Original Research Article

Evaluation of nephroprotective properties of acqueous extracts of Morus mesozygia Linn Stapfkidney function parameters ofon Streptozotocin-Induced Diabetic Wistar Rats treated with *Morus mesozygia* Linn. *Stapf.*, Leaf extracts

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ABSTRACT

Introduction

Aim: To assess the effect of *Morus mesozygia Linn. Stapf.,* leaf extracts on the kidneys 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: **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 2019A 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) 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. They were sacrificed after 30 days and blood samples were collected for the estimation of kidney function parameters using both electrochemical and spectrophotometric methods. Histological analysis was done and 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 urea and creatinine levels, (from 13.93 ± 0.71 to 18.19 ± 1.24 mmol/l and from 154.4±13.03 to 173.8 ± 3.34 mmol/l) respectively. However, the administration of various concentrations of *Morus mesozygia Linn. Stapf., aqueous, methanolic and ethanolic* leaf extracts showed significant ($p<.05$) decreases in the values of both parameters. Electrolytes values were also affected by the induction and treatments.

Conclusion: Reno-protective effect of *Morus mesozygia Linn. Stapf.,* leaf extracts may be due to the presence of tannins. However, further studies to elucidate the molecular mechanisms are required.

Keywords: Kidney function parameters, Streptozotocin-Induced, Diabetic Wistar Rats, Morus mesozygia Linn. Stapf., Leaf extracts

Comment [P1]: Where is tanin coming from. Did you do phytochemical screening?

1. INTRODUCTION

The kidney is a specialized organ for the filtration and transportation of unwanted substances out from the body in form of urine. It is also known to perform essentially the process of homeostasis [1] and also regulates the reabsorption of substances such as water, bicarbonates, sodium, potassium and haemoglobin that the body needs for its various function [2-5]. When these substances such as glucose after a carbohydrate meal are not utilized or reabsorbed through the kidney, this causes an elevation of glucose in the blood stream leading to cases of hyperglycemia of type two diabetes mellitus.

Diabetic nephropathy is considered as one of the most serious microvascular complications of diabetes mellitus (DM) and a common cause of end-stage renal disease (ESRD) [6]. Studies have shown that about 40% of patients with DM have varying degrees of Diabetic nephropathy, and approximately 25% of them will progress to uremia [6-7]. Currently, diabetic nephropathy is considered as a chronic inflammatory disease caused by various metabolic disorders. In past decades, it has long been considered a kind of glomerular disease, and tubular-interstitial damage is regarded as a secondary change [8].

However, in recent years, investigators believe that the impairment of renal tubules precedes the occurrence of glomerular lesions [9-12], and renal tubulointerstitial abnormalities are more likely to be the early modification of DN [12]. Additionally, Vallon et al. [9] indicated that the glomerular filtration rate was determined by the balance of forces between primary tubular and primary vascular events. Taken together, these findings may have a huge influence on the early diagnosis, effective treatment and prognosis of DN. According by the assessment carried out by Bailey et al. [13], results revealed about 40 percent of individuals with elevated blood glucose cases levels resulting into cases of chronic kidney diseases as well as type two diabetic kidney disease (DKD), an equivalent of well over 29 million individuals in the United States of America.

Studies reporting the use of different extracts of *Morus mesozygia Linn. Stapf.* leaves in the management of streptozotocin-induced diabetes in rats are scarce, therefore, the current study aims to assess the effect of *Morus mesozygia Linn. Stapf.,* leaf extracts on the kidneys 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 14 days 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 was 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 [14].

Comment [P2]: Is this commecial feed or you made yours. Give composition of feed

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

Comment [P3]: Were they young leaves, mature leaves, and at what time of the day were they collected?

UNDER PEER REVIEW

of Port Harcourt with the herbarium voucher number (UPFH 0125) and was submitted at the department's herbarium.

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, it was stored and labelled into an air tight container prior to use.

Comment [P4]: Condition of drying-room temperature, under dark condition etc? indicate

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 was 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 [6] 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.

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.

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.

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 250mg/dl or 13mmol/L confirmed the diabetic state [15]. The diabetic male rats were picked and used for the study design.

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2.5 Administration of *Morus mesozygia* linn. (African mulberry) for Treatment

After the rats were confirmed diabetic at above 13mmol/L, blood samples were collected from the tail end of the rat. The assay of the blood glucose levels was carried out by the glucose-oxidase principle [16]. Finetest^{TM} test strips and FineTest Auto Coding^{TM} 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 acclamatized 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 kidney function tests and glucose respectively.

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2.7.2 Experimental Analysis

2.7.2.1 Determination of Plasma Urea ~~[17]~~

The determination of plasma urea using the Bertholot's enzymatic method is such that urea in plasma is hydrolyzed to ammonia in the presence of the enzyme 'urease [17]'. The quantified color of the ammonia produced is directly proportional to the concentration of urea in the sample produced. This is measured spectrophotometrically against a reagent blank to obtain the concentration of the urea in the test sample.

2.7.2.2 Determination of Plasma Creatinine by Jaffe's Colorimetric Method [18]

Creatinine when in an alkaline solution will react with picric acid to form a colored complex of picrates. The intensity of the color developed was directly proportional to the concentration of the creatinine in the sample measured as read at 490nm.

2.7.2.3 Determination of Sodium [19]

The principle of determination of sodium was based on the selection of chromogen whose ability is based directly on its chromophore capacity as measured on its concentration and absorbance of the presence of sodium in the test sample**.**

2.7.2.4 Determination of Bicarbonate [20]

Serum bicarbonates reacts with excess standard hydrochloric acid while the remaining hydrochloric acid is back titrated with standard sodium hydroxide with the use of phenol red as an indicator.

2.7.2.5 Determination of Chloride by Ion-Selective Electrode [19]

Chloride ions react with the combination of mercurous thiocyanate to produce mercury per chlorate and thiocyanate. The presence of a red complex as a result of the formation of thiocyanate with ferric ions was formed in the presence of nitric acid.

2.7.2.6 Determination of Potassium by Ion-Selective Electrode [19]

The concentration of potassium was measured by the inclusion of sodium tetraphenyl boron in an exact mixture suitable to produce a colloidal suspension. The presence of the turbidity formed is such that was proportional to the concentration of the potassium in the test sample.

2.7.2.7 Histopathological Analysis of the Kidneys

The kidneys 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 slides were studied for histopathological lesions., the photomicrographs were made 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 ($p<0.05$).

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Ethical consideration:

Comment [P5]: Indicateif this study was approved by the IRB for use of animal and whatOECD guidelines was used tor the toxicity studies?

3. RESULTS AND DISCUSSION

Your table 1, 2, 3 results not intepreted.-, plate 1

Table 1: Electrolytes, Creatinine and Urea of Rats treated orally with 400mg/kg, 200mg/kg for 30 days with aqueous leaves of *Morus mesozygia Linn. Stapf.* Extracts compared with 100mg/kg in Dosages of Metformin Standard with Non-Treated Controls

	K+(mmol/L)	*CL- (mmol)*	*Na+ (mmol/L)*	*HCO3- (mmol/L)*	*Cr (mmol/L)*	*Urea (mmol/L)*
GRP1NC	4.34 ± 0.71[2]	44.6±1.52[2]	143.4±3.36	23.4 ±2.07[2]	154.4±13.03	13.93 ± 0.71[2]
GRP2PC	5.98 ± 0.13	49.8±1.3	152± 2.44	19.4 ± 1.52	173.8 ± 3.34	18.19 ± 1.24[2]
GRP3	4.20 ± 0.76[2]	41.4±1.14	137.8±5.89[2]	28.8 ±1.30[2]	153 ± 23.27	11.81 ± 0.78[2]
GRP5	4.18 ± 0.23[2]	42.8±1.92[2]	138.8±1.30[2]	25.2 ±2.48[2]	161.8±12.98	16.29 ± 1.12
GRP6	4.38 ± 0.23[2]	42 ± 1.58[2]	135± 3.80[2]	25 ± 3.39[2]	137 ± 6.16	11.28 ± 0.43
GRP9	3.53 ± 0.72[2]	38.8±5.16[2]	133.4±4.44[2]	26.2 ±2.16[2]	166.4 ± 10.4	11.55 ± 0.93[2]
p-values	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.1429	< 0.0001
F-values	13.15	18.32	10.52	17.01	1.84	35.15

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

Table 2: Electrolytes, creatinine and urea parameters of Rats treated orally for 30 days with 400mg/kg, 200mg/kg in doses of Methanolic Leaves of *Morus mesozygia Linn. Stapf.* Extracts compared with 100mg/kg of Metformin Drug and Non-Treated Controls

	K+(mmol/L)	*CL- (mmol)*	*Na+ (mmol/L)*	*HCO3- (mmol/L)*	*Cr (mmol/L)*	*Urea (mmol/L)*
GRP1NC	4.34 ± 0.71[2]	44.6 ± 1.52	143.4 ± 3.36[2]	23.4 ± 2.07	154.4±13.03	13.93 ± 0.71[2]
GRP2PC	5.98 ± 0.13	49.8 ± 1.3	152± 2.44	19.4 ± 1.52	173.8±3.34[2]	18.19 ± 1.24[2]
GRP11	4.16 ± 0.65[2]	42.8 ± 3.11[2]	135.4 ± 3.84[2]	24 ± 1.58[2]	145 ±15.28[2]	11.97 ± 0.46[2]
GRP13	4.38 ± 0.23[2]	42 ± 1.58[2]	135 ± 3.80[2]	25 ± 3.39[2]	137 ± 6.16	11.28 ± 0.43[2]
GRP12	2.84 ± 0.63[2]	39.2 ± 2.94[2]	140.8 ± 4.43[2]	26.2 ± 2.59[2]	154.8 ± 20.5	11.88 ± 0.48[2]
GRP9	3.53 ± 0.72[2]	38.8 ± 5.16[2]	133.4 ± 4.44[2]	26.2 ± 2.16[2]	166.4 ± 10.4	11.55 ± 0.93[2]
p-values	< 0.0001	< 0.0001	< 0.0001	0.0009	0.0016	< 0.0001
F-values	17.29	9.513	17.14	60.36	5.547	4.009

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

Table 3: Electrolytes, creatinine and urea parameters of Rats treated orally for 30 days with 400mg/kg, 200mg/kg in Doses of Ethanolic Leaves of *Morus mesozygia Linn. Stapf.* Extracts compared with Non-Treated Controls

	K+(mmol/L)	*CL- (mmol)*	*Na+ (mmol/L)*	*HCO3(mmol/L)*	*Cr (mmol/L)*	*Urea (mmol/L)*
GRP1NC	4.34 ± 0.71[2]	44.6 ± 1.52	143.4 ± 3.36	23.4 ± 2.07[2]	154.4 ± 13.03	13.93 ± 0.71[2]
GRP2PC	5.98 ± 0.13	49.8 ± 1.3	152± 2.44	19.4 ± 1.52	173.8 ± 3.34	18.19 ± 1.24

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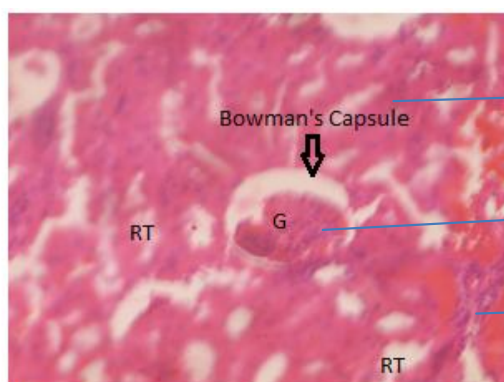
GRP4	3.58 ± 1.09^2	38.8 ± 0.84^2	105.6 ±51.65^2	22.6 ± 1.34	152.2 ± 19.43^2	11.93 ± 0.60^2
GRP7	3.96 ± 0.23^2	43.4 ± 3.2^2	159 ± 5.7	24.6 ± 2.41^2	161.4 ± 4.56	11.79 ± 0.18^2
GRP8	3.59 ± 0.89^2	42.2 ± 2.38^2	139.6 ± 6.98^2	21.8 ± 1.92	165 ± 5.70	11.91 ± 1.25^2
GRP9	3.53 ± 0.72^2	38.8 ± 5.16^2	133.4 ± 4.44^2	26.2 ± 2.16^2	166.4 ± 10.4	11.55 ± 0.93^2
p-values	< 0.0001	< 0.0001	< 0.0001	0.0003	0.0465	< 0.0001
F-values	8.606	10.74	29.96	7.306	2.676	30.33

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



Showing normal prominent nuclear arrangement

containing the Glomerular cells, Messengial matrix

Renal tubule lined with simple epithelium

Plate 1: Group 1(Negative Control) , H&E. MAG. X400. KIDNEY. DOSE: Distilled water: NO TREATMENT SUBSTANCE: NEGATIVE CONTROL Normal Histology reading of the kidney with the Glomeruli (G) containing the Glomerular cells, Messengial matrix, Glomerular capillaries, Bowman's capsule(BC) which appeared patent as well as the Renal tubules (RT)

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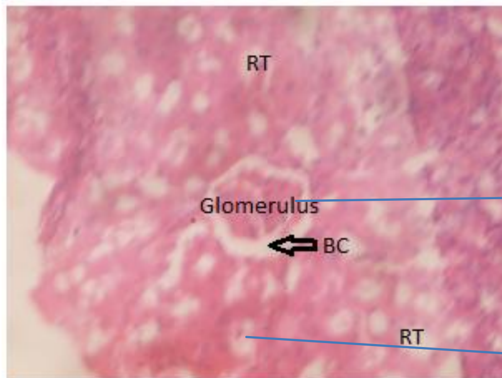

Plate 2: Group 2(PC), H&E.mag. x400. Kidney. dose: 0.0mg/kg. Treatment substance: nil. positive control. normal histological result of the kidney which revealed the glomeruli (g), bowman's capsular space (BC) and renal tubules (RT)

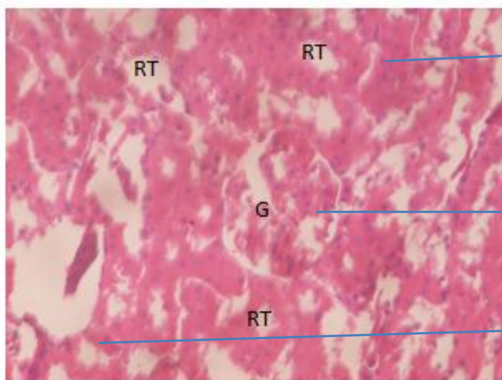

Plate 3: Group 5. H&E. MAG. X400. KIDNEY. DOSE:200mg/kg. Diabetic. Treatment with aqueous leaf extract of *MMLS.*(ORAL) for 30 days. The plate revealed the histology of the kidney' with Glomeruli(G), Bowman's capsule (BC), and Renal tubules (RT)

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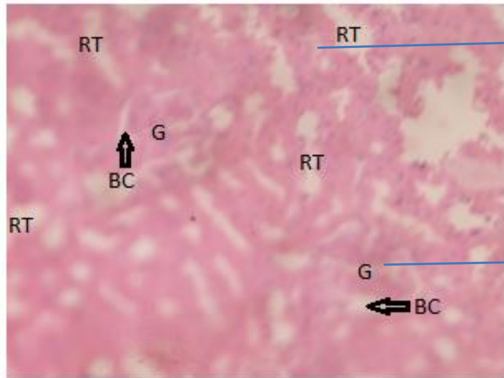

Dilation of the tubules and glomeruli, mesenchymal and nodular sclerosis

Loss of endocytic vesicles as well as presence of cast in the tubules

670

Plate 4: Group. 3. H&E. MAG. X400. KIDNEY. DOSE: 400mg/kg. Administered with aqueous leaf extract of *MMLS*, alone. Normal Glomeruli (G), BC and RT

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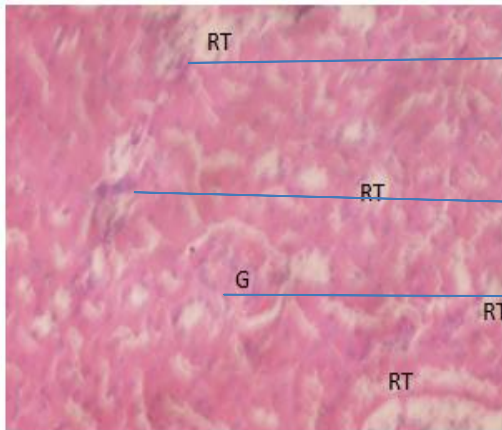


Showing shrinkage of tubular and inflammation

Section showing nodular glomerulosclerosis and hypercellularity

Showing mesangial expansion and proliferation with increase in the glomerular capillary thickening

659

Plate 5: Group 11. H&E. MAG. X400. KIDNEY. DOSE: 400mg/kg. Administered substance: was with methanolic leaf extract of *MMLS*.(ORAL) 30 days.The plate revealed a 'Distorted Kidney' with the Glomeruli (G). Renal tubules (RT)

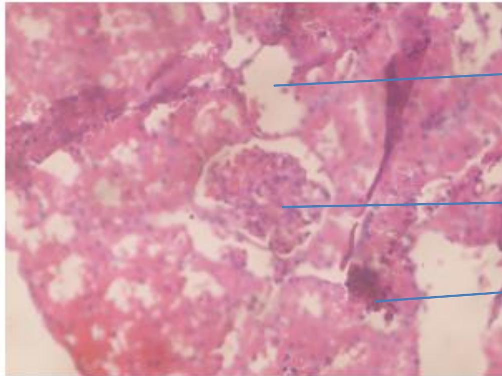


Plate 6: Grp 12.H&E. MAG. X400. KIDNEY.DOSE: 200mg/kg. Diabetic. Treatment with methanolic leaf extract of *MMLS*.(ORAL) 30 days. Normal kidney histolical result with the Glomeruli (G), Bowman's capsule (BC) and Renal tubules(RT)

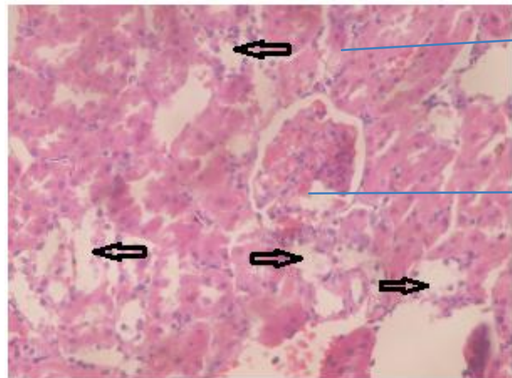
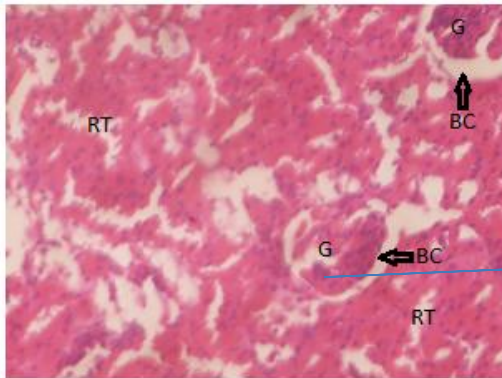


Plate 7: Group 8. H&E. MAG. X400. KIDNEY.DOSE: 200mg/kg. Diabetic. Treatment with ethanolic extract of *MMLS* (ORAL) for 30 days. The plate revealed a mildly distorted Glomeruli (G), Bowman's capsule (BC) and Renal tubule (RT)

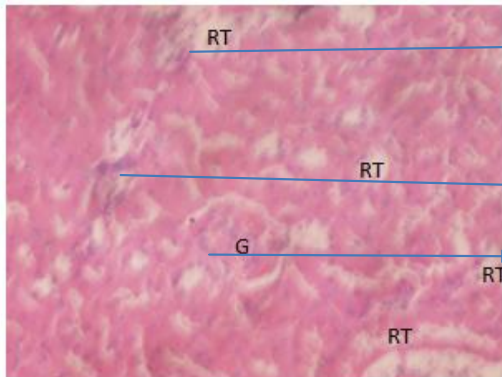


Normal glomerulus, normal basement membrane

Showed increase in cellular regeneration with prominent nuclear rearrangement

658

Plate 8: Group 6. H&E. MAG. X400. KIDNEY. DOSE: 400mg/kg. Diabetic, treatment substance: was with aqueous leaf *MMLS.*(ORAL) The result revealed a 'Normal kidney' with the Glomeruli(G), Bowman's capsule (BC) and Renal tubules(RT)



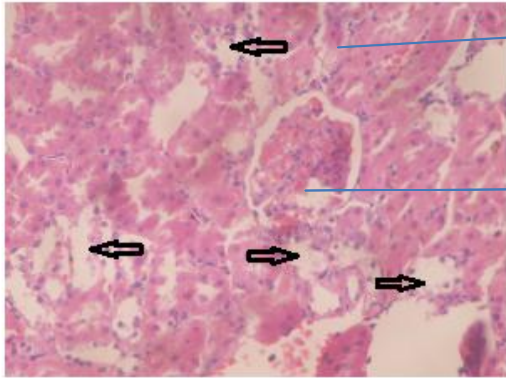
Showing shrinkage of tubules and inflammation

Section showing nodular glomerulosclerosis and hypercellularity

Showing mesangial expansion and proliferation with increase in the glomerular capillary thickening

659

Plate 9: Group 13. H&E. MAG. X400. KIDNEY. DOSE: 400mg/kg. Diabetic. treatment substance: was with methanolic leaf extract of *MMLS.*(ORAL) 30days, Result revealed a 'Distorted Kidney' with the Glomeruli (G). Renal tubules (RT) all distorted

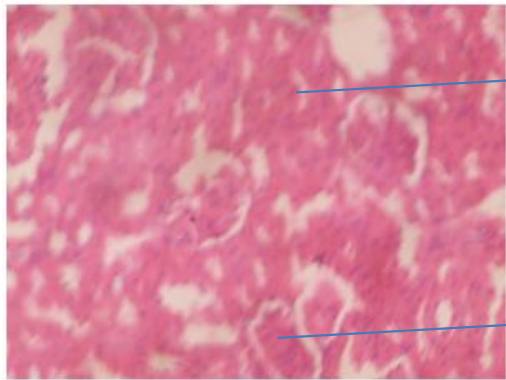


Showed cellular regeneration with the nucleus intact

Revealed a distorted bowman capsule with the renal tubules

661

Plate 10: Group 7. H&E. MAG. X400. KIDNEY.DOSE:400mg/kg. Diabetic, treatment with ethanolic leaf extract of *MMLS.*(ORAL) .The plate revealed a distorted Glomeruli (G), Bowman's capsule (BC), Renal tubules (RT)(Arrowed)



Kidney tissue showing normal cellular architecture.

Cell membrane disruption and cytotoxicity

669

Plate 11: Grp 9. H&E. MAG. X400. kidney. dose: 100mg/kg (ORAL). Treatment with metformin. The result revealed a normal Glomeruli (G), (BC) and RT

In this study, we established a diabetic nephropathy rat model by intraperitoneal injection of STZ and assessed the reno-protective effect of oral administration of aqueous, methanolic and ethanolic extracts of *Morus mesozygia Linn. Stapf.* leaves for 30 days. In Tables 1-3, it was observed that induction of diabetes with streptozotocin caused a significant increase in

serum potassium, chloride, bicarbonate and urea levels. Creatinine levels were also observed to have increased, but not to a statistically significant amount. The increase in creatinine and urea is suggestive of acute kidney injury, occasioned by either the direct effect of streptozotocin on the kidney tissues or as a metabolic consequence of the induced diabetes (diabetic nephropathy).

We also observed that after treating the rats with the different extract types and concentrations of *Morus mesozygia Linn. Stapf.* leaves, it caused a significant decrease in creatinine and urea. There were significant increases in bicarbonates ($HCO3^-$) and sodium (Na+), when 400mg/kg of ethanolic leaf extract (LMD) was administered orally in 30 days' treatment to the diabetic rats followed by methanolic then aqueous leaf extracts and the positive control. This nephron-protective functioning of the 400mg/kg of methanolic leaf extracts on the kidney may be due to the presence of tannins. Ethanolic leaf extract of *Morus mesozygia Linn. Stapf.* leaves were reported to have exhibited highest tannin yield (9.29%) followed by aqueous (4.84%) then methanolic (4.51%) on phytochemical analysis, [21]. These reductions in creatinine and urea may suggest that the 400mg/kg ethanolic leaf extract had a great impact on kidney functioning in the process of filtration and reabsorption of electrolytes. Tannins have been reported to be an astringent, nephron-protective in a mechanism of action that has the tannins pulling cells together of the gallic esters of glucose in a manner that seem to extract the 'unwanted from the wanted'. The findings of a significant decrease in urea levels are in tandem with the reports of Tohid *et al.* [22], that reported a significant reduction in urea levels. The metformin standard drug alone did not show greater improvements when compared with the combinatorial regiment of both the leaf and metformin in combination. This revealed a synergistic effect the combinatorial therapy has over the mono-therapy treatment.

Histological findings correlated with the biochemical results (Plates 1-10). The kidneys (plate 7) showed cellular regeneration with prominent nuclear rearrangement in the 30 days' treatment with 200mg/kg of ethanolic leaf extracts of *Morus mesozygia Linn. Stapf.* The results also showed (plate 10) cellular regeneration with prominent nuclear rearrangement intact in the 30 days' treatment with 400mg/kg of ethanolic leaf extracts of *Morus mesozygia Linn. Stapf.* The kidneys (plate 9) showed a section with nodular glomerulosclerosis and hypercellularity with mesangial expansion and proliferation with increase in the glomerular capillary thickening.

4. CONCLUSION

Significant reductions in urea and creatinine in the diabetic experimental rat model treated with high dose of methanolic leaf extract which is indicative of the glomerular filtration capacity, this nephron-protective effect may be due to the presence of tannins. However, further studies to elucidate the molecular mechanisms are required.

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.

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