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Evaluation of Antidiabetic Potentials of *Morus mesozygia* Linn. Stapf., Leaf Extracts in Streptozotocin-Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NN, EOW and NB designed the study, wrote the protocol. Author MTJ wrote the draft of the manuscript, managed the analyses and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study therefore was to evaluate the antidiabetic potentials of *Morus mesozygia* Linn. Stapf., Leaf extrcts in Streptozotocin-Induced Diabetic Rats.
Study Design: The study is an experimental case-controlled study.
Place and Duration of Study: This study was carried out at the 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) was from 250 mg/dl or 13 mmol/L and above.
Results: The results showed that there were significant decreases ($p<0.05$) in, FBG, increased insulin and increased C-peptide and increased HOMA-IR concentration of induced diabetic male

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rats treated orally with 400 mg/kg of *MMLS*., when compared with the values of the diabetic male rats in treated orally with 200 mg/kg of *MMLS*., and non-significant decrease($p<0.05$) in FBG, HOMA-IR increased insulin, increased C-peptide concentrations in the diabetic male rats treated orally for 30 days with 400 mg/kg in dose of methanolic leaves extracts of *MMLS*., in the group treated with 400 mg/kg methanol, when compared to the concentration of diabetic male in treated orally for 30 days with 200 mg/kg of methanolic leaves extracts of *MMLS*. Compared likewise with that treated with 100mg/kg of metformin standard drug. There were also significant decrease ($p<0.05$) in FBG, HOMA-IR, C-peptide and increased insulin concentrations in diabetic male rats treated with 200 mg/kg in dose of ethanolic and methanolic leaves extracts of *MMLS*., when compared with the values in the diabetic male rats treated orally with 400 mg/kg in dose of ethanolic and methanolic leaves extracts of *MMLS*., compared with values of diabetic male rats treated orally with 100mg/kg in dose of metformin standard drugs compared with the controls.

Conclusion: From the findings of this study, we conclude that streptozotocin increased FBG levels, while the Methanolic, ethanolic and aqueous extracts of *Morus mesozygia Linn. S* improved FBG, C-peptide, insulin and HOMA levels in a dose-dependent manner, with the methanolic extract having the best ameliorative effect, probably due to its more phytochemical composition.

Keywords: Antidiabetic; Morus mesozygia leaves; Streptozotocin-induced; diabetic; rats.

1. INTRODUCTION

One of the biggest and cost-effective diseases globally that increasingly drains the budget of the private health sector annually is diabetes mellitus Diabetes mellitus has been acknowledged as one of the world's leading causes of disability and mortality [1]. In 2017 alone, an estimate of 424.9 million adults worldwide as provided by the International Diabetes Federation, were sufferers of diabetes mellitus, what is alarming is that this estimate by the year 2040 is expected to rise to 776 million individuals living with diabetes. It has been reported that about 50 percent of these individuals do not know they have diabetes, a scourge in annual expenditure for healthcare delivery globally has risen from 727 billion USD in the year 2017 with a massive 776 million USD that is expected to be budgeted on for the year 2040, this increase is expected for treatment due to both an incidence of insulin resistance as well as the prevalence of type 2 diabetes mellitus which has been the most prevalent due to unhealthy feeding habits and also sedentary lifestyle which reduces life expectancy with an overt that comes from the complications of diabetes mellitus [1].

Diabetes mellitusis a metabolic disorder that arises due to the dysfunction of the beta cells with a decrease in insulin insufficiency that leads to hyperglycemia as well as the action of endogenous insulin [2] and certain complications comes with it such as microvascular (diabetic neuropathy, diabetic retinopathy as well as diabetic nephropathy) and macro vascular (atherosclerosis, ischemic heart disease as well as stroke). The International Diabetes Federation has reported an estimation of the prevalence of diabetes mellitus from the year 2017 with well over 424.9 million individuals and by the year 2040 an increased data of over 776 million individuals are estimated to suffer from the burden this disease brings. This alarming increase has been associated with sedentary lifestyle; obesity due to an unhealthy change in diet [1].

The African mulberry *(Morus mesozygia Linn. Stapf.)*., an herb, is also an African species of the Morus genus plant amongst its temperate species such as Morus alba has been reported by the western Yoruba tribes of the Nigerian people to have medicinal value that include treatments of ulcer, veneral diseases as well as certain stomach pains. [3] Reported the increasing trends in the usefulness of plants as medicinal remedies in disease conditions to having some certain degree of antioxidant properties, especially flavonoids [4]. Some drugs in circulation that are prescribed to ailing individuals are also plant derivatives. The aim of this study therefore was to evaluate the antidiabetic potentials of *Morus mesozygia Linn. Stapf.,* Leaf extrcts in Streptozotocin-Induced Diabetic 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 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 [3].

2.2 Plant Collection and Authentication

Morus mesozygia Linn.(family Moraceae) fresh leaves samples were collected 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 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.

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 [5] 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 Diabetes Induction 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 [6]. 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 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 [7]. 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 allowed to incubate by acclimatizing for two weeks prior to the progression of the study. They were randomly separated into 13 groups of 5 rats 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 400 mg/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 40 mg/kg body weight of streptozotocin and treated with 400 mg/kg body weight of aqueous leaf extract

Group Six: 5 male rats were induced intraperitoneally with a single dose of 40 mg/kg body weight of streptozotocin and treated orally with 200 mg/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 400mg/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 for Laboratory Analysis

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 Ethylene diamine tetra acetic acid (EDTA) anticoagulant bottles for hematological analysis.

2.8 Experimental Analysis

2.8.1 Determination of blood glucose concentration [8]

The determination of blood glucose concentration in this assay adopted the principle of 'Electro-Chemistry' a reaction described by [8].

2.8.1.1 Principle

Glucose is assayed for after anauto-encoding chemical reaction between the embedded electrodes in the test stripes as a result of the electric current present in the poles of the electrodes. The principle works solely on the reaction of glucose oxidase with the blood glucose to form gluconic acid that further reacts with ferrocyanide that enhances the generation of current directly proportional to the amount of glucose.

2.8.1.2 Procedure

Sample of the blood of the rats was taken from 8am to 10am on fasting**.** The tail of the rat was swabbed with cotton wool and pricked with a clean lancet, the first drop of blood was wiped off and the second drop collected for reading using a glucometer, to avoid false- positive results.

2.8.2 Determination of insulin [9]

The kit for the determination of insulin was obtained from Cal Biotech, United States of America.

2.8.2.1 Principle

The principle of the assay was based on Antigen-Antibody binding and was detected using Enzyme Linked Immunosorbent Assay. The micro-Elisa is modified in a way that the solid phase indirectly competes with the enzymes.

2.8.2.2 Laboratory procedure

The method as described by the manufacturer in their protocol was followed.

Briefly, 25 µl of sample was introduced into the various wells and 100 µl of insulin conjugate reagent was added to the wells and mixed.

The mixture in the plate was placed in an incubator and was set to incubate at 25^{o}C and allowed to stand for one hour.

After on hour, plates were washed using the wash buffer provided in the kit and blotted on an absorbent paper.100µl of TMB substrate was added to wells and incubated for another 15 minutes at 25^{o}C.

The reaction was stopped using a stop solution and the plate was read using an Elisa reader set at an absorbance of 450nm.

2.8.3 Determination of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) [10]

This was measured by the multiplication of fasting blood glucose and fasting plasma insulin divided by 22.5.

The formulae is mathematically expressed as

$$\text{HOMA-IR} = \frac{\text{FBG (mg/dl) X FPI(mIU/ml)}}{22.5}$$

2.8.4 Determination of C- Peptide Hormone [11]

The C-Peptide kit used was bought from Cal Biotech, in the United States of America.

2.8.4.1 Principle

The principle of the assay was based on Antigen binding to Antibody and detected usingan enzyme.

2.8.4.2 Laboratory procedure

The procedure followed all the guidelines as instructed from the beginning to the ending of the manufacturer's guide.

50µl of sample was loaded on pre-coated plates and 100µl of enzyme conjugate was added to each well. The plate was gently mixed for 20 seconds and allowed to incubate for one hour at 25^{o}C.
Wells were washed using the wash buffer provided in the kit and this was followed by the addition of TMB substrate.50µl of stop solution was added into each well after the plate was incubated for 15 minutes and the absorbance read on the reader at 450nm.

2.9 Statistical Analysis

Statistical evaluation was made possible with the application of Graph pad prism (version). 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

The results of C-peptide, Insulin, FBG, HOMA-IR from Table 1 revealed that the diabetic male

albino rats orally treated with 400 mg/kg of aqueous leaves extracts showed a better ameliorative fasting blood glucose lowering capacity on the pancreas as well as an increased insulin resistant exhibited nature of acting antihyperglycemic when compared to the results the diabetic male albino rats treated orally with 200mg/kg of aqueous extracts of *MMLS* also when compared with the C-peptide, Insulin, FBG, HOMA-IR index of the diabetic male rats treated orally with 100mg/kg in dosage of the standard metformin drug as well as those of the non- treated control groups.

This beta cell function as well as the hypoglycemic nature exhibited by the oral administration of the 400mg/kg dose of aqueous leaves extract of *MMLS* of the male diabetic albino rats treated for 30 days. From the result of [12], a Gas chromatographic mass spectrometry (GC-MS) analysis of *Morus mesozygia Linn. Stapf.,* leaf extracts showed the presence of phytol (12.511%), a branched chained acyclic diterpenes that are reported to be antidiabetic [13], an heterodimer found in the chlorophyl part of a plant as geranylgeraniol, that once metabolized in humans and mammals, gets converted into its natural precursor form of rexinoid, a substance that when in the liver gets converted into phytanic acid a gamma sub-type (PPARγ)has been reported by reported by [14] to ameliorate insulin resistance; that stimulates muscle beta oxidation and further enhances the uptake of glucose into cell membranes thereby suppressing the action of hepatic glucose production and inhibiting the action of TNF-α [15] The antihyperglycemic results explained from Table 1 above did not collaborate with the work done by [16] they assessed the role of saponin from the root bark of the *Berberis vulgaris Linn* plant by feeding the streptozotocin wistar rats with 25mg/kg of the extracts within day 1 and 21 days of treatment with an estimated of glycemic index drop from 73.1% to 76.03% compared with the control group; they reported that this hypoglycemic outcome may be due to the presence of saponins in the root bark of the plant owing that might have had a lowering effect of insulin resistance on the beta cells.

Table 1. C-Peptide, Insulin, Fasting Blood Glucose and HOMA-IR parameters of Streptozotocin induced diabetic male rats treated orally for 30 days with 400 mg/kg, 200 mg/kg of aqueous leaves of *Morus mesozygia Linn. Stapf.* Extracts compared with controls

Groups	C-Peptide (ng/ml)	Insulin (mIU/ml)	FBG (mmol/L)	HOMA
GRP1NC	4.06 ± 0.58	9.75 ± 0.52	5.7± 0.1	2.47 ± 0.13
GRP2PC	1.95 ± 0.39	2.91 ± 0.32	13.76 ± 4.65[1]	1.8 ± 0.67
GRP3	2.31 ± 1.29	2.57 ± 1.36	5.54 ± 0.15[2]	0.63 ± 0.33
GRP5	14.51±3.46[1,2,3]	22.79 ± 7.07[1,2,3]	12.06 ± 3.63[1,3]	11.8 ± 2.13[1,2,3]
GRP6	2.75 ± 0.91[5]	3.01 ± 1.28[5]	11.04 ± 3.59	1.48 ± 0.48[5]
GRP9	14.29±5.45[1,2,3,6]	22.95 ± 7.42[1,2,6]	12.54 ± 4.39	10.06± 4.75[1,2,3,6]
p-values	< 0.0001	< 0.0001	0.0011	< 0.0001
F-values	24.54	26.56	5.863	18.87

Number superscripts (1,2,3….) indicate significance at p<0.05 (Tukey's post hoc test), with each number signifying the corresponding group

Table 2. C-peptide, insulin, fasting blood glucose and HOMA-IR parameters of streptozotocin induced diabetic male rats treated orally for 30 days with methanolic leaves of *Morus mesozygia Linn. Stapf.* Extracts compared with controls

Groups	C-Peptide (ng/ml)	Insulin (mIU/ml)	FBG(mmol/L)	HOMA
GRP1NC	4.06 ± 0.58	9.75 ± 0.52	5.7± 0.1	2.47 ± 0.13
GRP2PC	1.95 ± 0.39	2.91 ± 0.32	13.76 ± 4.65[1]	1.8 ± 0.67
GRP11	9.49 ± 0.12[1,2]	5.32 ± 0.73	5.56 ± 0.11[2]	1.31 ± 0.16
GRP13	4.65 ± 0.11	6.06 ± 1.82	11.14 ± 3.45	3.21 ± 1.41
GRP12	10.26 ± 2.07[1,2]	16.51 ± 3.95[2,4,13]	11.66 ± 3.80	8.6 ± 3.81[1,4]
GRP9	14.29 ± 5.45[1,2,13]	22.95 ± 7.42[1,2,11]	12.54 ± 4.39	10.06 ± 4.75[1,11,13]
p-values	< 0.0001	< 0.0001	0.0013	< 0.0001
F-values	15.51	25.65	5.687	14.2

Number superscripts (1,2,3….) indicate significance at p<0.05 (Tukey's post hoc test), with each number signifying the corresponding group

The phytochemical analysis of the methanolic leaves extracts of *Morus mesozygia Linn. Stapf.,* leaf extracts by [12] revealed the presence of bioactive agents such as alkaloids, flavonoids, saponins and tannins. Table 2 revealed a decrease in FBG and an increased concentration of C-peptide, insulin and HOMA-1R indices in the diabetic rats treated orally for 30 days with 200mg/kg of methanolic compared with a decreased C-peptide and insulin concentration and a low HOMA-IR index of the diabetic rats treated with 400mg/kg of the methanolic leaves extracts of *MMLS*., revealing a poor glycemic index and control of the diabetic condition. The experimental diabetic rats treated with 200mg/kg of methanolic leaves extracts exhibited good diabetic characteristics of glycemic index by the decreased FBG and increased insulin, C-peptide with a good HOMA-IR., this could be possibly because of the high presence of flavonoids quantitated by [12] with a weight of 21.72%.

Flavonoids have been reported as a collection of poly-phenolic metabolites that show anti diabetic properties, widely found as glycosylated derivatives. The proposed mechanism of action as anti- diabetic may be one which works synergistically with its antioxidative properties and beneficial in the management of diabetes, this may be by increasing the central consumption of glucose and preventing the the glucose transporter activity from the intestine. The results also indicated a significant decrease ($p<0.05$) in C-peptide of the diabetic rats treated with 200mg/kg of ethanolic leaves extracts when compared with those of the diabetic rats treated with 400mg/kg of ethanolic extracts compared to the increased C-peptide levels of the diabetic male rats treated with 100mg/kg of metformin.

This decreased C-peptide levels may be due to the autoimmune destruction of the pancreatic beta islets because C-peptide contributes to the fine tunings of the metabolism of the physiological conditions of tissues to enhance insulin sensitivity in the peripheral circulation.

Table 3. C-Peptide, $$ and HOMA-IR parameters of Streptozotocin induced diabetic male rats treated orally for 30 days with 400 mg/kg, 200 mg/kg in doses of ethanolic leaves of *Morus mesozygia Linn. Stapf.* Extracts compared with non-treated controls

Groups	C-Peptide (ng/ml)	Insulin (mIU/ml)	FBG(mmol/L)	HOMA
GRP1NC	4.06 ± 0.58	9.75 ± 0.52	5.7± 0.1	2.47 ± 0.13
GRP2PC	1.95 ± 0.39	2.91 ± 0.32	13.76 ± 4.65[1]	1.8 ± 0.67
GRP4	7.2 ± 0.23	14.77 ± 4.13[2]	5.54 ± 0.15[2]	3.63 ± 0.45
GRP7	16.81 ± 5.20[1,2]	22.39 ± 7.37[1,2,7]	12.22 ± 3.93[1,4]	11.9 ± 0.56[1,2,4]
GRP8	2.78 ± 0.78[13]	18.23 ± 1.24[5,7]	12.34 ± 4.10[1,4]	1.61 ± 0.92[7]
GRP9	14.29 ± 5.45[1,2,4,7,8]	22.95 ± 7.42[1,2,8]	12.54 ± 4.39	10.06 ± 4.75[1,2,4,8]
p-values	< 0.0001	< 0.0001	0.0012	< 0.0001
F-values	20.05	18.77	5.801	14.06

Number superscripts (1,2,3….) indicate significance at p<0.05 (Tukey's post hoc test), with each number signifying the corresponding group

Table 4. C-peptide, insulin, fasting blood glucose, HOMA-IR parameters of streptozotocin induced diabetic male rats treated orally for 60 days with 400 mg/kg aqueous leaves extracts, 200 mg/kg aqueous leaves extracts of *Morus mesozygia Linn. Stapf.*, in comparison with 100 mg/kg of metformin drug compared with controls

Groups	C-Peptide (ng/ml)	Insulin (mIU/ml)	FBG	HOMA
GRP1NC	3.67 ± 0.83	9.00 ± 0.70	5.52 ± 0.20	2.21 ± 0.20
GRP2PC	3.38 ± 0.54	2.77 ± 0.46[1]	13.76 ± 4.82[1]	1.75 ± 0.73
GRP3	2.12 ± 1.10	2.51 ± 1.37[1]	5.34 ± 0.11[2]	0.60 ± 0.33
GRP9	9.70 ± 1.86[1,2,3]	13.38 ± 3.99[2,3]	11.72 ± 3.45[1,3]	6.72 ± 2.49[1,2,3]
GRP6	1.91 ± 0.45[5]	2.17 ± 0.92[1,5]	10.64 ± 3.25	1.01 ± 0.56
GRP9	11.01 ± 2.52[1,6]	17.68 ± 5.14[1,2,3,6]	10.5 ± 3.19	7.89 ± 2.68[1,2,3,5,6]
p-values	< 0.0001	< 0.0001	0.0007	< 0.0001
F-values	39.08	28.16	6.27	20.35

Number superscripts (1,2,3….) indicate significance at p<0.05 (Tukey's post hoc test), with each number signifying the corresponding group

Table 5. C-peptide, insulin, fasting blood glucose, HOMA-IR parameters of streptozotocin induced diabetic male rats treated orally for 60 days with 400 mg/kg methanolic leaves Extracts, 200 mg/kg doses of methanolic extracts of *Morus mesozygia Linn. Stapf.*, in comparison with 100 mg/kg of metformin drug compared with non-treated controls

	C-Peptide (ng/ml)	Insulin (mIU/ml)	FBG	HOMA
GROUP1NC	3.67 ± 0.83	9.00 ± 0.70	5.52 ± 0.20	2.21 ± 0.20
GROUP 2PC	3.38 ± 0.54	2.77 ± 0.46[1]	13.76 ± 4.82[1]	1.75 ± 0.73
GROUP 11	9.16 ± 2.49[1,2]	5.09 ± 0.98	5.5 ± 0.1[2]	1.24 ± 0.23
GROUP 13	3.51 ± 0.81	4.20 ± 1.75[2]	10.96 ± 3.33	2.23 ± 1.22[11]
GROUP 12	6.31 ± 2.88	9.05 ± 2.57	11.5 ± 3.79	4.69 ± 2.25
GROUP 9	11.01 ± 2.52[1,2,13]	17.68 ± 5.14[1,2,11,]	10.5 ± 3.19	7.89 ± 2.68[1,2,11,13]
p-values	< 0.0001	< 0.0001	0.0012	< 0.0001
F-values	14.22	23.14	5.797	13.28

Number superscripts (1,2,3….) indicate significance at p<0.05 (Tukey's post hoc test), with each number signifying the corresponding group

Table 6. C-peptide, insulin, fasting blood glucose, HOMA-IR parameters of streptozotocin induced diabetic male rats treated orally for 60 days with 400 mg/kg ethanolic leaves Extracts, 200 mg/kg dose of ethanolic extracts of *Morus mesozygia Linn. Stapf.*, in comparison with 100 mg/kg of metformin drug compared with non-treated controls

Groups	C-Peptide (ng/ml)	Insulin (mIU/ml)	FBG	HOMA
GRP1NC	3.67 ± 0.83	9.00 ± 0.70	5.52 ± 0.20	2.21 ± 0.20
GRP2PC	3.38 ± 0.54	2.77 ± 0.46	13.76 ± 4.82[1]	1.75 ± 0.73
GRP4	14.04 ± 4.14[1,2]	12.39 ± 4.25[2]	5.68 ± 0.10	3.11 ± 1.02[2]
GRP7	11.51 ± 4.10[1,2]	15.69 ± 6.10[2]	12.1 ± 3.82[1,2,4]	8.46 ± 4.57
GRP8	1.96 ± 0.81[4,7]	1.96 ± 1.03[4,7]	12.26 ± 4.04[1,2,4]	1.11 ± 0.78[1,4,7]
GRP9	11.01 ± 2.52[1,2,8]	17.68 ± 5.14[1,2,8]	10.5 ± 3.19	7.89 ± 2.68[2,8]
p-values	0.0001	0.0001	0.0012	0.0001
F-values	19.01	14.43	5.926	9.881

Number superscripts (1,2,3….) indicate significance at p<0.05 (Tukey's post hoc test), with each number signifying the corresponding group

The insulin levels of the diabetic rats in Group 8 treated orally with 200mg/kg of ethanolic leaves compared to those of the diabetic male rats treated with 400mg/kg of ethanolic leaves extracts are stabilized and within limits probably because after a period of 30 days of treatment, the beta cells may have had some form of repairs, results from a decrease in its FBG level may have also played a role at the intake of 200mg/kg over 30 days treatment period, likewise was a decrease in its HOMA-IR compared to those of the diabetic rats treated with 400mg/kg that showed increased HOMA-IR index.

Increase in HOMA-IR index as reported by [17] has shown great increase in HOMA-IR to be correlated with insulin insensitivity.

4. CONCLUSION

From the findings of this study, we conclude that streptozotocin increased FBG levels, while the Methanolic, ethanolic and aqueous extracts of *Morus mesozygia Linn. S* improved FBG, C-peptide, insulin and HOMA levels in a dose-dependent manner, with the methanolic extract having the best ameliorative effect, probably due to its more phytochemical composition.

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.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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