Original Research Article

Assessment of Antioxidant Effects of Aqueous, Ethanolic and Methanolic Extracts of *Morus mesozygia Linn. Stapf.,* Leaves in Streptozotocin-Induced Diabetic Rats

ABSTRACT

Aim: The aim of this study was therefore to assess the antioxidant effects of aqueous, ethanolic and methanolic extracts of *Morus mesozygia Linn. Stapf.,* Leaves 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) were from 250mg/dl or 13mmol/L and above.

Results: The results showed that there were significant increase in the levels of superoxide dismutase(SOD,411.8±1.49) ng/ml, total antioxidant status (TAS,75.25±0.42) mU/ml, total oxidant status (TOS,353.51± 6.07) mU/ml activity, an oxidative stress index of 4.69±0.05 and a reduced concentration of malondaldehyde (MDA of 19.0± 1.49mmol/L) when rats were treated with 400mg/kg of aqueous leaves of *Morus mesozygia Linn. S.,* when compared with those of rats treated with 200mg/kg of aqueous leaf extracts of *MMLS*. Other methods of extractions (methanolic and ethanolic), also improved the antioxidant statuses of the diabetes induced and treated rats.

Conclusion: Methanolic, ethanolic and aqueous extracts of *Morus mesozygia Linn. S* ameliorated oxidative stress, in Streptozotocin-induced diabetic rats, with the methanolic extract showing the most potent effect.

Keywords: ***Antioxidant Aqueous, Ethanolic, Methanolic, Morus mesozygia Leaves, Streptozotocin-Induced, Diabetic, Rats***

1. INTRODUCTION

A major problem of the metabolic syndrome an individual with type 2 diabetes mellitus have is oxidative stress; which have been reported to be due to insulin resistance. In type 2

diabetes, the impermeability of glucose into the cellular membrane as a result of the defect of the beta cells of Langerhans has increased the activity of the reactive oxygen species (ROS), which are known to produce free radicals that increase the establishment of the impairment in many tissues via mitochondria electron transport chain leading to a shift in enzymes activity of the body's defense mechanism. The insulin resistance is reported to be the sole cause of the mechanism behind endothelial dysfunction of smooth muscles as well as organ dysfunction that cause further complications leading to a serious health situation in diabetics [1].

Oxidative stress is the state of the presence of an enormous amount of endogenous oxidative species known as 'Reactive Oxygen Species' such as free radicals of superoxides, which includes hydrogen peroxides, nitric oxides and proxy nitrite as these radicals are known to cause the impairment of the endogenous antioxidant defense system [2][3][4]. These species are known to be responsible for cell damage and also manipulate signal pathways of the deoxyribonucleic acid (DNA) cell structure of the mitochondria, lipids, peroxisomes and protein structures [5].
The signaling effects of oxidative stress are known to have certain functions such as in transcriptional control as well as cell cycle regulation [2][6] with an interaction between the NADPH oxidases the redox enzymes and endogenous mitochondrial reactive oxygen species such as the hydrogen peroxides leading to macrovascular damages.
Diabetes mellitus patients have been reported to have an increase in free radical formation. This establishment of free radicals is made possible by certain processes such as glycation that initiate in the vascular linings oxidative reactions which is significant of diabetic angiopathy and its relevant pathogenesis [7]. The complications that arise from this pathogenesis is an hyperglycemia- induced over production of superoxide which has been described as the causative factor to the development of high glucose concentrations that cause hyperglycemic damages such as microvascular complications (such as diabetic retinopathy, diabetic neuropathy, diabetic nephropathy) and microvascular complications (such as stroke, congestive heart failure;, atherosclerosis).Further diagnosis made in the clinical laboratories with patient's sample revealed increased levels of lipids, proteins, DNA bases causing damages done to the DNA component.

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. [8] reported the increasing trends in the usefulness of plants as medicinal remedies in disease conditions to having some certain degree of antioxidant properties than the aforementioned antioxidants. Some drugs in circulation that are prescribed to ailing individuals are plant derivatives. The aim of this study was therefore to assess the antioxidant effects of aqueous, ethanolic and methanolic extracts of *Morus mesozygia Linn. Stapf.,* Leaves 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 [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, OsunState, 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 [9] 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 [10]. 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 [11]. 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 allowed to incubate by acclimatizing for two weeks prior to the progression of the study. 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 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.

2.7.1 Experimental Analysis

2.7.1.1 Determination of Total Oxidant Status (TOS) [12]

Principle

The principle was based on Sandwich-Enzyme linked immunosorbent assay launched between labelled sample and horseradish conjugated antibody specific to total oxidant status.

Procedure

The reagent was brought to room temperature (19-25^{o}C) before the laboratory procedure commenced. The protocol from the manufacturers was followed from the onset to the finish of the test.
Briefly, samples were diluted (1:5), 10µl of sample was added to the wells, 40µl of dilution buffer was also added. 100µl of Horseradish peroxidase conjugate was added into all the wells and incubated at 37^{o}C for 60 minutes.
After the incubation, wells were washed five times according to the manufacturer's instruction.
Chromogen A and B were added and incubated in the dark at 37^{o}C for 15 minutes. Thereafter, stop solution was added into all the wells to stop the reaction. Absorbance was read using a microplate reader at 450nm.

2.7.1.2 Determination of Total Antioxidant Status (TAS) [12]

Principle

The principle was based on Sandwich- Enzyme linked immunosorbent assay with a pre-coated horseradish peroxidase conjugate on the micro ELISA plate that is specific to super oxidase dismutase.

Laboratory Procedure

The procedure followed stringent protocol as laid on the manufacturer's guide.40µl of the sample dilution buffer was added to the wells, likewise was 10µl of the sample in a dilution factor of 1:5. The mixture was gently shaken.
100µl of Horseradish peroxidase conjugate was added to each of the wells excluding the blank well. The plate was allowed to incubate at 37^{o}C for 15 minutes. The wells were

washed with diluted distilled water from wash bottle, excess water was discarded and made to dry.
50µl chromogen solution A and B was added to the mixture which turned blue. The mixture changed into yellow on the addition of the stop solution. Absorbance was read at 450nm.

2.7.1.3 Calculation of OSI [12]

Oxidative Stress Index further explains the degree of oxidative stress**.**
Calculations for the determination of OSI is expressed as the ratio of the total oxidant status (TOS) level by total antioxidant status.
That is $\frac{\text{TOS}}{\text{TAS, (arbitrary unit) expressed as } \frac{\text{TOS (umol } H_2O_2 \text{ Eq/L)}}{\text{TAS (umol Trolox Eq/L) [12]}}}$

2.7.1.4 Determination of Superoxide Dismutase [13]

The superoxide dismutase kit was obtained fromAssay Solution in the United States of America. The experiment followed all the specifications as stipulated by the manufacturer from the beginning to the end of this assay.

Principle

The principle was based on Sandwich- Enzyme linked immunosorbent assay method, an antigen- antibody binding as described by [13].

Laboratory Procedure

40µl of the sample dilution buffer and 10ul of the sample were added to all the wells in a dilution factor of 5.
The samples were loaded to the bottom and allowed to mix properly.100µl of horseradish peroxidase conjugate reagent was added to each of the wells excluding the blank well. The mixture was covered with the membrane sealant provided and allowed to incubate for 60^{o}C for 15 minutes. A 20- fold solution of distilled water was used to wash the solution after which it was reserved.
The mixture after discarding off excess liquid was uncovered, it was washed with the wash buffer provided five times for 30 seconds and allowed to drain, this procedure was repeated five more times and finally allowed to dry. 50µl of chromogen solution A and chromogen solution B were added to all the wells and kept in the dark to incubate for 37^{o}C for 15 minutes which developed a blue color in the wells. 50µl of the stop solution was added after 15 minutes to stop the reaction which enhanced the color developed, the final color developed was yellow. The plate was read at an absorbance of 450nm.

2.7.1.5 Determination of Malondialdehyde

The estimation of malondialdehyde was carried using the method described by [12]

Principle

The principle is based on the quantification of a powerful light-absorbing and fluorescing adduct in a continuous reaction with thiobarbituric acid (TBA).

Laboratory Procedure

0.8ml of serum was added to the mixture of TBA, Thiobarbituric acid, hydrochloric acid in equal volumes. The mixture was boiled for ten minutes with the aid of a water bath. After the sample was boiled, it was allowed to cool and centrifuged for ten minutes. The absorbance was read at 532nm.

2.8 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 ($p<0.05$).

4 Results and Discussion

The results from Table 1 revealed an increased level of superoxide dismutase concentration (SOD,411.8±1.49) ng/ml, (TAS,75.25±0.42) mU/ml, (TOS,353.51± 6.07) mU/ml, OSI index of 4.69±0.05 and a reduced concentration of MDA of 19.0± 1.49mmol/L that were treated with 400mg/kg of aqueous leaves of *Morus mesozygia Linn. S.,* in Group 5 when compared to the levels of SOD (411.38±0.71)ng/ml, (TAS,67.54±54) mU/ml, (TOS,319.37±24.69), OSI index of 5.10±1.71 and an MDA of 21.11±1.86mmol/L of the male diabetic rats in Group 10 treated with 200mg/kg of aqueous leaf extracts of *MMLS*., This showed that 400mg/kg of aqueous leaves could be a better dose that has the ability to suppress the levels of oxidative stress in type 2 diabetes mellitus probably because of the presence of the following polyphenols analyzed during the phytochemical analysis (not shown). The highly presence of Flavonoids as a bioactive agent may be responsible for the antioxidative activity shown in the reduction of MDA concentration when 400mg/kg of aqueous leaves extracts were administered orally to the diabetic rats compared with that of the non-treated. This was similar to the reports of [14] that observed antioxidant activities such as Quercertin (QT), Quercertin-3-O-glucose-6"-acetate (QT-G,QT-GA), Rutin(RT) on diabetic induced experimental rat models. This again was similar with the work done by [15] who administered 500mg/kg of aqueous leaves extracts of *Morus alba Linn*., in male albino rats and observed a significant alleviation of SOD concentration that played an inductive role in the scavenging of toll- like receptors and the activation of pathways of vaious oxidative reactive oxygen species which are responsible for certain phagocytic actions that causes damages to the cells

The significant decreased concentration of MDA in Group 5 on Table 1 of the diabetic rats treated with 400mg/kg of aqueous leaves extracts was significant due to increased SOD and TAS that induced oxidative stress on the pancreas by the process of lipid peroxidation when compared with the MDA levels of the diabetic male rats in Group 6 treated with a lower dosage of 200mg/kg of aqueous leaves extracts. This antioxidative effect exhibited by the 400mg/kg dose of aqueous leaves extracts of *MMLS* could be possible due to the high presence of flavonoids, a bioactive phenolic compound that might have worked on an action mechanism against lipid peroxidation capable of initiating oxidative stress with a resultant effect of acting as an antioxidative agent against vascular and cardiovascular diseases that can be classified as risk factors to type two diabetes. This work was in agreement with the work done by [16] who administered diabetic rats with aqueous leaves extracts of mulberry leaves of *Morus indica Linn*. that decreased lipid peroxidation as a result of decreased levels of MDA and further alleviate hyperglycemia; thus on the other hand, hyperglycemia can lead to glycosylation of antioxidant activities like the enzyme superoxide dismutase [17]. The non-diabetic group administered orally with 400mg/kg aqueous leaves extracts also showed

increased significant difference of SOD, TAS, and decreased MDA concentrations when compared with those of the diabetic populations, this can be indicative that this dosage has a potent capacity of combating oxidative stress in any sub population. The Turkey's Multiple Comparison Test showed a great significant difference between the oxidative parameters of the positive controls when compared with that of the normal controls.

Table 1 Oxidative Profiles of Streptozotocin Induced Diabetic Male Rats Treated with Aqueous Extract of *Morus mesozygia Linn. Stapf.* Leaf Extracts

Groups	SOD (ng/ml)	TAS (mU/ml)	TOS (mU/ml)	OSI	MDA (mmol)
GRP1NC	412.47 ± 1.32	58.81 ± 15.38	363.55 ± 7.58	6.39 ± 2.15	20.51 ± 1.75
GRP2PC	409.42 ± 1.42[1]	43.69 ± 4.04	336.05 ± 13.16	7.72 ± 0.53	36.94 ± 0.27[1]
GRP3	414.24 ± 2.15[1]	109.84 ± 78.99	349.74 ± 32.12	6.64 ±1.34	15.59 ± 1.84[1,2]
GRP5	411.8 ± 1.49	75.25 ± 0.42	353.51 ± 6.07	4.69 ± 0.05	19.0 ± 1.49[1]
GRP6	411.38 ± 0.71[3]	67.54 ± 18.95	319.37 ± 24.69[2]	5.10 ± 1.71	21.1 ± 1.86[1,3]
GRP9	414.26±0.11[1,6]	111.62 ± 12.95[1]	368.66± 14.74[6]	3.35 ± 0.55	15.43 ± 2.24[All]
p-values	< 0.0001	0.0217	0.004	0.1863	< 0.0001
F-values	9.12	3.267	4.692	1.646	110
Remark	S	S	S	NS	S

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

The results from Table 2 revealed a significant increase in SOD, TAS, decreased TOS and OSI levels in the male diabetic rats treated orally for 30 days with 200mg/kg of methanolic leaves extracts of *MMLS*., revealing no that the increase in SOD, TAS maintained the integrity of the pancreas against damage due to oxidative stress by ROS when compared with the SOD concentration of the diabetic rats treated orally with 400mg/kg of methanolic leaves that showed decreased SOD, TAS, increased TOS and OSI that might be due to oxidative damage. The antioxidative nature of 200mg/kg in treatment of these diabetic rats might be due to the high presence of flavonoids which in its action in mechanism might have scavenged the reactive oxygen species that are responsible for oxidative stress in the diabetes. This was in agreement with the work carried out by [18] who investigated myocardial infarction in streptozotocin induced male rats on a low dosage of methanolic *Morus alba Linn*. in an *in vivo* method and found out that the low dosage essentially decreased the activities of reactive oxygen species. This result however did not agree with the work done by [18] who treated diabetic rats with 500mg/kg in high dose and found out

that there were decrease in the cardio-protective activities of antioxidative enzymes of superoxide dismutase and catalases.

Table 2 Oxidative Profiles of Streptozotocin Induced Diabetic Male Rats Treated with Methanolic Extract of *Morus mesozygia Linn. Stapf.* Leaf Extracts

Groups	SOD(ng/ml)	TAS (mU/ml)	TOS (mU/ml)	OSI	MDA (mmol)
GRP1NC	412.47 ± 1.32	58.81 ± 15.38	363.55 ± 7.58	6.39 ± 2.15	20.51 ± 1.75[1]
GRP2PC	409.42 ± 1.42[1]	43.69 ± 4.04	336.05 ± 13.16	7.72 ± 0.53	36.94 ± 0.27
GRP11	413.52 ± 1.63[2]	61.68 ± 55.03	353.06 ± 53.06	4.09±0.34[1,2]	19.56 ± 4.46[2]
GRP13	414.0 ± 1.27[2]	86.68 ± 6.74	367.70 ± 1.46	4.25± 0.35[2]	17.73 ± 0.33[2]
GRP12	416.7 ± 0.95[1,2,11]	124.54±19.11[1,2,11]	361.18 ± 1.61	2.96±0.57[1,2]	22.83 ± 3.99[2]
GRP9	414.26± 0.11[2,12]	11.62±12.95[1,2,11]	368.66± 14.74	3.35±0.55[1,2]	15.43 ± 2.24[2]
p-values	< 0.0001	0.0001	0.2656	< 0.0001	< 0.0001
F-values	19.38	8.026	1.834	13.05	39.91
Remark	S	S	NS	S	S

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

Table 3 revealed a correl of decreasing lipid peroxidation as MDA levels with increased levels of SOD, increased, TAS decreased TOS from the results of the diabetic male albino rats treated with 200mg/kg of ethanolic leaves were observed when compared to the increased MDA, decreased SOD, TAS and increased TOS levels of the diabetic rats treated with 400mg/kg of ethanolic rats. This decreased in MDA exhibited by treatment with 200mg/kg of ethanolic leaves may be due to phytochemical presence of Flavonoids, Quercetin is the most studied type of flavonol, which might have shown a significant protective and anti-inflammatory activities exerted on the metabolic, pathological derangement of glucose metabolism by scavenging nitrogen reactive species and lipid peroxidation on cell membranes according by the reports of [19].The results from these findings did not agree with those of [18], who treated diabetic rats with 500mg/kg of methanolic extracts of Mulberry leaves and observed the dosage might have imparted upon on the SOD, CAT, GPx and GSH concentrations.

Table 3 Oxidative Profiles of Streptozotocin Induced Diabetic Male Rats Treated with Ethanolic Extract of *Morus mesozygia Linn. Stapf.* Leaf Extracts

Groups	SOD (ng/ml)	TAS (mU/ml)	TOS (mU/ml5)	OSI	MDA (mmol)
GRP1NC	412.47 ± 1.32	58.81 ± 15.38	363.55 ± 7.58	6.39 ± 2.15	20.51 ± 1.75
GRP2PC	409.42 ± 1.42	43.69 ± 4.04	336.05 ± 13.16	7.72 ± 0.53	36.94 ± 0.27[1]
GRP4	411.22 ± 1.03	145.6 ± 81.31[1]	383.84 ± 28.91[2]	3.87 ± 2.82[2]	17.20 ± 0.41[2]
GRP7	413.75 ± 0.71	56.32 ± 2.23[4]	340.62 ± 8.04[4]	6.05 ± 0.40	22.99 ± 7.59[2]
GRP8	416.69 ± 0.36	154.24±47.43[1,2,7]	365.54 ± 13.08	2.67±1.26[1,2,7]	2.97 ± 1.25[1,2,4,7]
GRP9	414.26 ± 0.11	111.62 ± 12.95[1]	368.66± 14.74[2]	3.35 ± 0.55[2]	15.43 ± 2.24[2,7]
p-values	< 0.0001	0.0002	0.0003	0.0002	< 0.0001
F-values	35.02	7.637	6.453	7.942	54.39
Remark	S	S	S	S	S

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

4. CONCLUSION

From the findings of this study, we conclude that Methanolic, ethanolic and aqueous extracts of *Morus mesozygia Linn. S* ameliorated oxidative stress in Streptozotocin-induced diabetic rats, with the methanolic extract (due to its more phytochemical constituents) and higher doses of the three extracts relatively showing better ameliorative effect.

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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