



## Evaluation of the effects of Aqueous and Ethanolic Extracts of *Morus mesozygia* Linn. Stapf., Leaves on Lipid Profile and Renal Indices of Streptozotocin- Induced Diabetic Rats

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### Abstract

*Diabetes mellitus as a metabolic disorder invades with characterized hyperglycemia as well as glycosuria that if not treated properly can lead to the amputation of the arms and limbs. The increase in medi-care cost and the side effects that comes from the use of orthodox drugs has made scientists to look into phytomedicine, the use of plant herbs as an alternative for treatment modalities. Morus mesozygia Linn Stapf., was the medicinal plant chosen for this research study. The study evaluated the effects of Morus mesozygia Linn. Stapf., Leaves and Twigs on lipid profile and renal indices in Streptozotocin-Induced Diabetic Rats. A total of fifty (50) male albino rats were used for the study. Two different extracted solvents; aqueous, and ethanolic leaves extracts were used. The male albino rats for this study were induced with a single dose of 40mg/kg b.wt, streptozotocin in 0.1M of citrate buffer, pH 4.5 intraperitoneally. The diabetic male rats were those whose fasting blood glucose (FBG) were from 250mg/dl or 13mmol/L and above. TCHOL, TG & HDL-C were analyzed by enzymatic method, LDL-C by Friedwald's calculation. Electrolytes ( $K^+$ ,  $Na^+$ ,  $Cl^-$ ,  $HCO_3^-$ ) were analyzed by ion-selective electrode. A significant decrease ( $p<0.05$ ) in TCHOL, TRIG, LDL and increased HDL levels when compared with those of the aqueous leaves and ethanolic leave extracts. The ethanolic leaves extract exhibited anti-hypercholesterolemic, anti-lipidemic activities more than the aqueous extract, this may be probably due to ethanol having a greater polarity index as well as exhibiting more components such as polyphenols, flavonoids,, tannins, saponins, alkaloids.*

**Keywords:** Aqueous, Ethanolic Extracts, *Morus mesozygia* Linn. Stapf., Leaves, Lipid Profile, Renal Indices Streptozotocin-Induced, Diabetic Rats.

### Introduction

One of the biggest 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<sup>[1]</sup>. 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.

According by the assessment carried out by<sup>[2]</sup>, 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.

For the purpose of proper diagnosis and assessment of *Morus Mesozygia Linn.* leaves in the cause of digestion and reabsorption in this research study, it is important to carry out laboratory investigations of certain electrolytes such as bicarbonates, sodium, potassium, as the kidney does this in a delicate sensory mechanism<sup>[3]</sup>. Therefore, the aim of the study was to assess the effect of *Morus Mesozygia Linn.* Leaves on the lipid profile and electrolytes of streptozotocin induced diabetic rats.

## Materials and Methods

### 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 (see appendix for parts preparation) 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 (Bahmani *et al.*, 2014).

### Plant Collection and Authentication

*Morus mesozygia Linn.*(family Moraceae) fresh leaves and 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, 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.

### Preparation of Plant Extract (Cold Maceration Extraction Method)

The *Morus mesozygia linn* leaves and 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 powdered leaves produced a total weight of 2.90kg, the powdered twigs weighed a total of 2.45kg; they were stored and labelled into their different air tight containers prior to use.

### Extraction of Powdered *Morus mesozygia linn* leaves using absolute Ethanol

Nine hundred and sixty grams (960g) of dried powdered *Morus mesozygialinn* leaves was put into a clean beaker, five liters (5L) of ethanol was suspended into the beaker, this was shaken severally on a shaker, it was mixed properly and was stored for 24 hours. It was macerated and filtered through a muslin cloth and again filtered out through a What Man's number one filter paper. The filtered extracts were concentrated (on low pressure) using the rotary evaporator equipment<sup>[4]</sup> after which they were dried on an evaporating dish at a temperature of 50°C to 60°C to a semi- solid form. A sticky semi-solid dark brownish substance was obtained. The extract was stored in a well corked universal bottle. The leaf ethanol extract yielded (18.65) g (Appendix I for

calculation) and was kept in a 4°C refrigerator prior to pharmacological investigations.

The same method was used in the aqueous extraction, but instead, 5L of distilled water was used.

### **Aqueous and Ethanolic Extract Dosage Calculation**

Based on the results from the Acute Toxicity test carried out, 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. That is, Average weight of rats in group 1= 150g.

### **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.

### **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 (It is important to put into consideration the citric acid monohydrate of 0.1M solution contains 21.01g/L. The Tri-sodium citratedehydrates of 0.1M solution contains 29.41g/L)

### **Diabetes Induction with Streptozotocin**

After two weeks of acclimatization, the diabetes was induced in the male albino rats with streptozotocin (STZ, Sigma Chemical Company, St. Louis, Milestone). STZ was intravenously (i.v.) 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<sup>[5]</sup>. The diabetic male rats were picked and used for the study design.

### **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 artery and orbital sinus of the rats. The assay of the blood glucose levels was carried out by the glucose-oxidase principle<sup>[6]</sup>. Finetest<sup>TM</sup> test strips and Fine Test Auto Coding<sup>TM</sup> 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.

### **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 twenty-five (26) groups and the research study was carried out as follows:

**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 with aqueous leaf extract only

**Group Four:** 5 male rats were given 400mg/kg body weight orally with 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.

#### **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 anticoagulant bottles for biochemical test which was allowed to sit for some minutes and spun at 3,000r.p.m for 5minutes to get the serum for the analysis of lipid profile.

#### **Determination of Serum Total Cholesterol**

The method used to analyze total cholesterol was as described by<sup>[7]</sup>.

#### **Triglycerides Determination**

The determination of triglycerides in the samples was done by the Glycerokinase peroxidase method, a colorimetric enzymatic method described by<sup>[8]</sup>.

#### **Determination of High Density Lipoprotein-Cholesterol**

The method used to analyze High Density Lipoprotein-Cholesterol was as described by<sup>[9]</sup>.

#### **Estimation of Low Density Lipoprotein-Cholesterol**

The estimation of Low density lipoprotein (LDL) was established using the formulae proposed by<sup>[10]</sup>:

$$\text{LDL -Cholesterol (mg/dl)} = \frac{\text{Total Cholesterol - High density lipoprotein- Triglycerides}}{5}$$

#### **Determination of Plasma Creatinine by Jaffe's Colorimetric Method**

The method used to analyze Creatinine was as described by<sup>[11]</sup>.

#### **Determination of Plasma Urea**

**Bertholot's Enzymatic Method** was used to analyze plasma urea.

#### **Determination of Sodium by Ion-Selective Electrode**

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.

#### **Determination of Bicarbonate by Ion-Selective Electrode**

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.

#### **Determination of Chloride by Ion-Selective Electrode**

Chloride ions react with the combination of mercurous thiocyanate to produce mercury perchlorate 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.

#### **Determination of Potassium by Ion-Selective Electrode**

The concentration of potassium was measured by the inclusion of sodium tetraphenylboron 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..

### Statistical Analysis

Statistical evaluation was made possible with the application of Graph pad prism (version 5.0). Data

generated were revealed as mean and standard deviations (Mean  $\pm$ SD) in addition to the use of ANOVA (Turkey's Multiple Comparative Test) since the comparism is within more than two group study. The level of significance was tested at ( $p < 0.05$ ).

### Results

**Table 4.34:** Mean and Standard Deviations of Lipid Profiles of Streptozotocin Induced Diabetic Male Rats Treated Orally for 30 days with Aqueous Leaves of *Morus mesozygia* Linn. Stapf. Extracts compared with controls.

	<i>CHOL</i> (mg/dl)	<i>TRIG</i> (mg/dl)	<i>HDL</i> (mg/dl)	<i>LDL</i> (mg/dl)
GRP1NC	205.78 $\pm$ 2.56	187.26 $\pm$ 5.21	182.35 $\pm$ 19.63	26.75 $\pm$ 2.72
GRP2PC	194.36 $\pm$ 1.19	182.57 $\pm$ 3.65	101.86 $\pm$ 10.06	54.01 $\pm$ 9.78
GRP3NDALO	198.98 $\pm$ 4.42	183.42 $\pm$ 3.56	109.9 $\pm$ 3.40	50.54 $\pm$ 9.09
GROP5DA400mg	197.45 $\pm$ 3.44	180.66 $\pm$ 3.41	94.26 $\pm$ 0.24	66.63 $\pm$ 2.83
GRP6DA200mg	222.71 $\pm$ 55.88	190.21 $\pm$ 5.67	180.88 $\pm$ 32.34	37.64 $\pm$ 1.73
GRP9MF100mg	194.33 $\pm$ 2.94	180.32 $\pm$ 1.90	163.89 $\pm$ 5.01	6.90 $\pm$ 2.89
p-values	0.3786	0.0047	< 0.0001	< 0.0001
F-values	1.116	4.546	32.42	67.02
<b>Tukey's Multiple Comparison Test</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>
Positive control group vs Negative control group	Ns	Ns	***	***
Positive control group vs Group3	Ns	Ns	***	***
Positive control group vs Group5	Ns	Ns	***	***
Positive control group vs Group6	Ns	Ns	Ns	Ns
Positive control group vs Group9	Ns	Ns	Ns	***
Negative control group vs Group3	Ns	Ns	Ns	Ns
Negative control group vs Group5	Ns	Ns	Ns	*
Negative control group vs Group6	Ns	Ns	***	**
Negative control group vs Group9	Ns	Ns	***	***
Group3 vs Group4	Ns	Ns	Ns	**
Group3 vs Group6	Ns	Ns	***	*
Group3 vs Group9	Ns	Ns	***	***
Group5 vs Group6	Ns	*	***	***
Group5 vs Group 9	Ns	Ns	***	***
Group6 vs Group 9	Ns	**	Ns	***

Note: GRP1- Negative control, GRP2PC – Positive control, GRP 3NDALO –Non Diabetic Aqueous Leaves Orally fed with 400mg of Aqueous leaves extract of(MMLS.) extract only, GRP5- Diabetic male rats treated orally with 400mg dose of Aqueous(MMLS.), GRP 6- Diabetic male rats treated with 200mg dose of Aqueous leaves extracts of (MMLS.), GRP 9- Diabetic male rats treated with 100mg dose of standard Metformin drug.

**Table 4.37:** Mean and Standard deviation of Potassium, Chloride, Sodium, Bicarbonate, Creatinine and Urea Parameters of Streptozotocin Induced Diabetic Male 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.

	<i>K+</i> (mmol/L)	<i>CL-</i> (mmol)	<i>Na+</i> (mmol/L)	<i>HCO3-</i> (mmol/L)	<i>Cr</i> (mmol/L)	<i>Urea</i> (mmol/L)
GRP1NC	4.34 ± 0.71	44.6 ± 1.52	143.4 ± 3.36	23.4 ± 2.07	154.4 ± 13.03	13.93 ± 0.71
GRP2PC	5.98 ± 0.13	49.8 ± 1.3	152 ± 2.44	19.4 ± 1.52	173.8 ± 3.34	18.19 ± 1.24
GRP3NDALO	4.20 ± 0.76	41.4 ± 1.14	137.8 ± 5.89	28.8 ± 1.30	153 ± 23.27	11.81 ± 0.78
GRP5DA400mg	4.18 ± 0.23	42.8 ± 1.92	138.8 ± 1.30	25.2 ± 2.48	161.8 ± 12.98	16.29 ± 1.12
GRP6DA200mg	4.38 ± 0.23	42 ± 1.58	135 ± 3.80	25 ± 3.39	137 ± 6.16	11.28 ± 0.43
GRP9DMF100mg	3.53 ± 0.72	38.8 ± 5.16	133.4 ± 4.44	26.2 ± 2.16	166.4 ± 10.4	11.55 ± 0.93
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
<b>Tukey's Multiple Comparison Test</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>
GROUP1 vs GROUP2	**	*	Ns	*	Ns	***
GROUP1 vs Group3	Ns	Ns	Ns	**	Ns	*
GROUP1 vs Group5	Ns	*	Ns	**	Ns	**
GROUP1 vs Group6	Ns	Ns	Ns	Ns	Ns	*
GROUP1 vs Group9	Ns	*	*	Ns	Ns	*
GROUP2 vs Group3	***	***	***	***	Ns	***
GROUP2 vs Group5	***	***	***	***	Ns	ns
GROUP2 vs Group6	***	**	**	***	Ns	ns
GROUP2 vs Group9	***	***	***	***	Ns	***
Group3 vs Group9	Ns	Ns	Ns	Ns	Ns	***
Group3 vs Group6	Ns	Ns	Ns	Ns	Ns	***
Group3 vs Group9	Ns	Ns	Ns	Ns	Ns	ns
Group5 vs Group6	Ns	Ns	Ns	Ns	Ns	ns
Group5 vs Group9	Ns	Ns	Ns	Ns	Ns	***
Group6 vs Group9	Ns	Ns	Ns	Ns	Ns	***

Note: GRP1- Negative control, GRP2PC – Positive control, GRP 3NDALO –Non Diabetic AqueousLeaves Orally fed with 400mg of aqueous leaves extract of(*MMLS.*) extract only,GRP5- Diabeticmale rats treated orally with 400mg dose of aqueous(*MMLS.*), GRP 6- Diabetic male rats treated with 200mg dose of Aqueous leaves extracts of (*MMLS.*), GRP 9- Diabetic male rats treated with 100mg dose of standard Metformin drug. (p<0.05 = Statistically Significant) (p> 0.05 = Not Statistically Significant)

**Table 4.46:** Mean and Standard Deviations of Lipid Profiles of Streptozotocin Induced Diabetic Male Rats Treated Orally for 30 days with 400mg/kg, 200mg/kg in Doses of Ethanolic Leaves of *Morus mesozygia* Linn. Stapf., Extracts Compared with 100mg/kg of metformin Standard and Non-Treated Controls.

	<i>CHOL</i> (mg/dl)	<i>TRIG</i> (mg/dl)	<i>HDL</i> (mg/dl)	<i>LDL</i> (mg/dl)
GRP1NC	190.78 ± 2.56	181.26 ± 5.21	182.35 ± 19.63	26.75 ± 2.72
GRP2PC	194.36 ± 1.19	182.57 ± 3.65	101.86 ± 10.06	54.01 ± 9.78
GRP5DET400mg	190.37 ± 4.56	141.64 ± 44.5	110.8 ± 7.25	59.05 ± 16.1
GRP7DET400mg	197.36 ± 1.97	154.44 ± 6.30	202.98 ± 26.04	21.95 ± 9.92
GRP8DET200mg	192.31 ± 0.93	184.34 ± 1.49	170.99 ± 46.45	62.18 ± 12.95
GRP9DMF100mg	194.33 ± 2.94	180.32 ± 1.90	163.89 ± 5.01	6.90 ± 2.89
p-values	< 0.0001	0.2693	< 0.0001	< 0.0001
F-values	21.03	1.373	14.4	24.81
<b>Tukey's Multiple Comparison Test</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>
Group 1 vs Group 2	*	Ns	***	**
Group 1 vs Group 5	***	Ns	***	***
Group 1 vs Group 7	***	Ns	Ns	Ns
Group 1 vs Group 8	***	Ns	Ns	***
Group 1 vs Group 9	***	Ns	Ns	Ns

Group 2 vs Group 4	Ns	Ns	Ns	Ns
Group 2 vs Group 7	Ns	Ns	***	***
Group 2 vs Group 8	Ns	Ns	**	Ns
Group 2 vs Group 9	Ns	Ns	**	***
Group 4 vs Group 7	**	Ns	***	***
Group 4 vs Group 8	Ns	Ns	**	Ns
Group 4 vs Group 9	Ns	Ns	*	***
Group 7 vs Group 8	Ns	Ns	Ns	***
Group 7 vs Group 9	Ns	Ns	Ns	Ns
Group 8 vs Group 9	Ns	Ns	Ns	***

Note: GRP1- Negative control, GRP2PC – Positive control, GRP 5NDMETHLO –Non Diabetic Ethanolic Leaves Orally fed with 400mg of ethanolic leaves extract of(MMLS.) extract only,GRP7- Diabeticmale rats treated orally with 400mg dose of ethanolic(MMLS.), GRP 8- Diabetic male rats treated with 200mg dose of ethanolic leaves extracts of (MMLS.), GRP 9- Diabetic male rats treated with 100mg dose of standard Metformin drug. (p<0.05 = Statistically Significant) (p> 0.05 = Not Statistically Significant)

**Table4.49:** Mean and Standard deviation of Potassium, Chloride, Sodium, Bicarbonate, Creatinine and Urea Parameters of Streptozotocin Induced Diabetic Male 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	44.6 ± 1.52	143.4 ± 3.36	23.4 ± 2.07	154.4 ± 13.03	13.93 ± 0.71
GRP2PC	5.98 ± 0.13	49.8 ± 1.3	152 ± 2.44	19.4 ± 1.52	173.8 ± 3.34	18.19 ± 1.24
GRP4NDET400mg	3.58 ± 1.09	38.8 ± 0.84	105.66 ± 51.65	22.6 ± 1.34	152.2 ± 19.43	11.93 ± 0.60
GRP7DET400mg	3.96 ± 0.23	43.4 ± 3.2	159 ± 5.7	24.6 ± 2.41	161.4 ± 4.56	11.79 ± 0.18
GRP8DET200mg	3.59 ± 0.89	42.2 ± 2.38	139.6 ± 6.98	21.8 ± 1.92	165 ± 5.70	11.91 ± 1.25
GRP9DMF100mg	3.53 ± 0.72	38.8 ± 5.16	133.4 ± 4.44	26.2 ± 2.16	166.4 ± 10.4	11.55 ± 0.93
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
<b>Tukey's Multiple Comparison Test</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>
Group 1 vs Group 2	*	Ns	Ns	*	Ns	***
Group 1 vs Group 4	Ns	*	***	Ns	Ns	Ns
Group 1 vs Group 7	Ns	Ns	***	Ns	Ns	*
Group 1 vs Group 8	Ns	Ns	Ns	Ns	Ns	Ns
Group 1 vs Group 9	Ns	*	*	Ns	Ns	*
Group 2 vs Group 4	***	***	***	Ns	*	***
Group 2 vs Group7	**	*	Ns	**	Ns	***
Group 2 vs Group 8	***	**	**	Ns	Ns	***
Group 2 vs Group 9	***	***	***	***	Ns	***
Group 4 vs Group 7	Ns	Ns	***	Ns	Ns	Ns
Group 4 vs Group 8	Ns	Ns	*	Ns	Ns	Ns
Group 4 vs Group 9	Ns	Ns	Ns	Ns	Ns	Ns
Group 7 vs Group 8	Ns	Ns	***	Ns	Ns	Ns
Group 7 vs Group 9	Ns	Ns	***	Ns	Ns	Ns
Group 8 vs Group 9	Ns	Ns	Ns	*	Ns	Ns

Note: GRP1- Negative control, GRP2PC – Positive control, GRP4NDMETHLO –Non Diabetic MethanolLeaves Orally fed with 400mg of ethanolic leaves extract of(MMLS.) extract only,GRP7- Diabeticmale rats treated orally with 400mg dose ofethanolic (MMLS.), GRP 8- Diabetic male rats treated with 200mg dose of ethanolic leaves extracts of (MMLS.), GRP 9- Diabetic male rats treated with 100mg dose of standard Metformin drug. (p<0.05 = Statistically Significant) (p> 0.05 = Not Statistically Significant)

**Table 4.76:** Mean and Standard deviations of Lipid Parameters of Streptozotocin Induced Diabetic Male Rats Treated orally for 60 days with 400mg/kg Aqueous Leaves Extracts, 200mg/kg Aqueous Levae Extracts of *Morus mesozygia* Linn. Stapf., in Comparism with 100mg/kg of Metformin Drug compared with controls.

	CHOL (mg/dl)	TRIG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
GROUP1NC	199.84 ± 0.96	186.77 ± 9.44	151.13 ± 33.67	24.76 ± 3.46
GROUP 2PC	190.73 ± 9.71	181.36 ± 8.04	127.87 ± 26.91	56.52 ± 11.06
GROUP 3NDAL400mg/kg	182.07 ± 3.13	182.08 ± 3.06	103.28 ± 3.40	49.72 ± 9.81
GROUP 5DAL400mg/kg	143.85 ± 26.59	158.66 ± 4.71	73.46 ± 11.19	51.21 ± 5.22
GROUP 6DAL200mg/kg	161.86 ± 7.24	151.21 ± 6.42	146.40 ± 7.13	28.12 ± 1.72
GROUP 9DMF100mg/kg	196.30 ± 3.84	162.11 ± 40.72	150.79 ± 31.22	16.89 ± 2.89
p-values	< 0.0001	0.0162	0.0004	< 0.0001
F-values	16.43	3.498	6.815	30.58
<b>Tukey's Multiple Comparison Test</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>
Group 1 vs Group 2	Ns	Ns	Ns	***
Group 1 vs Group 3	Ns	Ns	Ns	***
Group 1 vs Group 5	***	Ns	**	***
Group 1 vs Group 6	***	*	Ns	Ns
Group 1 vs Group 9	Ns	Ns	Ns	Ns
Group 2 vs Group 3	Ns	Ns	Ns	Ns
Group 2 vs Group 5	***	Ns	*	Ns
Group 2 vs Group 6	*	Ns	Ns	***
Group 2 vs Group 9	Ns	Ns	Ns	***
Group 3 vs Group 5	***	Ns	Ns	Ns
Group 3 vs Group 6	Ns	Ns	Ns	***
Group 3 vs Group 9	Ns	Ns	Ns	***
Group 5 vs Group 6	Ns	Ns	**	***
Group 5 vs Group 9	***	Ns	**	***
Group 6 vs Group 9	**	Ns	Ns	Ns

Note: NC(Negative Control), PC (Positive control), NDAL400mg/kg (Non-Diabetic given orally 400mg/kg of Aqueous leaves), DAL400mg/kg (Diabetic rats treated with Aqueous leaf 400mg/kg), DAL200mg/kg (Diabetic rats treated orally with 200mg/kg) (p<0.05=significant)

**Table 4.79:** Potassium, Chloride, Sodium, Bicarbonate, Creatinine, and Urea Parameters of Streptozotocin Induced Diabetic Male Rats Treated orally for 60 days with 400mg/kg of Aqueous Leaves and 200mg/kg Extracts of *Morus mesozygia* Linn. Stapf., in Comparism with 100mg/kg of Metformin Drug compared with controls.

	K+(mmol/L)	CL- (mmol)	Na+ (mmol/L)	HCO3- (mmol/L)	Cr (mmol/L)	Urea (mmol/L)
GROUP1NC	3.88 ± 0.67	42.8 ± 1.92	140.4 ± 2.30	21.4 ± 2.07	146.8 ± 7.85	13.33 ± 0.98
GROUP 2PC	5.94 ± 0.15	49.4 ± 1.14	146.6 ± 5.89	19.6 ± 1.67	143 ± 6.16	16.28 ± 2.23
GROUP 3NDAL400mg	3.97 ± 0.65	40.4 ± 1.14	142 ± 16.01	27.2 ± 1.48	150.8 ± 24.10	11.54 ± 1.12
GROUP 5DAL400mg	3.5 ± 0.36	38.8 ± 1.30	136.2 ± 7.19	28.6 ± 1.67	166.8 ± 5.40	17.05 ± 0.30
GROUP 6DAL200mg	4.1 ± 0.15	41.2 ± 2.17	139.4 ± 0.89	25.2 ± 2.48	161.8 ± 12.98	17.00 ± 0.46
GROUP9DMF100mg	3.53 ± 0.71	38.8 ± 5.16	133.4 ± 4.44	26.2 ± 2.16	166.4 ± 10.43	11.55 ± 0.93
p-values	< 0.0001	< 0.0001	0.1696	< 0.0001	0.0215	< 0.0001
F-values	15.61	12.03	1.715	15.88	3.275	24.75
<b>Tukey's Multiple Comparison Test</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>
Group 1 vs Group 2	***	**	Ns	Ns	Ns	**
Group 1 vs Group 3	Ns	Ns	Ns	**	Ns	Ns
Group 1 vs Group 5	Ns	Ns	Ns	***	Ns	***
Group 1 vs Group 6	Ns	Ns	Ns	Ns	Ns	***
Group 1 vs Group 9	Ns	Ns	Ns	**	Ns	Ns
Group 2 vs Group 3	***	***	Ns	***	Ns	***
Group 2 vs Group 5	***	***	Ns	***	Ns	Ns
Group 2 vs Group 6	***	***	Ns	**	Ns	Ns
Group 2 vs Group 9	***	***	Ns	***	Ns	***
Group 3 vs Group 5	Ns	Ns	Ns	Ns	Ns	***
Group 3 vs Group 6	Ns	Ns	Ns	Ns	Ns	***
Group 3 vs Group 9	Ns	Ns	Ns	Ns	Ns	Ns
Group 5 vs Group 6	Ns	Ns	Ns	Ns	Ns	Ns
Group 5 vs Group 9	Ns	Ns	Ns	Ns	Ns	***
Group 6 vs Group 9	Ns	Ns	Ns	Ns	Ns	***

Note: NC(Negative Control), PC (Positive control), NDAL400mg/kg (Non-Diabetic given orally 400mg/kg of Aqueous leaves), DAL400mg/kg (Diabetic rats treated with Aqueous leaf 400mg/kg), DAL200mg/kg (Diabetic rats treated orally with 200mg/kg) (p<0.05=significant)



**Table 4.88:** Mean and Standard Deviations of Lipid Parameters of Streptozotocin Induced Diabetic Male Rats Treated orally for 60 days with 400mg/kg Ethanolic Leaves Extracts, 200mg/kg Dose of Ethanolic Levaees Extracts of *Morus mesozygia Linn. Stapf.*, in Comparism with 100mg/kg of Metformin Drug compared with Non-Treated controls.

	CHOL (mg/dl)	TRIG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
GRP1NC	199.84 ± 0.96	186.77 ± 9.44	151.13 ± 33.67	24.76 ± 3.46
GRP2PC	190.73 ± 9.71	181.36 ± 8.04	127.87 ± 26.91	56.52 ± 11.06
GRP4NDET400mg	186.73 ± 6.66	140.76 ± 44.54	103.98 ± 5.95	56.58 ± 15.90
GRP7DET400mg	142.36 ± 36.47	97.04 ± 21.23	153.92 ± 13.26	14.09 ± 6.26
GRP8DET200mg	172.31 ± 33.41	154.34 ± 36.75	165.18 ± 48.65	55.58 ± 10.51
GROUP 9	196.30 ± 3.84	162.11 ± 40.72	150.79 ± 31.22	16.89 ± 2.89
p-values	0.0002	0.0078	0.1253	0.0001
F-values	5.267	4.102	1.937	23.72
<b>Tukey's Multiple Comparison Test</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>
Group 1 vs Group 2	Ns	Ns	Ns	***
Group 1 vs Group 4	Ns	Ns	Ns	***
Group 1 vs Group 7	**	**	Ns	Ns
Group 1 vs Group 8	Ns	Ns	Ns	***
Group 1 vs Group 9	Ns	Ns	Ns	Ns
Group 2 vs Group 4	Ns	Ns	Ns	Ns
Group 2 vs Group 7	*	*	Ns	***
Group 2 vs Group 8	Ns	Ns	Ns	Ns
Group 2 vs Group 9	Ns	Ns	Ns	***
Group 4 vs Group 7	*	Ns	Ns	***
Group 4 vs Group 8	Ns	Ns	Ns	Ns
Group 4 vs Group 9	Ns	Ns	Ns	***
Group 7 vs Group 8	Ns	Ns	Ns	***
Group 7 vs Group 9	**	Ns	Ns	Ns
Group 8 vs Group 9	Ns	Ns	Ns	***

Note: NC-Negative Control, PC-Positive Control, NDET400mg-Non-Diabetic fed orally with ethanolic extracts of MMLS., DET400mg-Diabetic fed orally with 400mg/kg dose of ethanolic extracts, DET200mg-Diabetic treated with 200mg/kg of ethanolic leaves extracts of MMLS., DMF100mg-Diabetic treated with 100mg/kg of Metformin Standard drug. (p<0.05)- Statistically significant)

**Table 4.91:** Potassium, Chloride, Sodium, Bicarbonate, Creatinine, and Urea Parameters of Streptozotocin Induced Diabetic Male Rats Treated orally for 60 days with 400mg/kg of Ethanolic Leaves and 200mg/kg Doses of Ethanolic Leaves Extracts of *Morus mesozygia Linn. Stapf.*, in Comparism with 100mg/kg of Metformin Drug Compared with Non-Treated Controls.

	K+(mmol/L)	CL- (mmol)	Na+ (mmol/L)	HCO3- (mmol/L)	Cr (mmol/L)	Urea (mmol/L)
GROUP1NC	3.88 ± 0.67	42.8 ± 1.92	140.4 ± 2.30	21.4 ± 2.07	146.8 ± 7.85	13.33 ± 0.98
GROUP 2PC	5.94 ± 0.15	49.4 ± 1.14	146.6 ± 5.89	19.6 ± 1.67	143 ± 6.16	16.28 ± 2.23
GROUP4NDETL400mg	2.81 ± 0.79	37.4 ± 1.94	124.4 ± 3.84	20.4 ± 1.67	138.6 ± 25.38	11.7 ± 0.63
GROUP 7DETL400mg	3.96 ± 0.23	42.4 ± 1.94	159 ± 5.70	24.6 ± 2.40	159 ± 5.83	11.79 ± 0.18
GROUP 8DETL200mg	3.59 ± 0.89	41 ± 1.22	139.6 ± 6.98	22.6 ± 2.70	165 ± 5.70	11.91 ± 1.25
GROUP 9DMF100mg	3.53 ± 0.71	38.8 ± 5.16	133.4 ± 4.44	26.2 ± 2.16	166.4 ± 10.43	11.55 ± 0.93
p-values	0.0001	0.0001	0.0001	0.0001	0.0004	0.0001
F-values	13.44	12.93	26.53	6.96	4.606	11.48
<b>Tukey's Multiple Comparison Test</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>	<b>Summary</b>
Group 1 vs Group 2	***	**	Ns	Ns	Ns	**
Group 1 vs Group 4	Ns	*	***	Ns	Ns	ns
Group 1 vs Group 7	Ns	Ns	***	Ns	Ns	ns
Group 1 vs Group 8	Ns	Ns	Ns	Ns	Ns	ns
Group 1 vs Group 9	Ns	Ns	Ns	*	Ns	ns
Group 2 vs Group 4	***	***	***	Ns	Ns	***
Group 2 vs Group 7	***	**	**	*	Ns	***
Group 2 vs Group 8	***	***	Ns	Ns	Ns	***
Group 2 vs Group 9	***	***	**	***	Ns	***
Group 4 vs Group 7	Ns	Ns	***	Ns	Ns	ns
Group 4 vs Group 8	Ns	Ns	**	Ns	*	ns
Group 4 vs Group 9	Ns	Ns	Ns	**	*	ns
Group 7 vs Group 8	Ns	Ns	***	Ns	Ns	ns
Group 7 vs Group 9	Ns	Ns	***	Ns	Ns	ns
Group 8 vs Group 9	Ns	Ns	Ns	Ns	Ns	ns

Note:NC-Negative Control, PC-Positive Control, NDET400mg-Non-Diabetic fed orally with ethanolic extracts of MMLS., DET400mg-Diabetic fed orally with 400mg/kg dose of ethanolic extracts, DET200mg-Diabetic treated with 200mg/kg of ethanolic leaves extracts of MMLS., DMF100mg-Diabetic treated with 100mg/kg of Metformin Standard drug. (p<0.05)- Statistically significant)

## Discussion

The significant increase in High density cholesterol (HDL-C), that was observed when the diabetic male albino rats that were treated orally with 200mg/kg aqueous leaves extracts of *MMLS.*, showed the antihyperlipidemic activities, the dosage of this extract has when compared to the HDL-C of the male diabetic albino in the group treated with 400mg/kg body weight of aqueous leaves extract of *MMLS.*, also when compared to the HDL concentration of the diabetic rats treated with 100mg/kg of metformin standard drug (Table 4.1); this might be due to the very high phytochemical presence of flavonoids. This was in agreement with the work done by<sup>[12]</sup> who analysed the effect of TCHOL and LDL in rats fed orally with aqueous leaves of *Morus alba Linn.* and had results that revealed increased concentrations HDL and also reported in addition that these results may have caused certain stimulated actions in the innate and acquired functionings of the immunity of the cell against certain attacks of killer cells that can cause cardiovascular events.

The non-significant concentrations (Table 4.2) in  $K^+$ ,  $Na^+$ ,  $HCO_3^-$ , and decreases in Creatinine (Cr), Chloride (Cl) and Urea concentrations of the induced diabetic male rats treated orally with 400mg/kg dose of aqueous leaves extracts of *MMLS.*, show certain nephron-protective as well as anti-nephrotoxicity<sup>[13]</sup> as compared to the increased levels of the afore-mentioned kidney parameters of in the diabetic rats treated orally with 200mg/kg dose of aqueous leaves extracts of *MMLS.* This could be as result of the presence flavonoids.<sup>[14]</sup> When compared with the concentrations in  $K^+$ ,  $Na^+$ ,  $HCO_3^-$ , Creatinine (Cr), Chloride (Cl) and Urea of the induced diabetic albino male rats treated orally with 200mg/kg of aqueous leaves extracts of *MMLS.* As reports have made emphases that increase in  $K^+$ ,  $Na^+$ ,  $HCO_3^-$ , Creatinine (Cr), Chloride (Cl) and Urea concentrations may result into diabetic nephropathies which are implicated in end-stage renal diseases that results in high mortality rate<sup>[15]</sup>. This work was similar with that carried out by<sup>[15]</sup>,

they reported a decrease in creatinine when *Morus alba Linn* fruit juice extracts was administered orally in amino glycosides induced renal damaged rats, they also reported that the decrease could be due to the polyphenolic contents of flavones isolated abundantly from flavonoids and that these flavones served as diuretics that have protective effects on the kidneys.

Table 4.3 revealed a decrease in total cholesterol (TCHOL), triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) as well as an increased concentration in high density lipoprotein (HDL-C) in the diabetic male rats treated with 400mg/kg of ethanolic leaves of *MMLS* when compared with the lipid profilings of the diabetic rats treated with 200mg/kg of ethanolic leaves extracts and those of the diabetic group treated with 100mg/kg of metformin standard drug and those of the non-treated group. This lipid lowering capacity exhibited by the dosage of 400mg/kg of ethanolic leaves extracts in diabetic rats may be due to the presence of the phytochemicals present which included: Saponins, Flavonoids, Tannins and Alkaloids. The cholesterol could have been decreased by the phytochemical presence of Saponins which has been reported to show certain anti-hypercholesterolemic activity it may exhibit by its ability in mechanism by the attachment to the receptors of perixosomes proliferator-activated sites that are responsible for stimulating the acceptance, usage and breakdown of fatty acids by up-regulation of genes involved in the fatty acid transport. This was similar to the work done by<sup>[16]</sup> that induced rats with alloxan and treated them with 400mg/kg in dosage of ethanolic *Corchorus olitorius* extracts for a period close to a month and observed a decrease in TCHOL, TG, LDL-C and an increase in concentration of HDL-C in the diabetic when compared with to the untreated group.

The result from Table 4.4 showed a decrease in concentration of creatinine and urea, a significant increase in  $K^+$  and  $HCO_3^-$  and the non-significant differences of the diabetic rats in Group 7 treated with 400mg/kg of ethanolic leaves of *MMLS.*,

when compared to the significant decrease of K<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> and the significant increase of creatinine and urea of the diabetic rats treated orally for 30 days with 200mg/kg of ethanolic leaves extract may be due to the possibly protective effect the 400mg/kg dosage of ethanolic leaves extracts has over the kidneys, this. The results of significant decrease in LDL, non-significant TCHOL, TRIG and increased HDL levels as exhibited by the diabetic rats' treated for 60 days with 200mg/kg of aqueous leaves on Table 4.5 is indicative of the antilipidemic effect of the dosage of this extracts on the endothelial lining and functioning of the arterial wall when compared with the increased LDL, and decreased HDL that the diabetic rats treated with 400mg/kg of aqueous leaves extracts of *MMLS* exhibited. This antioxidative effects may be due to the presence of flavonoids. This was similar to the work of<sup>[16]</sup> who observed decrease in LDL and an increase of HDL in *Morus alba* against the invasion of macrophages.

The significant decreased urea and creatinine and the non-significant differences of K<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup>, HCO<sub>3</sub><sup>-</sup> exhibited by the diabetic rats treated with 200mg/kg of aqueous leaves extracts of *MMLS* on Table 4.6 indicated that the glomerular filtration rate of the kidney with the reabsorption of the electrolytes balanced for the elimination of toxic wastes when compared with the increased urea and creatinine of the diabetic rats treated with 400mg/kg dosage of aqueous leaves extracts. This nephrotoxic protection of the 200mg/kg dosage may be due to the presence of flavonoids. This concurs with the work of<sup>[17]</sup> who reported decrease in urea and creatinine as a result of the extracts from the fruits of *Morus alba* L. that served as a form of diuretics on renal functioning and clearance.

Table 4.7 revealed a significant decrease of TCHOL, TRIG, LDL and an increase in HDL levels of the diabetic rats treated for 60 days with 400mg/kg of ethanolic leaves. This is indicative of antihypercholesteromic effect as well as antilipidemic effect this dose has in lowering

cholesterol and transporting it easily into cell membranes, as a result of the increased HDL compared with that of the diabetic rats treated with 200mg/kg. This antihypercholesteromic effect exhibited may be due to saponins. Saponins may have acted in an action mechanism of attaching its perioxosome proliferator receptors on lipid sites thereby regulating the upward movement of glucose into the islets cells. This work did not agree with that of<sup>[18]</sup> that investigated the impart of 400mg/kg of ethanolic extracts of *Corchorus olitorius* in diabetic alloxan induced rats and observed decreased TCHOL, TRIG, LDL within a period less than 30 days.

The significant decrease of urea and creatinine is indicative of renal protective effect of the diabetic rats treated with 200mg/kg ethanolic leaves of *MMLS* on Table 4.8 when compared with increased urea and creatinine of the diabetic rats treated with 400mg/kg of ethanolic leaves. This renal protective effect of the 200mg/kg ethanolic leave extract may be due to the presence of tannins. This agreed with the work done by<sup>[18]</sup>.

### Conclusion

The combinatorial therapy of the aqueous and metformin standard drug at 400mg/kg body weight showed a more antidiabetic potential when compared to that of metformin drug alone. The ethanolic leaves extract exhibited anti-hypercholesterolemic, anti-lipidemic activities more than the aqueous extract, this may be probably due to ethanol having a greater polarity index as well as exhibiting more components such as polyphenols, flavonoids,, tannins, saponins, alkaloids.

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