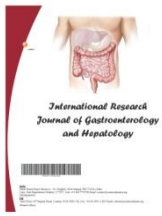

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Assessment of the Effects of *Morus mesozygia* Linn. *Stapf.,* Leaf Extracts on the Liver of Streptozotocin-Induced Diabetic Wistar Rats

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Aim: To assess the effect of *Morus mesozygia Linn. Stapf.,* leaf extracts on the liver of Streptozotocin-Induced Diabetic Wistar Rats.
Study Design: Case-controlled experimental study.
Place and Duration of Study: Biochemistry Research Laboratory, University of Port Harcourt, Rivers State, Nigeria, between June 2018-April 2019.
Methodology: A total of 65 male albino rats that weighed between 150g to 200g were used for this research study. Three different extracted solvents; aqueous, ethanolic and methanolic leaves extracts were administered to different groups of the rats. The male albino rats for this study were induced with a single dose of 40mg/kg b.wt, intraperitoneally of streptozotocin in 0.1M of citrate buffer, pH 4.5. The diabetic male rats were those whose fasting blood glucose (FBG) levels were from 250 mg/dl or 13 mmol/L and above. The rats were divided into different experimental groups based on the treatment regimen; administration of the herb was done orally after intraperitoneal injection of streptozotocin. The rats were sacrificed after 30 days and blood samples were collected for the estimation of liver function parameters using spectrophotometric method. Histological analysis was done and the slides were stained using H & E staining technique. Graph pad prism (version 5.01) was used for statistical analysis and p values less than 0.05 were considered statistically significant.
Results: The results showed that intraperitoneal induction of diabetes with streptozotocin caused a significant increase ($p<.05$) in the activity of AST, ALT and ALP (5.92 ± 0.49 to 11.88 ± 1.2 U/L),

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(2.69 ± 0.43 to 4.43 ± 0.53 U/L) and (22.6 ± 4.78 to 36.04 ± 2.68 U/L) respectively. However, treatment with 200 and 400mg/kg of aqueous, methanolic and ethanolic extracts of *Morus mesozygia Linn. Stapf* leaves caused a significant reduction in the activity of these enzymes.
Conclusion: *Morus mesozygia Linn. Stapf* leaves extracts showed strong hepatoprotective effect, particularly the methanolic and ethanolic extracts. Further studies to unravel the molecular mechanism involved is hereby recommended.

Keywords: Morus mesozygia Linn. Stapf.; leaf extracts; liver; streptozotocin-induced diabetic rats.

1. INTRODUCTION

The liver has been described to be the largest organ in the body. It is also known to receive oxygen rich blood from the hepatic artery while the liver receives blood from the other parts of the body such as the intestines from the portal veins, it is an organ that performs detoxification of waste metabolites such as drug metabolites, and as a result is susceptible to damages caused by these compounds or their metabolites, especially when their concentrations exceed the detoxifying capacity of the liver [1]. Plant extracts given orally to experimental rats have been reported to be of possible toxicity, therefore, the following biochemical liver enzyme tests are important to analyze for hepatocellular damage: alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), amino peptidase, 5-nucloetidase (5' NTD), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT) and a host of others are essential for such assessment. Aminotransferases are enzymes that have been metabolized from proteins that are further used to synthesize processes of amino acids, they are otherwise known as transaminases and are known for their unique functioning in catalyzing transaminase reaction that has to do with an alpha keto acid and an amino acid [2].

These enzymes are of clinical relevance in as much as their levels are elevated in plasma due to moderate or excessive cell turn over in the liver, due to toxicity elicited by viral, bacterial or chemical aetiology The distinct clinical significance of the assessment of aspartate aminotransferase is that extreme values are significant for injuries that have been sustained on the liver. In a case where the levels of serum AST is greater than ALT, this signifies that there is hepatic necrosis [3]. Higher concentrations of ALP are commonly exhibited in the liver and bones of most infants as a result of several bone actions, likewise it is produced in the third trimesters of pregnant women [4]. Elevated concentrations of ALP are indicative of bone and liver disease.

There are evidences that STZ causes both direct organ toxicity and diabetes, and both actions can affect many organs [5] including liver [6]. In addition, previous studies have reported "the STZ-induced diabetes mellitus and its association with specific diabetic complications and disturbances in various tissues, such as diabetic nephropathy and cardiovascular diseases, but only limited data is available on the possible association between diabetic complications and liver function" [7]. The aim of this study was to assess the effect of *Morus mesozygia Linn. Stapf.,* leaf extracts on the liver of Streptozotocin-Induced Diabetic Wistar Rats.

2. MATERIALS AND METHODS

2.1 Animal Preparation

"All male albino rats of (150g to 200g) in weight were purchased from the University of Port Harcourt. They were used throughout the course of this research work and were made to acclimatize for 14days under standard laboratory conditions, fed with pelleted rat chow (Top Feed Finisher Mash, Nigeria) and tap water *ad libitum*. The rats were fed with high fatty feeds which were commercially prepared with margarine and sucrose in combination with the pelleted chow to initiate obesity, recent studies have reported that high fatty diets give out free radicals that contribute to the impairment of beta cells hence hyperglycemia and its subsequent complications" [8].

2.2 Plant Collection and Authentication

"Morus mesozygia Linn. (Family Moraceae) fresh leaves samples were collected by Dr. Oladele, A.T. in the month of July, 2018 from an abandoned, fallow- farmland at Ile –Ife, Ilesha Road, Ile-Ife, Osun State, South-Western Nigeria and was authenticated by plant botanist, Dr.

Oladele A.T.at the Department of Forestry and Wildlife Management, University of Port Harcourt with the herbarium voucher number (UPFH 0125) and the fresh leaves were submitted at the department's herbarium" [9].

2.2.1 Preparation of plant extract (Cold maceration extraction method)

The *Morus mesozygia linn* leaves were washed with distilled water and air dried separately for seven days and milled into fine powder with the use of a milling machine, the powdered leaves produced a total weight of 2.90kg, they were stored and labelled into an air tight container prior to use.

2.2.1.1 Extraction of powdered Morus mesozygia linn leaves using distilled water, absolute ethanol and methanol

"Nine hundred and sixty grams (960g) of dried powdered *Morus mesozygia linn* leaves were put into a clean beaker, five liters (5L) of distilled water, ethanol and methanol separately and were suspended into the beaker, they were shaken severally on a shaker, they were mixed properly and stored for 24hours. They were macerated and filtered through a muslin cloth and again filtered out through a Whatman's number one filter paper. The filtered extracts were concentrated (on low pressure) using the rotary evaporator equipment [10] after which they were dried on an evaporating dish at a temperature of 50^{o}C to 60^{o}C to a semi- solid form". "A sticky semi-solid dark brownish substance was obtained. The extracts were stored in a well corked universal bottle. The leaf extracts were kept in a 4^{o}C refrigerator prior to pharmacological investigations" [9].

2.2.2 Aqueous and ethanolic extract dosage calculation

"Based on the results from the Acute Toxicity test carried out, (not shown) doses adopted for this research study that was administered orally into the rats were 200mg/kg (low dose dose) and 400mg/kg (high) respectively. The average weights of the experimental rats in each of the groups were taken as these were used to calculate the doses of the extracts that were administered" [9].

2.2.3 Metformin dosage administration

"The metformin round tablet brand of Sandox tablet of 500mg was crushed and dissolved in normal saline containing 0.9% of sodium chloride (weight per volume) sodium citrate for the oral administration into the fasted diabetic rats as desired doses of 100mg/kg used by Metformin direct calculation of animal dose from human dose" [9].

2.3 Citrate Buffer Solution Preparation

The citrate buffer solution is a combination of citric acid salt and sodium citrate salt. About 1.47grams of the sodium citrate salt was measured and dissolved in 50ml of distilled water, this was followed by weighing 1.05gram of citric acid salt which was dissolved in 50ml of distilled water. The mixtures were thoroughly stirred to enable it evenly mixed together and a PH meter was used to check and adjust the pH buffer to 4.5.

2.4 Induction of Diabetes with Streptozotocin

"After two weeks of acclimatization, diabetes was induced in the male albino rats with streptozotozin (STZ, Sigma Chemical Company, St. Louis, Milestone). STZ was intraperitoneally (i.p.) administered in a dose of 40mg/kg dissolved in citrate buffer (0.1M, pH 4.5). Blood glucose concentrations were measured by Fine Test glucometer (Johnson & Johnson) after 48 hours and subsequently throughout the experiment after diabetes induction and glucose concentrations exceeded 250 mg/dl or 13mmol/L confirmed the diabetic state" [11]. The diabetic male rats were picked and used for the study design.

2.5 Administration of *Morus mesozygia* Linn. (African Mulberry) for Treatment

"After the rats were confirmed to be diabetic at above 13mmol/L, blood samples were collected from the tail end of the rats. The assay of the blood glucose levels was carried out by the glucose-oxidase principle" [12]. Finetest™ test strips and FineTest Auto Coding™ Premium Glucometer, INFOPIA Company, Limited, Korea) was used for the determination of the blood glucose levels of the animals and the results expressed as mmol/L. The administration of the *Morus mesozygia linn*. for the leaf aqueous and ethanol extracts were administered by the use of oral gavage method.

2.6 Study Design

The rats were acclimatized for two weeks prior to the treatments. They were randomly separated into 13 groups of 5 rat each as shown below:

- **Group One**: 5 male rats were given pellet feeds and water *ad libitum*, this served as the 'Negative Control' group
- **Group Two**: 5 male rats were induced intraperitoneally with a single dose of 40mg/kg body weight of streptozotocin and were fed with pellets and water *ad libitum*, this served as the 'Positive Control' group.
- **Group Three**: 5 male rats were given 400mg/kg body weight orally of aqueous leaf extract only
- **Group Four**: 5 male rats were given 400mg/kg body weight orally of ethanolic leaf extract only
- **Group Five:** 5 male rats were induced with a single dose of 40mg/kg body weight of streptozotocin and treated with 400mg/kg body weight of aqueous leaf extract
- **Group Six:** 5 male rats were induced intraperitoneally with a single dose of 40mg/kg body weight of streptozotocin and treated orally with 200mg/kg body weight of aqueous leaf extract
- **Group Seven**: 5 male rats were induced intraperitoneally with a single dose of 40mg/kg body weight of streptozotocin and treated orally with 400mg/kg body weight of ethanolic leaf extracts
- **Group Eight:** 5 male rats were induced intraperitoneally with a single dose of 40mg/kg body weight of streptozotocin and treated orally with 200mg/kg body weight of ethanolic leaf extracts.
- **Group Nine:** 5 male rats were induced intraperitoneally with a single dose of 40mg/kg body weight of streptozotocin and treated orally with 100mg/kg body weight of metformin standard drug.
- **Group Ten**: 5 male rats were induced intraperitoneally with a single dose of 40mg/kg body weight of streptozotocin and treated orally with 200mg/kg body weight of aqueous leaf extract and 100mg/kg of metformin.
- **Group Eleven**: 5 male rats were given 400mg/kg body weight orally with methanolic leaf extract only
- **Group Twelve**: 5 male rats were induced intraperitoneally with a single dose of 40mg/kg body weight of streptozotocin and treated orally with 200mg/kg body weight of methanolic leaf extracts
- **Group Thirteen**: 5 male rats were induced intraperitoneally with a single dose of 40mg/kg body weight of streptozotocin and treated orally with 400mg/kg of methanolic leaf extracts

2.7 Collection of Sample and Laboratory Analysis

2.7.1 Sample collection

The rats were kept on fasting for 6 hours prior to the process of euthanasia, they were also weighed before the process started. Blood samples were collected for analysis into plain and fluoride oxalate bottles for the estimation of liver function tests and glucose respectively.

2.7.2 Experimental analysis

2.7.2.1 Determination of liver function parameters

The determination of blood glucose concentration in this assay adopted the principle of 'Electro-Chemistry' a reaction described by Kuhn, [13] and Liver Enzymes were estimated by Reitzman and Frankel Method, [14], using spectrophotometric method.

2.7.2.2 Histopathological analysis of the liver

The liver of two rats per group were harvested and transferred into a sterile universal container containing 10% formol saline fixative for preservation. They were sliced (in sizes of about 3mm x 3mm and allowed to dehydrate in various concentrations of ethanol firstly in ascending grades (50%,70%,90%,95%) and then finally in 100% absolute alcohol. They were cleared with xylene passed twice for a period of 1 to 2 hours. This was then followed by embedding the tissues in a molten paraffin wax in constant temperatures of 56 to 60 degree Celsius and allowed to cool in a solid L-shaped form, the tissues were then placed inside the block and sectioned (after having a consistent form) with a Shandon AS Rotary microtome. The sectioned tissues were then stained with Hematoxylin and Eosin (H & E) and the slides were studied for histopathological lesions. The photomicrographs were made with the use of an Olympus microscope CX31, model CX31RTSF, Tokyo, Japan.

2.8 Statistical Analysis

Statistical evaluation was made possible with the application of Graph pad prism (version 5.01). Data generated were revealed as mean and standard deviations (Mean ± S.D) in addition to the use of ANOVA (Tukey's Multiple Comparative Test) since the comparison is within more than two group study. The level of significance was tested at ($p<0.05$).

3. RESULTS AND DISCUSSION

This study evaluated the effect of *Morus mesozygia Linn. Stapf.,* leaf extracts on the liver of Streptozotocin-Induced Diabetic Wistar Rats. Table 1 (Aqueous leaf), 2 (Methanolic leaf) and 3 (Ethanolic leaf) of the different plant extracts showed the liver enzyme levels in the 30 days' treatment of the diabetic rats. The plant concentration of 400mg/kg of ethanolic leaf revealed a significant decrease in AST, ALT and ALP when the diabetic rats were treated orally, followed by aqueous then methanolic extracts. This reduction in liver enzymes showed the hepato-protective effect the 400mg/kg in body weight of ethanolic leaf extract possess in the diabetic rat model. This hepato-protective effect of the 400mg/kg of ethanolic leaf extract may be due to the presence of flavonoids 12.71%, [15]. Flavonoids was reported to act as hepato-protective agent as it helps to scavenge a complex series of reactions that are initiated by lipid peroxidation which is a generalization of hydrogen from fatty acid that dismisses the complete breakdown of the polyunsaturated fatty acid molecules with the formation of aldehydes such as in malondialdehyde that can cause liver damage. This was similar to the reports of Salemi et al. [16] who reported decrease in liver enzymes.

Table 1. Mean and standard deviations of serum Aspartate Transaminase (AST), Serum Alanine Transaminase (ALT) and Serum Alkaline Phosphatase (ALP) parameters of streptozotocin induced diabetic male rats treated orally for 30 days with 400mg/kg, 200mg/kg of aqueous leaves of *Morus mesozygia Linn. Stapf.* extracts compared 100mg/kg of metformin standard drug with non-treated controls

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
GRP1NC	5.92 ± 0.49^{2}	2.69 ± 0.43^{2}	22.6 ± 4.78^{2}
GRP2PC	11.88 ± 1.2	4.43 ± 0.53	36.04 ± 2.68
GRP3	4.75 ± 0.90^{2}	2.16 ± 0.21^{2}	19.15 ± 3.71^{2}
GRP5	6.11 ± 0.41^{2}	1.79 ± 0.47^{2}	23.39 ± 8.75^{2}
GRP6	5.94 ± 0.47^{2}	2.75 ± 0.45^{2}	30.02 ± 3.37
GRP9	4.27 ± 0.52^{2}	2.44 ± 0.70^{2}	28.2 ± 6.36
p-values	< 0.0001	< 0.0001	0.0006
F-values	71.22	17.22	6.45
Remark	S	S	S

Key: S – significant at p<0.05 for ANOVA and ^{2} signifies significant when compared with Group 2

Table 2. Mean and standard deviations of serum Aspartate Transaminase (AST), Serum Alanine Transaminase (ALT) and Serum Alkaline Phosphatase (ALP) parameters of streptozotocin induced diabetic male rats treated orally for 30 days with 400mg/kg, 200mg/kg of methanolic leaves of *Morus mesozygia Linn. Stapf.* extracts compared 100mg/kg metformin drug and non-treated controls

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
GRP1NC	5.92 ± 0.49^{2}	2.69 ± 0.43^{2}	22.6 ± 4.78^{2}
GRP2PC	11.88 ± 1.2	4.43 ± 0.53	36.04 ± 2.68
GRP11	4.97 ± 1.00^{2}	1.77 ± 0.17^{2}	36.78 ± 5.90
GRP13	5.41 ± 0.76^{2}	2.59 ± 0.38^{2}	31.42 ± 5.61
GRP12	5.22 ± 0.30^{2}	2.49 ± 0.77^{2}	23.84 ± 3.08^{2}
GRP9	4.27 ± 0.522^{2}	2.44 ± 0.70^{2}	28.2 ± 6.36
p-values	< 0.0001	< 0.0001	0.0003
F-values	64.19	13.57	7.319
Remark	S	S	S

Key: S – significant at p<0.05 for ANOVA and ^{2} signifies significant when compared with Group 2

Table 3. Mean and standard deviations of serum Aspartate Transaminase (AST), Serum Alanine Transaminase (ALT) and Serum Alkaline Phosphatase (ALP) parameters of streptozotocin induced diabetic male rats treated orally for 30 days with 400mg/kg, 200mg/kg in doses ethanolic leaves of *Morus mesozygia Linn. Stapf.* extracts compared with controls

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
GRP1NC	5.92 ± 0.49[2]	2.69 ± 0.43[2]	22.6 ± 4.78[2]
GRP2PC	11.88 ± 1.2	4.43 ± 0.53	36.04 ± 2.68
GRP4	8.29 ± 2.84[2]	2.03 ± 0.22[2]	20.06 ± 1.39[2]
GRP7	4.50 ± 0.59[2]	1.52 ± 0.53[2]	20.5 ± 3.51[2]
GRP8	5.87 ± 0.73[2]	2.09 ± 0.49[2]	22.84 ± 1.72[2]
GRP9	4.27± 0.52[2]	2.44 ± 0.70[2]	28.2 ± 6.36[2]
p-values	< 0.0001	< 0.0001	< 0.0001
F-values	22.68	19.56	12.8
Remark	S	S	S

Key: S – significant at p<0.05 for ANOVA and [2] signifies significant when compared with Group 2

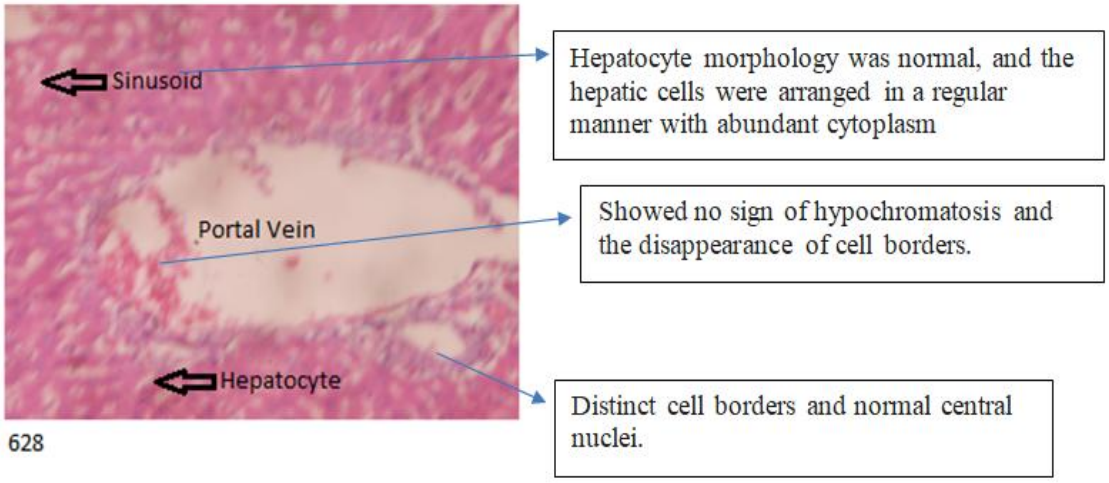

Plate 1. Group 1 (NEG. CONTROL). H&E. MAG X400. LIVER.DOSE: Distilled water. Normal portal vein that is patent with a normal hepatocyte and sinusoid

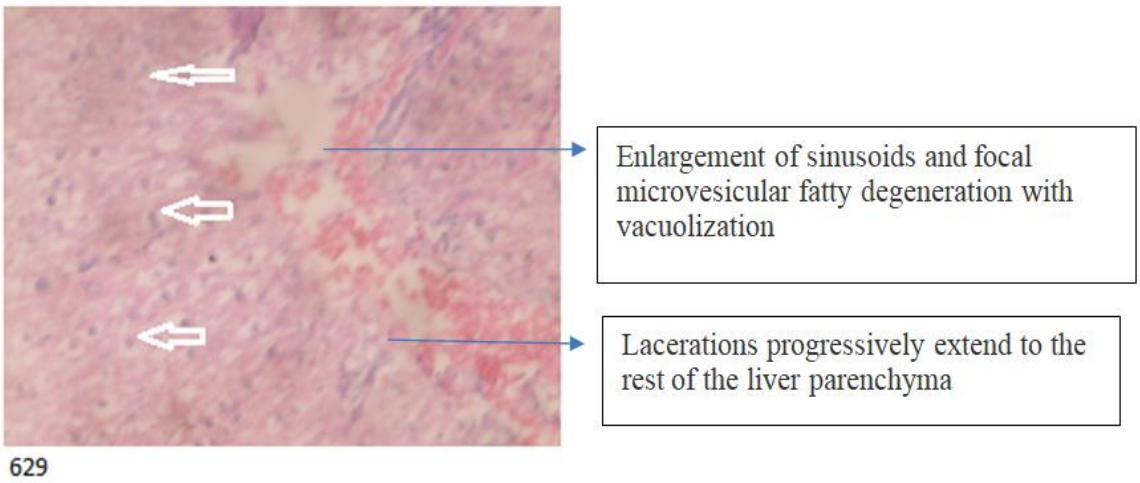

Plate 2. Group 2 (Positive Control).H&E. MAG. X400. LIVER. Diabetic. DOSE: 40mg/kg of STZ in 0.1M Sodium Citrate. Distorted Hepatocyte, distorted microvascular steantosis

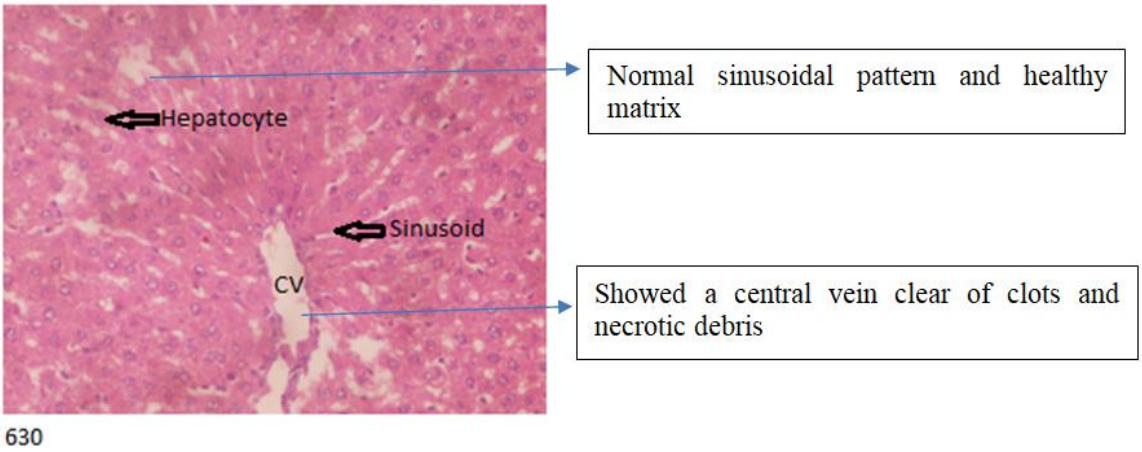

Plate 3. Group 5. H&E. MAG. X400. LIVER. DOSE: 400mg/kg (ORAL). Diabetic, treatment with aqueous leaf extract of *MMLS*

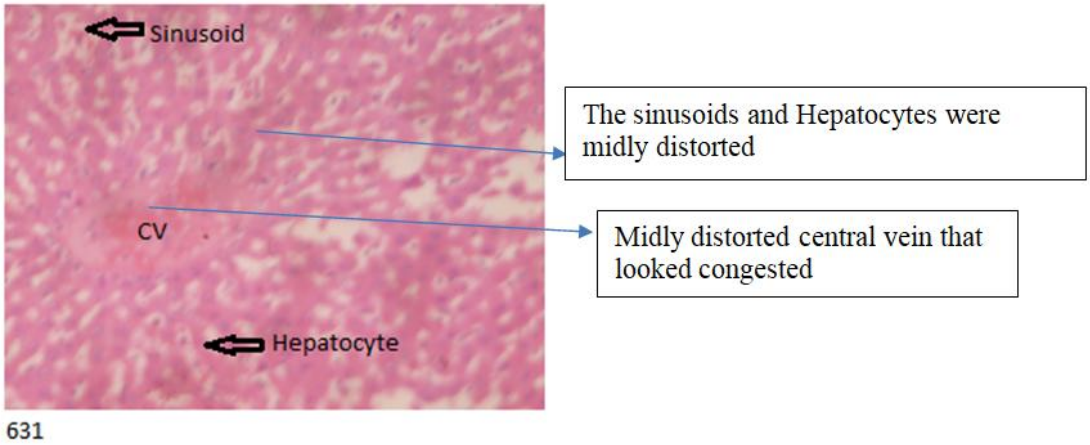

Plate 4. Group 6. H&E. MAG.X400. LIVER. DOSE: 200mg/kg (ORAL). Diabetic. Treatment with aqueous leaf extract of *MMLS*. Mildly distorted central vein that looked congested. The sinusoids and hepatocyte were mildly distorted

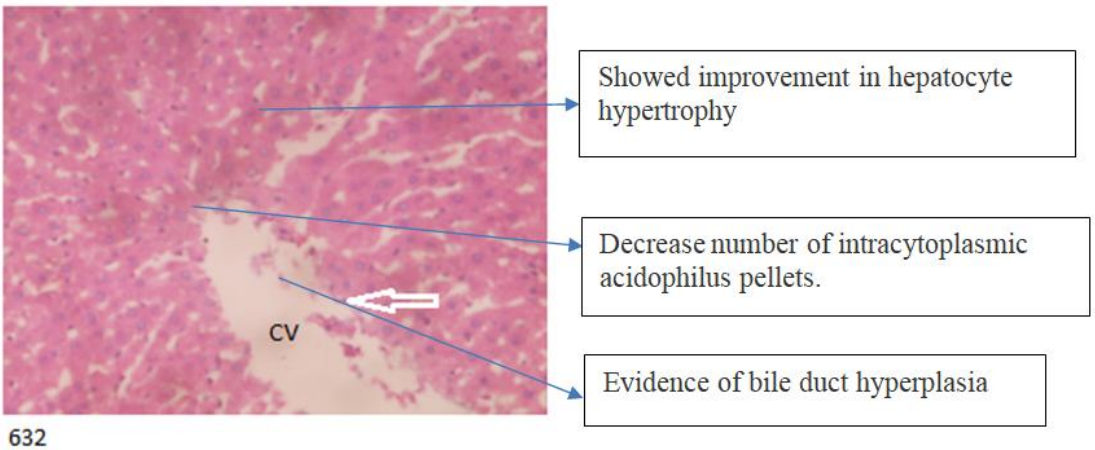

Plate 5. Group 13. H&E. MAG. X400. LIVER. Diabetic. DOSE:400mg/kg(ORAL). Treatment was with methanolic leaf extract of *MMLS*

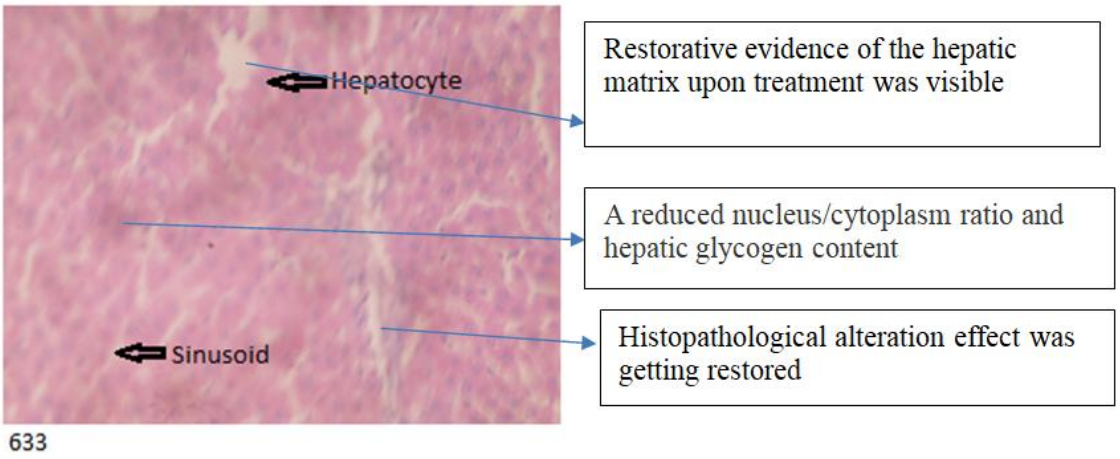

Plate 6. Group 12. H&E., MAG. X400. LIVER. DOSE:200mg/kg(ORAL). Diabetic. Treatment with methanolic leaf extract of *MMLS*. Normal liver

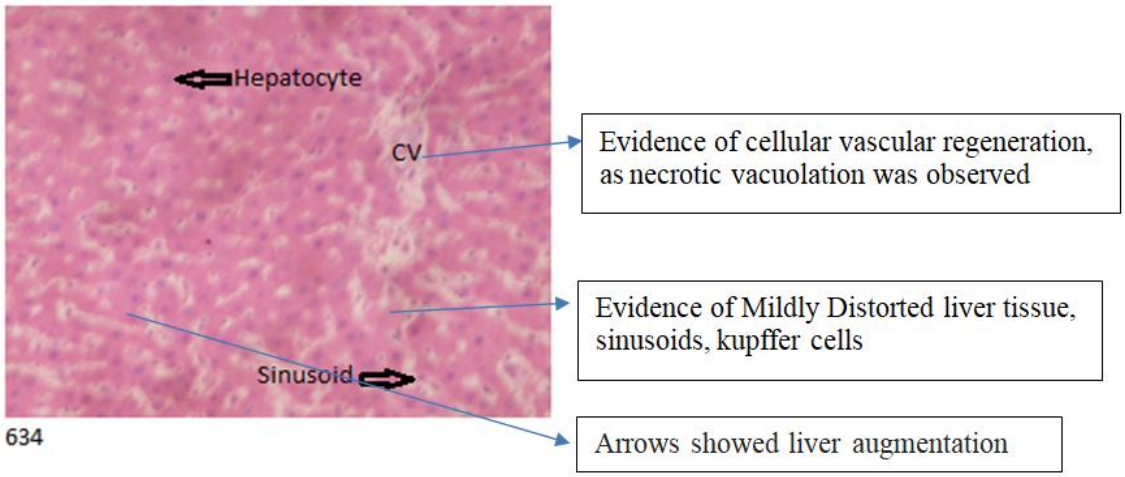

Plate 7. Group 7 H&E. MAG. X400. LIVER. Diabetic. DOSE: 400 (ORAL). Treatment with ethanolic leaf extract of *MMLS*. Mildly Distorted liver, sinusoids, kupffer cells

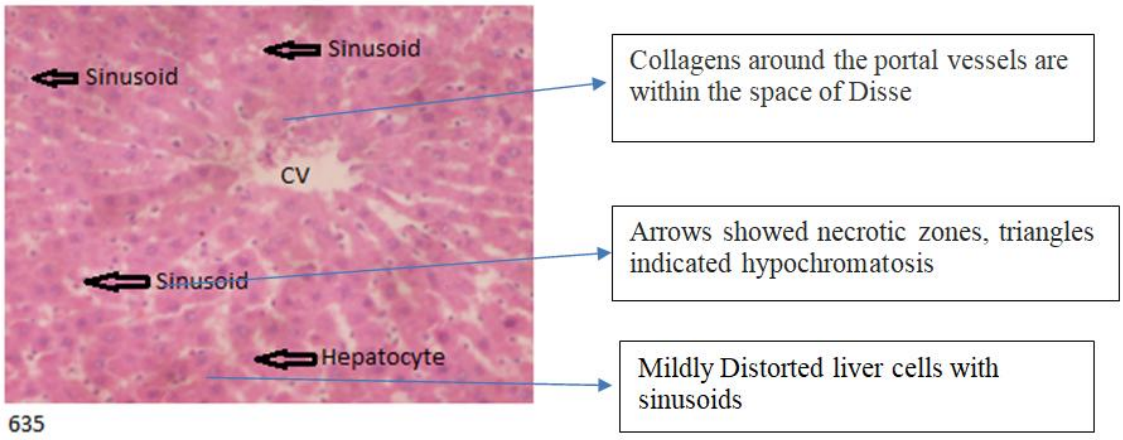

Plate 8. Group 8. H&E. MAG. X400. LIVER DOSE: 200mg/kg(ORAL). Diabetic. Treatment was with ethanolic leaf extract of *MMLS*

The results (Tables 1-3) showed the different extract types on liver enzymes, however, there were improvements in reductions of liver enzymes in 30 days' treatment of diabetes in the rat experimental model when 400mg/kg body weight of aqueous leaf (Table 1) extracts were administered orally to the rats followed by ethanolic then methanolic leaf extracts showing decrease in liver enzyme activity. This further reduction of liver enzymes is indicative that 400mg/kg of aqueous leaf in 30 days was able to protect the hepatocytes from liver damage thus providing hepato-protective effect on the liver organ. This was similar to the reports of Sung & Chang [17] who stated that reductions in AST and ALP. However, the combinatorial of aqueous leaves and metformin showed improvement in reductions of liver enzymes than the mono-therapy of metformin alone compared also with that of the positive control.

Histopathological changes were observed in the liver after 30 days' treatment of the diabetic experimental rats with aqueous, methanolic, ethanolic leaves of *Morus mesozygia Linn. Stapf.* (African Mulberry). The histopathological revelations from the liver when 400mg/kg of ethanolic plant extract was administered in the 30 days' treatment of the diabetic rats showed (Plate 7) evidences of cellular vascular regeneration of necrosis vasculation.

The liver (Plate 5) after treatment with 400mg/kg/ body weight of methanolic leaf plant extract showed improvement on the hepatocyte with evidence of bile duct hyperplasia and also a decreased number of intracytoplasmic acidophilus pellets. This was similar to the findings of Ayman et al. [18] who stated recovery of the hepatocytes after treatment. The liver (Plate 7) revealed evidence of the cellular vascular regeneration as necrotic vascuolation was observed. This was similar to the reports of Elisana et al. [19] but this did not agree with the reports of Tohid et al. [20].

4. CONCLUSION

The result depicted a significant decrease in AST, ALT and ALP levels in the diabetic rats treated with high dosages of ethanolic leaf extracts, this hepato-protective effect may be due to the presence of flavonoids reported to act as inhibitors of complex series of reactions from reactive oxygen species and lipid peroxidation that are responsible for liver damage by activating the liver cells linked to glucogenetic metabolism.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that Principles of laboratory animal care were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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