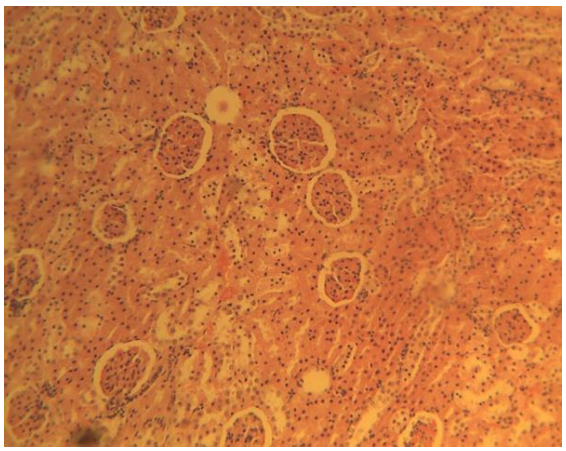

Plate 5: Section of the kidney collected from group 5, showing numerous tubules of the cortex undergoing, degenerative and necrotic changes (arrow). Glomeruli, (V)(G), Pyknotic nucleus (arrow). HandEx100

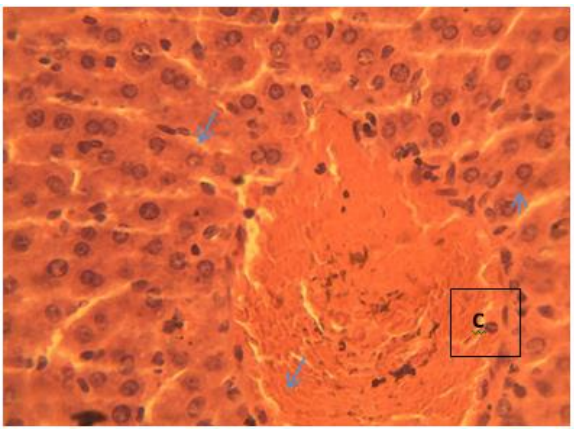

Plate 8: Photomicrograph of the liver from group 3 showing the normal hepatic histo-architecture Normal hepatocytes arranged in chords (arrow) can be seen around the central vein (C). HandEx4000

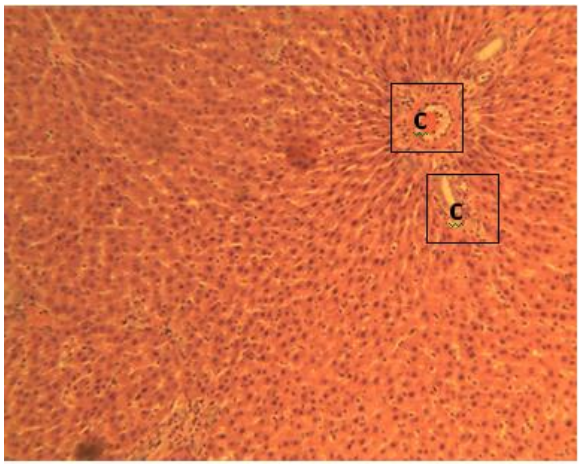

Plate 9: Photomicrograph of the liver collected from group 4 showing the normal hepatic histo-architecture. Normal hepatic arranged in radiating chords around the central cytopl-veins can be observed. Normal components of asms. the portal area can also be observed. Hepatic artery (A), Bile duct (B), Central vein (V). HandEx100

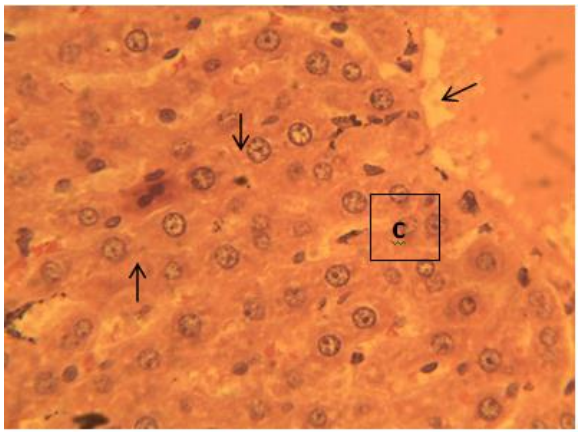

Plate 10: Photomicrograph of the liver collected from group 5 showing a mild to moderate, widespread vacuolar degeneration of the hepatocytes (arrow). The hepatocytes appear slightly swollen and contain numerous s minute vesicles (clear spaces) in their Central vein (C). HandEx100

In histopathology, hepatic chords are separated by the hepatic sinusoids and radiate towards the periphery of the hepatic lobules where they join the portal triads which are made up of normal hepatic artery, hepatic vein and bile ducts. The group that was administered the higher dose shows a mild to moderate, widespread vacuolar degeneration of the hepatocytes (arrow). The hepatocytes appear slightly swollen and contain numerous minute vesicles (clear spaces) in their cytoplasms. Sections of the liver collected from the animals in group 5 which was treated with the highest dose of the test extract showed changes consistent with hepatotoxicity. Mild to moderate cellular swelling were observed, involving all the described anatomic zones of the hepatic lobule (centrilobular, mid-zonal and periportal zones). The hepatic chords consisted of hepatocytes with swollen, micro-vessiculated cytoplasm. The swollen hepatocytes tend to occlude the hepatic sinusoids.

CONCLUSION

This research has provided information on the major bioactive constituents of the *S. mombin* aqueous leaf extract. It consists mainly of flavonoids, saponins, alkaloid, tannins and cyanogenic glycosides, which have considerable therapeutic values. The study also showed the increase in levels of AST, ALT, ALP in rats given the extracts, and histopathological of both kidney and liver changes associated with the studied extract in rats. This study however showed that the toxicity of *S. mombin* leaf extract is dose dependent. This study has shown the toxicological evaluation of aqueous leaf extract of *S. mombin* using albino rat.

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