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Antioxidant Potentials of Aqueous and Ethanolic Extracts of *Morus mesozygia Linn Stapf* Twigs in Streptozotocin-Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to assess the antioxidant effects of aqueous, ethanolic and methanolic extracts of *Morus mesozygia Linn. Stapf.* Twigs 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 and 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 twig 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. They were then divided into different groups and treated with different concentrations of aqueous, ethanolic and methanolic extracts of the plant material. At the end of treatment period, the rats were kept on fasting for 6 hours prior to the process of euthanasia, they were sacrificed and blood samples were collected through cardiac puncture for analysis into lithium heparin bottle for the estimation of oxidative stress markers, malondaldehyde (MDA), total oxidant

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status (TOS), oxidative stress index (OSI), and antioxidant enzymes, superoxide dismutase (SOD), total antioxidant capacity (TAS). Statistical analysis was done using GraphPad prism (version 6.1) software. Data generated were represented as mean and standard deviations (Mean ±S. D). Level of significant at Tukey's Multiple Comparative Test was tested at $p<0.0001$. Charts were made possible with the application of Minitab version 2019.

Results: The results showed that there were significant increases in the levels of superoxide dismutase (SOD, 414.2±1.30) ng/ml, total antioxidant status (TAS, 82.97±7.71) mU/ml, total oxidant status (TOS, 355.02± 14.02) mU/ml activity, a reduced oxidative stress index of 4.29±0.26 and concentration of malondialdehyde (MDA of 18.67± 0.26mmol/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*. there was a significant increases and decreases respectively. Other methods of extractions (methanolic and ethanolic), also improved the antioxidant statuses of the diabetes induced and treated rats after treatment of the extracts.

Conclusion: The three extracts of *Morus mesozygia Linn. S* showed tremendous antioxidant effects against Streptozotocin-induced diabetic rats, with the methanolic extract showing the most potent effect.

Keywords: Antioxidant potentials, aqueous; ethanolic extracts; Morus mesozygia Linn. Stapf.; twigs; streptozotocin-induced diabetic rats.

1. INTRODUCTION

The mortality and morbidity effect that arises due to the metabolic complications of diabetes mellitus has created a significant menace on public health. However due to the increase in obesity as a result of unhealthy feeding habits as well as sedentary lifestyle amongst individual's insulin resistance with hyperglycemia, has been reported as a significant study that lead to the increase in the prevalence of type 2 diabetes mellitus compared to the other types of diabetes mellitus in Nigeria [1]. Adeloye et al. [2] carried out a meta-analysis involving individuals between ages 20 to 79 years and observed an increase of 285 million individuals with diabetes mellitus in 2010 and 415 million in 2015 [3]. It has also been reported that about 1 in 11 individuals which is an equivalent of 80% of individuals all over the world live with diabetes, [3].

A prevalent data finding on a recent research was compared in urban Africa with some parts of the developed countries, the outcome revealed enormous data of individuals living with the burden of diabetes mellitus against in the urban against those in the developed countries, these findings was suggestive of a linkage between environmental migration fluctuations, the aperture of the way of life of the westerners, urbanization intensification and diet changes. A study was conducted by Adeloye et al. [2] in the southern parts of Nigeria and this derived the largest pool of data of the prevalence of individuals suffering with type 2 diabetes mellitus (at 8.5%, 95% confidence interval, then at 5.1% it grew up to 11.9%) lined up next in geopolitical zones with highest prevalence of type two diabetes was North-East and the South East areas (at 4.6%, 95% confidence interval then at 0.3% it rose up to 8.8% and (at 3.7% in 95% confidence interval, the data grew from 3.3% to 4.2 correspondingly.

Type 2 diabetes mellitus as a chronic metabolic disease is characterized by the formation of free radicals that predominantly leads to oxidative stress. These free radicals are defined as extremely combative atoms or molecules that possess one or more impaired electrons that are in the outermost part of the shell that are often in interactions with various other molecules [4].

A state of imbalance in relationship to the elimination of free radicals that alter the activities of endogenous anti-oxidative enzymes such as the Glutathione Peroxidases (GPx), Superoxide Dismutases (SOD), Catalases (CAT) and a host of others is termed 'Oxidative Stress'. These free radicals have been reported to cause an increase in levels of mitochondrial energy production, protein glycosylation, glucose oxidation and increasing lipid peroxidation of reactive oxygen species (ROS) as well as free radicals are known to produce a significant implication in the degeneration of diseases such as in persistent hyperglycemia as in type 2 diabetes mellitus, cardiovascular diseases, cancers [4].

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. Bahmani et al. [5] also, 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. A study by Joshua et al. [6], it was also reported that *Morus mesozygia Linn. Stapf.* leaves possess strong antioxidant effect. Some drugs in circulation that are prescribed to ailing individuals are plant derivatives.

The aim of this study was to evaluate the antioxidant potentials of aqueous and ethanolic extracts of *morus mesozygia linn. stapf.,* twigs 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 [5].

2.2 Plant Collection and Authentication

Morus mesozygia Linn. (family Moraceae) fresh twigs 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* twigs 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 powderedtwigs 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 twig using Distilled water, absolute Ethanol and Methanol

Nine hundred and sixty grams (960g) of dried powdered *Morus mesozygia linn* twig 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 severely 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 [7] 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 twig extracts were kept in a 4^{o}C refrigerator prior to pharmacological investigations.

2.2.2 Aqueous and ethanolic and methanolic 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) 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) and 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 [8]. 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 [9]. 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 twig aqueous, ethanol and methanolic 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 twig extract only.

Group Four: 5 male rats were given 400mg/kg body weight orally of methanolic twig extract only.

Group Five: 5 male rats were given 400mg/kg body weight orally of ethanolic twig extract only.

Group Six: 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 twig extract.

Group Seven: 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 twig extract.

Group Eight: 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 methanolic twig extracts.

Group Nine: 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 twig extracts.

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 of ethanolic twig extracts.

Group Eleven: 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 twig extracts.

Group Twelve: 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 Thirteen: 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 twig extract and 100mg/kg of metformin.

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 lithium heparin bottle for the estimation of oxidative stress markers and antioxidants.

2.7.1 Experimental analysis

2.7.1.1 Determination of total oxidant status (TOS) [10]

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) [10]

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 [10]

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 TOS

TAS, (arbitrary unit) expressed as TOS (umol H_2O_2 Eq/L)

TAS (umol Trolox Eq/L) [10]

2.7.1.4 Determination of superoxide dismutase [11]

The superoxide dismutase kit was obtained from Assay 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 [11].

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 [10]

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 Comparison 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 revealed a significant increase ($p<0.05$) in SOD, TAS, decrease ($p=0.0002$) in TOS, decrease ($p<0.05$) in OSI and decrease ($p<0.05$) in MDA concentration of the diabetic male rats in Group 7 treated orally with 200mg/kg body weight in dose for 30 days of aqueous twigs *MMLS*., when compared with the concentration of SOD, TAS, increased TOS, OSI and MDA in the diabetic male rats in Group 6 treated orally with 400mg/kg of aqueous twigs extracts of *MMLS*., (Table 1 and 2). Likewise compared with that of Group 12 and the positive control. There was significant decrease ($p<0.05$) in SOD, TAS, decreased TOS, OSI and MDA concentration of the non-diabetic male rats administered orally with 400mg/kg of twig aqueous extracts in Group 3 when compared to those of Group 7,6, 12 and the positive and negative controls.

The result indicated a significant increase ($p<0.05$) in SOD, TAS, decrease ($p=0.0002$) in TOS, decrease ($p<0.05$) in OSI and MDA concentrations of the diabetic male rats in Group 8 treated orally for 30 days with 400mg of methanolic twigs *MMLS*., when compared with the decreased concentration of SOD, TAS, increased TOS, OSI and MDA of the diabetic male rats in Group 9 treated orally for 30 days with 200mg/kg in dose of methanolic twigs extracts of *MMLS*., (Table 2 and 3). Likewise compared with those of Group 12 of the male diabetic rats treated orally with 100mg/mg of standard metformin drug and the positive controls. There was a significant increase ($p<0.05$) in SOD, TAS, decrease ($p=0.0002$) in TOS, decrease ($p<0.05$) in OSI and MDA concentrations of the non-diabetic male rats administered orally with 400mg/kg in dose of twig methanolic extracts in Group 4 when compared to the concentrations of Group 8, 9, 12, and positive controls.

There was also a significant increase ($p<0.05$) in SOD and TAS, decrease ($p<0.05$) in TOS, OSI and MDA concentrations of the diabetic male rats in Group 11 treated orally for 30 days with 200mg of ethanolic twigs *MMLS*., when compared with the concentration of decreased SOD and TAS, increased TOS, OSI and MDA concentrations in the diabetic male rats in Group 10 treated orally for 30 days with 400mg/kg in dose of ethanolic twigs extracts of *MMLS*., (Table 3 and 4) compared with the levels in Group 12 of the male diabetic rats treated orally with 100mg/mg of standard metformin drug and

Table 1. Treatment with 400mg/kg, 200mg/kg in doses of aqueous twig of *Morus mesozygia Linn Stapf,* extract compared with non-treated controls

	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	43.69 ± 4.04	336.05 ± 13.16	7.72 ± 0.53	36.94 ± 0.27
GRP3NDAT400mg	409.34 ± 2.38	39.78 ± 1.17	359.14 ± 5.08	9.03 ± 0.32	14.35 ± 0.83
GRP6DAT400mg	414.2 ± 1.30	82.97 ± 7 .71	355.02 ± 14.06	4.29 ± 0.26	18.67 ± 0.26
GRP7DAT200mg	414.99 ± 1.28	150.86 ± 11.11	377.86 ± 9.57	2.50 ± 0.13	18.24 ± 0.35
GRP12DMF100mg	414.26 ± 0.11	111.62 ± 12.95	368.66 ± 14.74	3.35 ± 0.55	15.43 ± 2.24
p-values	< 0.0001	< 0.0001	0.0002	< 0.0001	< 0.0001
F-values	14.79	98.1	7.904	36.7	229

Table 2. Multiple comparison test for treatment with 400mg/kg, 200mg/kg in doses of aqueous twig of *Morus mesozygia Linn Stapf.* extract compared with non-treated controls

	SOD (ng/ml)	***TAS (mU/ml)***	***TOS (mU/ml)***	***OSI***	***MDA (mmol)***
Tukey's Multiple Comparison Test	**Summary**	**Summary**	**Summary**	**Summary**	**Summary**
Group 1 vs Group 2	*	Ns	**	Ns	***
Group 1 vs Group 3	*	Ns	Ns	**	***
Group 1 vs Group 6	Ns	**	Ns	*	Ns
Group 1 vs Group 7	Ns	***	Ns	***	Ns
Group 1 vs Group 12	Ns	***	Ns	***	***
Group 2 vs Group 3	Ns	Ns	*	Ns	***
Group 2 vs Group 6	***	***	Ns	***	***
Group 2 vs Group 7	***	***	***	***	***
Group 2 vs Group 12	***	***	**	***	***
Group 3 vs Group 6	***	***	Ns	***	***
Group 3 vs Group 16	***	***	Ns	***	***
Group 3 vs Group 12	***	***	Ns	***	Ns
Group 6 vs Group 7	Ns	***	*	Ns	Ns
Group 6 vs Group 12	Ns	**	Ns	Ns	**
Group 7 vs Group 12	Ns	***	Ns	Ns	*

*Key: NS-Non significant, *=Significant at p<0.05, **= significant at p<0.01, ***= significant at p<0.001*

Table 3. Treatment with 400mg/kg, 200mg/kg in doses of Methanolic Twig of *Morus mesozygia Linn Stapf,* extracts compared with non-treated controls

	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	43.69 ± 4.04	336.05 ± 13.16	7.72 ± 0.53	36.94 ± 0.27
GRP4NDMT400mg	418.66 ± 3.28	133.84 ± 26.21	491.6 ± 104.9	2.78 ± 1.41	21.57 ± 0.57
GRP8DMT400mg	415.2 ± 0.83	163.16 ± 28.66	389.79 ± 17.19	2.44 ± 0.42	18.66 ± 0.12
GRP9DMT200mg	410.34 ± 1.02	129.47 ± 2.92	349.79 ± 20.96	2.69 ± 0.11	19.01 ± 1.90
GRP12MTMF100mg	414.26 ± 0.11	111.62 ± 12.95	368.66 ± 14.74	3.35 ± 0.55	15.43 ± 2.24
p-values	< 0.0001	< 0.0001	0.0002	< 0.0001	< 0.0001
F-values	21.2	33.79	7.738	20.49	142.8

Table 4. Multiple comparison test treatment with 400mg/kg, 200mg/kg in doses of Methanolic Twig of *Morus mesozygia Linn Stapf,* extracts compared with non-treated controls

	SOD (ng/ml)	*TAS (mU/ml)*	*TOS (mU/ml)*	*OSI*	*MDA (mmol)*
Tukey's Multiple Comparison Test	**Summary**	**Summary**	**Summary**	**Summary**	**Summary**
Group 1 vs Group 2	Ns	Ns	Ns	Ns	***
Group 1 vs Group 4	***	***	**	***	ns
Group 1 vs Group 8	Ns	***	Ns	***	ns
Group 1 vs Group 9	Ns	***	Ns	***	ns
Group 1 vs Group 12	Ns	**	Ns	**	***
Group 2 vs Group 4	***	***	***	***	***
Group 2 vs Group 8	***	***	Ns	***	***
Group 2 vs Group 9	Ns	***	Ns	***	***
Group 2 vs Group 12	**	***	Ns	***	***
Group 4 vs Group 8	*	Ns	*	Ns	*
Group 4 vs Group 9	***	Ns	***	Ns	ns
Group 4 vs Group 12	**	Ns	**	Ns	***
Group 8 vs Group 9	**	Ns	Ns	Ns	ns
Group 8 vs Group 12	Ns	**	Ns	Ns	*
Group 9 vs Group 12	*	Ns	Ns	Ns	**

*Key: NS-Non significant, *=Significant at p<0.05, **= significant at p<0.01, ***= significant at p<0.001*

Table 5. Treatment with 400mg/kg, 200mg/kg of ethanolic twig of *Morus mesozygia Linn Stapf,* extracts compared with non-treated controls

	SOD (ng/ml)	***TAS (mU/ml)***	***TOS (mU/ml)***	***OSI***	***MDA (mmol)***
GROUP1NC	412.47 ± 1.32	58.81 ± 15.38	363.55 ± 7.58	6.39± 2.15	20.51 ± 1.75
GROUP 2PC	409.42 ± 1.42	43.69 ± 4.04	336.05± 13.16	7.72± 0.53	36.94 ± 0.27
GRP5DET400mg/kg	414.34 ± 1.43	179.24± 21.83	406 ± 20.0	4.0 ± 1.32	20.78 ± 2.51
GRP10NDET200mg/kg	412.92 ± 1.4	216.03± 23.81	447.97± 28.21	2.07 ± 0.11	16.66 ± 2.07
GRP11DET200mg/kg	413.32 ± 0.19	245.77± 94.47	408.16± 22.84	2.11 ± 1.49	13.49 ± 0.66
GRP12MFD100mg/kg	414.26 ± 0.11	111.62± 12.95	368.66± 14.74	3.35 ± 0.55	15.43 ± 2.24
p-values	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F-values	12.1	9.041	22.15	8.156	112.8

Table 6. Multiple comparison test treatment with 400mg/kg, 200mg/kg of Ethanolic Twig of *Morus mesozygia Linn Stapf,* extracts compared with non-treated controls

	SOD (ng/ml)	***TAS (mU/ml)***	***TOS (mU/ml)***	***OSI***	***MDA (mmol)***
Tukey's Multiple Comparison Test	Summary	Summary	Summary	Summary	Summary
Group 1 vs Group 2	**	Ns	Ns	Ns	***
Group 1 vs Group 5	Ns	Ns	*	Ns	ns
Group 1 vs Group 10	Ns	**	***	**	*
Group 1 vs Group 11	Ns	**	*	**	***
Group 1 vs Group 12	Ns	Ns	Ns	Ns	**
Group 2 vs Group 5	***	*	***	*	***
Group 2 vs Group 10	***	**	***	***	***
Group 2 vs Group 11	***	***	***	***	***
Group 2 vs Group 12	***	Ns	Ns	**	***
Group 5 vs Group 10	Ns	Ns	*	Ns	*
Group 5 vs Group 11	Ns	Ns	Ns	Ns	***
Group 5 vs Group 12	Ns	Ns	*	Ns	**
Group 10 vs Group 11	Ns	Ns	*	Ns	ns
Group 10 vs Group 12	Ns	Ns	***	Ns	ns
Group 11 vs Group 12	Ns	*	*	Ns	ns

*Key: NS-Non significant, *=Significant at p<0.05, **= significant at p<0.01, ***= significant at p<0.001*

decreased SOD and TAS, increased TOS, OSI and MDA in the positive controls. There was a significant increase ($p<0.05$) in SOD, TAS, decrease ($p<0.05$) in TOS, OSI and MDA concentration of the non-diabetic male rats administered orally for 30 days with 400mg/kg in dose of twig methanolic extracts alone in Group 5 when compared to the levels in the diabetic male rats in Group 11, 10, 12, the negative and positive controls.

Tables 1 (Aqueous twig), 2 (Methanolic twig) and 3 (Ethanolic twig) explained oxidative stress in the 30 days' treatment of the induced- diabetic rat model but the 400mg/kg of methanolic twig (Table 2) plant extracts revealed greater increase in total antioxidative status (TAS) and decrease in MDA in treatment of diabetes in the experimental rats for 30 days followed by the ethanolic then aqueous twig (Table 1) extracts. This anti-oxidant effect of the high dosage of methanolic twig extract may be due the extract having the highest yield of flavonoids (22.99%), ethanolic twig (18.10%) then the aqueous twig extract (6.22%). This was similar to the reports of Ying et al. [12] that stated a reduction of MDA and increase in SOD.

This anti-oxidative effect is indicative of the high flavonoid yield of methanolic twig extract, followed by ethanolic twig then aqueous twig (unpublished). Flavonoids have been reported to be anti-oxidative in a mechanism that describes their scavenging properties of reactive oxygen species against lipid peroxidation by increase in MDA concentrations. This was in consonance with the reports of Saefudin et al. [13]. It has been reported that some medicinal plants show a neuroprotective effect due to the presence of antioxidants [14]. For example, extracts from young leaves and mulberry twig are rich in oxyresveratrol are utilized in the treatment of neurodegenerative diseases. Most studies, did not find the toxic effects of mulberry preparations. Reported beneficial health, therapeutic and antioxidant properties of mulberry products are most likely associated with the presence of bioactive compounds, including flavonoids, phenolic acids, iminosugars, tannins, polysaccharides and alkaloids [15].

4. CONCLUSION

The three extracts of *Morus mesozygia Linn. S* showed tremendous antioxidant effects against Streptozotocin-induced diabetic rats, with the methanolic extract showing the most potent effect.

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.

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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