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ASSESSMENT OF THE LEVELS OF TISSUE NECROTIC FACTOR – ALPHA (TNF-A) AND SOME HAEMATOLOGICAL PARAMETERS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS TREATED WITH AQUEOUS AND ETHANOLIC EXTRACTS OF *MORUS MESOZYGIA LINN. STAPF.*, LEAVES

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ABSTRACT
The levels of certain inflammatory cytokines are often higher in people with type 2 diabetes. Pro-inflammatory cytokines are the initiators of inflammation that begin the processes of beta cell apoptosis due to insulin resistance. The increase in medi-care cost and the side effects that comes from the use in orthodox drugs for the treatment of diabetes 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 choice of plant for this research study. The study evaluated the Investigation of Morus mesozygia Linn. Stapf., Leaves on Tissue necrotic factors-alpha (TNF-α) and some haematological parameters (haemoglobin concentration, Red Blood Cell count, White Blood Cell count, platelet count, Neutrophil, Lymphocytes, Eosinophil, and Monocyte counts) in Streptozotocin-Induced Diabetic Rats. A total of fifty (50) male albino rats were used for this research study. Two different extracted solvent; aqueous, and ethanolic leaves extracts 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. There were significant decreases ($p<0.05$) in TNF-α of the diabetic rats treated with aqueous leaves extracts of 400mg/kg of MMLS., when compared with those treated with 200mg/kg of aqueous extracts. There were significant increase ($P<0.05$) in TNF-α, in the diabetic rats treated with 400mg/kg of ethanolic leaves extracts compared with those treated with 200mg/kg. There was a significant decrease($p<0.05$) in TNF-α, in the diabetic rats treated with aqueous leaves in combination with metformin when compared with that of the diabetic rats treated with metformin alone. The result also indicated a significant increase in haemoglobin concentration, Red Blood Cell, White Blood Cell, platelet count, Neutrophil, Lymphocytes, decreased Eosinophil, and increased Monocyte counts of the diabetic male rats treated with 400mg/kg of aqueous leaves extracts of MMLS when compared with the decrease in concentration of HB, and counts of RBC, WBC, platelet, Neutrophil, Lymphocytes, Eosinophil, Monocyte counts of the diabetic male rats treated orally for 30 days could be as a result of the presence of the phytochemical constituents of flavonoids

KEYWORDS: *Aqueous, Ethanolic Extracts, Morus mesozygia Linn. Stapf., Leaves,* Tissue Necrotic Factor – Alpha (TNF-α) and Some Haematological Parameters *Streptozotocin-Induced, Diabetic Rats.*

INTRODUCTION
The levels of certain inflammatory cytokines are often higher in people with type 2 diabetes. Pro-inflammatory cytokines are the initiators of inflammation that begin the processes of beta cell apoptosis due to insulin resistance. Examples include interleukins (IL)-1, tumor necrosis factor alpha (TNF-alpha). These cytokines or simply put inflammatory interleukins are secreted from T-helper cells and macrophages. However, it has been reported that another form of an effector molecule, an interleukin known as Interleukin- 1Beta (IL-1B) has a form of implication in the processes of inflammatory response the type two diabetes suffer that leads to beta apoptosis (Welsh *et al*., 2005).

Tumor necrotic factor alpha is a small protein like molecular biomarker that can detect the integrity of a tissue on how well its differentiation and degree of apoptosis is seen especially in areas of inflammation that has to do with certain diseases such as in diabetes, cardiovascular events as well as cancers (Badowska-Kozakiewicz, 2013).

Hematological investigations are useful in the assessment of the extent to which blood cells have been destroyed in blood (Togun *et al*., 2007). The measurement of these hematological parameters stands as a standard or indicator of an experimental animal that is exposed to toxic substances that give rise to pathological situations.

Isaac *et al*., (2013) reported that experimental animals that show good blood composition may show good routine. For clinical assessment, full blood count is vital as it helps to give a clear information of an individual's health status. The various facets of full blood count include: Hemoglobin concentration. Red blood count, Platelets, Leucocytes and Percentage differential leucocytes count.

The impermeability of glucose into the cellular membrane as a result of the defect of the beta cells of Largenhans has increased the activity of the reactive oxygen species (ROS), which are known to produce free radicals that increases the establishment of the impairement 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 well as organ dysfunction that cause further complications leading to a serious health situation the diabetics suffer (Fiorentino *et al*., 2013).

The complications that come from insulin resistance results to chronic hyperglycemia due to the generation of nitrogen and reactive oxygen species as reported by several authors. These overproduction (Lopez-Alarcon & Denicola, 2013) or insufficient removal of reactive oxygen as well as nitrogen species have been reported to be the culprits in the development of hyperglycemia and its complications such as microvascular and macrovascular dysfunctions, causing severe damages to membrane lipids, cellular proteins and nucleic acids that can literally result to further chronic complications (de M Bandeira S *et al*., 2013).

The modalities that can be acquired to sustain the healthcare of the diabetics due to the aforementioned complications and side effects from the ingestion of orthodox drugs may be embraced with optimism if scientists look inward towards the traditional methods of alleviating disease conditions as it had long been reported that subsequent use of drugs as therapeutic measures for the pharmacological treatment of disease began long ago with the use of herbs, these are readily available and less cost effective (Al-Snafi, 2017).

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. This research will focus on the species '*Morus mesozygia linn'* to investigate its activities on glucose and tumor necrosis factor alpha with the use of the leaves and twigs particularly in streptozotocin induced-diabetic male albino rats.

Type 2 diabetes mellitus have been reported to have been linked to obesity and equally the formation of chronic low grade inflammation brought about by metabolic collection of diseases, immune system long term unavailability of protection due to certain free radicals the white blood cells is being attacked upon (interleukins) and likewise the intake of excess food leading to obesity (Guzman-Flores & Lopes-Briones, 2012; Shu *et al*., 2012).

Guarner & Rubio-Ruiz (2014) reported an association of inflammatory processes having some alterations in the eyes, kidneys, arteries due to complications from type 2 diabetes mellitus.

The report of Hotamisligil *et al*., (1993) and also studies of inflammation as well as insulin resistance showed a relationship in connection with a high concentration of tumor necrosis factor- alpha (TNF-α) in a diabetic rat model compared with its control. Likewise, reported also was that also of certain studies of inflammatory cytokines for example, interleukin IFN-y and IL-1B in diabetic rat models that exhibited obesity as a result of diabetes mellitus which induced insulin modulation (Mathias, 2013; Ouchi *et al*., 2011).

Recent reviews by Chang *et al*., (2016) reported a correlation between T-lymphocytes and pathogenesis of type two diabetes mellitus.

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 feds 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, 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.

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 powered 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 24hours. 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 (Gulcin, 2005) 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 extract was stored in a well corked universal bottle. The leaf ethanol extract yielded (18.65) g) and was kept in a 4^{o}C refrigerator prior to pharmacological investigations.

Extraction of Dried Morus mesozygia Leaves using Distilled Water

Nine hundred and sixty grams (960g) of dried sample *Morus mesozygia* leaves was measured and poured into dry clean beaker, 5 liters (5L) of distilled water was dispensed into the beaker and the mixture was shaken severally, macerated then the filtrate filtered out using a muslin cloth and again filtered through a what man number one filter paper. This was kept under a low rotary evaporator of 40^{o}C to dry (Gulcin, 2005). The semi-solid dark brownish yield of the water extract yielded (17.58)g. It was kept in 4^{o}C prior to pharmacological studies.

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 streptozotozin (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 (Damasceno e*t al.*, 2012). 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 (Beach & Turner, 1958). 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.

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

Haematological Determination
The haematological investigations that were carried out for the purpose of this research include the following: Haemoglobin (Hb), Platelets, total White Blood Cells (WBCs), Red Blood Cells (RBCs) and Differential white cell count. These test for the blood samples were all carried out in Ethylene Diamine Tetra Acetic (EDTA) bottles at the Biochemistry Research Laboratory, University of Port Harcourt, Rivers State, Nigeria.

Determination of Hematological Parameters by Coulter's Principle of Impedence (Coulter, 1956)
The hematological method that was used to determine the cell counts of the RBCs, WBCS, Differential white cell counts, Platelets and Hemoglobin concentration was based on the Coulter's principle of the Hematological Auto Analyzer.

Principle
The Coulter's electrical principle of Impedence works on the use of the whole blood in the EDTA anticoagulant bottle aspirating the factions of the blood counts into two portions mixed with the diluent. A dilution factor of with the Tuke's solution and blood sample in a dilution in 1:20 of the white blood cell diluent was used. The diluent contained in composition, 2% glacial acetic acid, a weak acid to lyse the red blood cells tinged with Gentian Violet to stain the nucleus of the white blood cells. One of the portions of the auto analyzer enales the red blood cells and the platelets counts to pass through its red cell aperture bath while the other portion (white blood count aperture) allows for the passage and counts of the white blood cells, differentials of the white cells as well as hemoglobin.

Determination of Tumor Necrosis Factor-Alpha (TNF-α)
Principle
The principle was based Sandwich- Enzyme Linked Immunosorbent Assay. That is the antibody was precoated to the micro ELISA plate that was specific for the rat TNF-α.

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 (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 4.1: Mean and Standard deviations of Tumor Necrosis Factor-α of Streptozotocin Induced Diabetic Male Rats Treated Orally for 30 days with 400mg/kg, 200mg/kg of Aqueous Leaves of *Morus mesozygia Linn. Stapf.* Extracts compared with controls.

	TNF (pg/ml)	Tukey's Test	Summary
GRP1NC	236.98 ±76.34	Group 1 vs Group 2	Ns
GRP2PC	370.27 ± 22.07	Group 1 vs Group3	Ns
GRP3NDALO	173.59 ± 37.44	Group 1 vs Group5	***
GRP5DA400mg	659.10 ± 7.07	Group 1 vs Group6	***
GRP6200mg	722.74 ± 56.12	Group 1 vs Group9	***
GRP9DMF100mg	699.37 ± 11.29	Group 2 vs Group3	Ns
p-values	< 0.0001	Group 2 vs Group5	*
F-values	16.18	Group 2 vs Group6	**
		Group 2 vs Group9	*
		Group3 vs Group5	***
		Group3 vs Group6	***
		Group3 vs Group9	***
		Group5 vs Group6	Ns
		Group5 vs Group9	Ns
		Group6 vs Group9	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- 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 4.2: Mean and Standard deviation of HB, RBC, WBC, Platelets, Neutrophils, Lymphocytes, Eosinophils And Monocytes Parameters of Streptozotocin Induced Diabetic Male Rats Treated Orally for 30 days with 400mg/kg, 200mg/kg of Aqueous Leaves of *Morus meso zygia Linn. Staph.* Extracts Compared with 100mg/kg of Metformin Standard Drug and Non-Treated Controls.

	Hb (swL)	*RBC (x1012/L)*	*WBC (x109/L)*	*PLATELET x109/L)*	*N(%)*	*L*	*E*	*M*
GRP1NC	14.44 ± 0.87	6.34 ± 0.21	8.64 ± 0.36	220.2 ± 58.37	22.4 ± 2.07	62 ± 4.47	4.2 ± 0.84	9 ± 1.22
GRP2PC	10.62 ± 0.51	4.08 ± 0.11	13.06 ± 1.11	269 ± 18.31	32.8 ± 2.12	54.8±3.11	1.4 ± 0.54	3.6 ± 0.54
GRP3NDALO	11.98 ± 1.87	5.05 ± 0.87	12.7 ± 2.14	308.8 ± 45.68	22 ± 3.08	61.8 ± 7.19	3.8 ± 0.76	6.8 ± 2.16
GRP5NDA400mg	14.18 ± 0.19	6.14 ± 0.04	10.94 ±0.55	274.8 ± 76.63	26.6 ± 2.96	55.6 ± 5.59	3.8 ± 1.30	5 ± 1.87
GRP6ND200mg	12.66 ± 1.25	5.9 ± 0.05	9.1 ± 0.26	255.6 ± 11.48	24.8 ± 3.96	59.2 ± 7.46	2.4 ± 0.89	7.8 ± 2.28
GRP9DMF100mg	12.7 ± 1.89	5.56 ± 0.65	11.12 ± 0.72	257.8 ± 14 04	20.6 ± 1.67	67± 4.69	2.4 ±0.54	6.8 ± 1.30
p-values	0.0008	< 0.0001	< 0.0001	0.1029	< 0.0001	0.0221	0.0003	0.0005
F-values	6.187	13.62	14.26	2.082	12.9	3.254	7.2	6.667
Tukey's Multiple Comparison Test	Summary	Summary	Summary	Summary	Summary	Summary	Summary	Summary
GROUP1 vs GROUP2	**	***	***	Ns	***	ns	***	***
GROUP1 vs Group3	Ns	**	***	Ns	Ns	ns	ns	ns
GROUP1 vs Group5	Ns	Ns	*	Ns	Ns	ns	ns	*
GROUP1 vs Group6	Ns	Ns	Ns	Ns	Ns	ns	*	ns
GROUP1 vs Group9	Ns	Ns	*	Ns	Ns	ns	*	ns
GROUP2 vs Group3	Ns	Ns	Ns	Ns	***	ns	**	ns
GROUP2 vs Group5	**	***	*	Ns	*	ns	**	ns
GROUP2 vs Group6	Ns	***	***	Ns	**	ns	ns	**
GROUP2 vs Group9	Ns	**	Ns	Ns	***	*	ns	ns
Group3 vs Group5	Ns	*	Ns	Ns	Ns	ns	ns	ns
Group3 vs Group6	Ns	Ns	***	Ns	Ns	ns	ns	ns
Group3 vs Group9	Ns	Ns	Ns	Ns	Ns	ns	ns	ns
Group5 vs Group6	Ns	Ns	Ns	Ns	Ns	ns	ns	ns
Group5 vs Group9	Ns	Ns	Ns	Ns	*	*	ns	ns
Group6 vs Group9	Ns	Ns	Ns	Ns	Ns	ns	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,GRP9- 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 4.3: Mean and Standard Deviations of Tumor Necrosis Factor-α, C-Peptide, Insulin, Fasting Blood Glucose and HOMA-IR 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.

	TNF (pg/ml)	Tukey's Multiple Comparison Test	Summary
GRP1NC	236.98 ±76.34	Group 1 vs Group 2	*
GRP2PC	370.27 ± 22.07	Group 1 vs Group 4	***
GRP4NDET	978.82 ± 67.45	Group 1 vs Group 7	***
GRP7DET400mg	827.38 ± 85.12	Group 1 vs Group 8	*
GRP8200mg	367.58 ± 63.18	Group 1 vs Group 9	***
GRP9DMF100mg	699.37 ± 11.29	Group 2 vs Group 4	***
p-values	< 0.0001	Group 2 vs Group 7	***
F-values	10.21	Group 2 vs Group 8	Ns
		Group 2 vs Group 9	***
		Group 4 vs Group 7	*
		Group 4 vs Group 8	***
		Group 4 vs Group 9	***
		Group 7 vs Group 14	***
		Group 7 vs Group 9	Ns
		Group 8 vs Group 9	***

Note: GRP1- Negative control, GRP2PC – Positive control, GRP 5NDETLO –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).

Table 4.4 Mean and Standard deviation of HB, RBC, WBC, Platelets, Neutrophils, Lymphocytes, Eosinophils And Monocytes 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.* Extract compared with Non-Treated Controls.

	Hb (swL)	*RBC (x1012/L)*	*WBC (x109/L)*	*PLATELET x109/L)*	*N(%)*	*L*	*E*	*M*
GRP1NC	14.44 ± 0.87	6.34 ± 0.21	8.64 ± 0.36	220.2 ± 58.37	22.4 ± 2.07	62 ± 4.47	4.2 ± 0.84	9 ± 1.22
GRP2PC	10.62 ± 0.51	4.08 ± 0.11	13.06 ± 1.11	269 ± 18.31	32.8 ± 2.12	54.8±3.11	1.4 ± 0.54	3.6 ± 0.54
GRP5NDET400mg	12.6 ± 0.12	5.72 ± 0.56	9.14 ±1.22	189.8 ± 23.2	24.8 ± 3.96	63.2 ± 6.97	3 ± 1	8.2 ± 1.78
GRP 7ND400mg	14.66 ± 0.56	5.92 ± 0.27	9.18 ± 1.80	247.8 ± 20.58	20 ± 1.58	61.6 ± 4.6	2.6 ± 1.14	9 ± 1.14
GRP8ND200mg	13.2 ± 1.29	5.9 ± 0.23	9.56 ± 0.94	276.6 ± 16.28	24 ± 2.34	65 ± 4.12	2.2 ± 0.83	7 ± 2.91
GRP 9DMF100mg	12.7 ± 1.89	5.56 ± 0.65	11.12 ± 0.72	257.8 ± 14 04	20.6 ± 1.67	67± 4.69	2.4 ±0.54	6.8 ± 1.30
p-values	0.0001	< 0.0001	< 0.0001	0.0007	< 0.0001	0.0117	0.009	0.0003
F-values	8.212	28.7	13.52	6.323	22.92	3.764	6.074	7.191
Tukey's Multiple Comparison Test	**Summary**	**Summary**	**Summary**	**Summary**	**Summary**	**Summary**	**Summary**	**Summary**
Group 1 vs Group 2	***	***	***	Ns	***	Ns	***	***
Group 1 vs Group 5	Ns	Ns	Ns	Ns	Ns	Ns	ns	ns
Group 1 vs Group7	Ns	Ns	Ns	Ns	Ns	Ns	ns	ns
Group 1 vs Group 8	Ns	Ns	Ns	Ns	Ns	Ns	*	ns
Group 1 vs Group 9	Ns	**	**	Ns	***	Ns	*	ns
Group 2 vs Group 4	Ns	***	***	**	***	Ns	ns	**
Group 2 vs Group 7	***	***	***	Ns	***	Ns	ns	***
Group 2 vs Group 8	*	***	***	Ns	***	*	ns	*
Group 2 vs Group 9	Ns	***	Ns	Ns	Ns	**	ns	ns
Group 4 vs Group 7	Ns	Ns	Ns	*	*	Ns	ns	ns
Group 4 vs Group 8	Ns	Ns	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	Ns	ns	ns
Group 7 vs Group 9	Ns	Ns	Ns	Ns	***	Ns	ns	ns
Group 8 vs Group 9	Ns	Ns	Ns	Ns	***	Ns	ns	ns

Note: GRP1- Negative control, GRP2PC – Positive control, GRP 5NDETLO –Non Diabetic Ethanolic Leaves Orally fed with 400mg of ethanolic leaves extract of *(MMLS.)* extract only, GRP7- Diabetic male 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)

Table 4.5: Mean and Standard Deviations of Tumor Necrosis Factor-α, C-peptide, Insulin. Fasting Blood Glucose, HOMA-IR Parameters of Streptozotocin Induced Diabetic Male Rats Treated orally for 60 days with 400mg/kg Aqueous Leaves Extracts, 200mg/kg Aqueous Leaves Extracts of *Morus mesozygia Linn. Stapf.*, in Comparism with 100mg/kg of Metformin Drug compared with controls.

	TNF (pg/ml)	Tukey's Multiple Comparison Test	Summary
GRP1NC	176.09 ± 47.7	Group 1 vs Group 2	Ns
GRP2PC	239.38 ± 94.93	Group 1 vs Group 3	Ns
GRP3NDAL400mg	172.05 ± 34.09	Group 1 vs Group 5	***
GRP5DAL400mg	639.53 ± 21.52	Group 1 vs Group 6	***
GRP6DAL200mg	692.46 ± 28.35	Group 1 vs Group 9	***
GRP9DMF100mg	694.03 ± 6.28	Group 2 vs Group 3	Ns
p-values	< 0.0001	Group 2 vs Group 5	**
F-values	19.41	Group 2 vs Group 6	***
		Group 2 vs Group 9	***
		Group 3 vs Group 5	***
		Group 3 vs Group 6	***
		Group 3 vs Group 9	***
		Group 5 vs Group 6	Ns
		Group 5 vs Group 9	Ns
		Group 6 vs Group 9	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 4.6: HB, RBC, WBC, Platelets, Neutrophils, Lymphocytes, Eosinophils And Monocytes Parameters of Streptozotocin Induced Diabetic Male Rats Treated orally for 30days with 400mg/kg Aqueous Leaves Extracts, 200mg/kg of Aqueous Leaves Extracts of *Morus mesozygia Linn. Stapf.* Extracts compared with100mg/kgof Metformin Drug compared with the controls.

	Hb (swL)	*RBC (x1012/L)*	*WBC (x109/L)*	*PLATELET x109/L)*	*N(%)*	*L*	*E*	*M*
GROUP1	13.65 ± 1.12	6.19 ± 0.17	8.22 ± 0.17	191 ± 46.16	22 ± 2	64.4 ± 4.56	3.8 ± 1.30	8 ± 1.22
GROUP 2	7.48 ± 0.58	3.55 ± 0.79	12.12 ± 1.5	187.39 ± 45.87	19.2 ± 1.48	65.8 ± 3.83	1.2 ± 0.44	2.6 ± 0.54
GROUP 3	11.19 ± 2.01	4.79 ± 0.76	11.91 ± 1.96	297 ± 49.71	17.2 ± 1.48	52 ± 18.62	3.4 ± 1.67	6 ± 2.26
GROUP 5	14.10 ± 0.10	6.08 ± 0.05	10.71 ± 0.58	244.2 ± 89.46	25.6 ± 2.97	52.8 ± 6.45	3.4 ± 0.89	4.4 ± 1.51
GROUP 6	12.11 ± 0.77	5.60 ± 0.61	8.7 ± 0.5	238.2 ± 17.34	23.4 ± 3.97	54.8 ± 8.31	2.8 ± 1.48	7.8 ± 1.30
GROUP 9	12.92 ± 1.03	5.63 ± 0.42	7.11 ± 0.89	227.8 ± 30.31	20.8± 1.48	61.4 ± 6.22	1.6 ± 0.89	4 ± 1.41
p-values	< 0.0001	< 0.0001	< 0.0001	0.0286	0.0002	0.1025	0.0089	< 0.0001
F-values	23.72	16.47	16.97	3.048	7.642	2.085	3.995	11.09
Tukey's Multiple Comparison Test	**Summary**	**Summary**	**Summary**	**Summary**	**Summary**	**Summary**	**Summary**	**Summary**
Group 1 vs Group 2	***	***	***	Ns	Ns	Ns	*	***
Group 1 vs Group 3	*	**	***	*	*	Ns	Ns	ns
Group 1 vs Group 5	Ns	Ns	*	Ns	Ns	Ns	Ns	**
Group 1 vs Group 6	Ns	Ns	Ns	Ns	Ns	Ns	Ns	ns
Group 1 vs Group 9	Ns	Ns	Ns	Ns	Ns	Ns	Ns	**
Group 2 vs Group 3	***	*	Ns	*	Ns	Ns	Ns	*
Group 2 vs Group 5	***	***	Ns	Ns	**	Ns	Ns	ns
Group 2 vs Group 6	***	***	***	Ns	Ns	Ns	Ns	***
Group 2 vs Group 9	***	***	***	Ns	Ns	Ns	Ns	ns
Group 3 vs Group 5	**	*	Ns	Ns	***	Ns	Ns	ns
Group 3 vs Group 6	Ns	Ns	**	Ns	**	Ns	Ns	ns
Group 3 vs Group 9	Ns	Ns	***	Ns	Ns	Ns	Ns	ns
Group 5 vs Group 6	Ns	Ns	Ns	Ns	Ns	Ns	Ns	*
Group 5 vs Group 9	Ns	Ns	***	Ns	*	Ns	Ns	ns
Group 6 vs Group 9	Ns	Ns	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 4.7: Mean and Standard Deviations of Tumor Necrosis Factor, C-peptide, Insulin. Fasting Blood Glucose, HOMA-IR Parameters of Streptozotocin Induced Diabetic Male Rats Treated orally for 60 days with 400mg/kg Ethanolic Leaves Extracts, 200mg/kg Dose of Ethanolic Extracts of *Morus mesozygia Linn. Stapf.*, in Comparism with 100mg/kg of Metformin Drug Compared with Non-Treated Controls.

	TNF (pg/ml)	**Tukey's Multiple Comparison Test**	**Summary**
GRP1NC	176.09 ± 47.7	Group 1 vs Group 2	Ns
GRP2PC	239.38 ± 94.93	Group 1 vs Group 4	***
GRP4ND400mg	924.57 ± 95.69	Group 1 vs Group 7	***
GRP7DET400mg	873.02 ± 9.24	Group 1 vs Group 8	*
GRP8DET200mg	332.53 ± 61,26	Group 1 vs Group 9	***
GRP9DMF100mg	694.03 ± 6.28	Group 2 vs Group 4	***
p-values	0.0001	Group 2 vs Group 7	***
F-values	12.08	Group 2 vs Group 8	Ns
		Group 2 vs Group 9	***
		Group 5 vs Group 7	Ns
		Group 5 vs Group 8	***
		Group4 vs Group 9	***
		Group 7 vs Group 8	***
		Group 7 vs Group 9	**
		Group 8 vs Group 9	***

Note:NC-Negative Control, PC-Positive Control, NDET400mg-Non-Diabetic fed orally with ethanolic extracts of MMLS., DET400mg-Diabtic 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 4.8: HB, RBC, WBC, Platelets, Neutrophils, Lymphocytes, Eosinophils And Monocytes Parameters of Streptozotocin Induced Diabetic Male Rats Treated orally for 60days with 400mg/kg Ethanolic Leaves Extracts, 200mg/kg of Ethanolic Leaves Extracts of *Morus mesozygia Linn. Stapf*. Extracts compared with 100mg/kg of Metformin Drug Compared with the Non-Treated Controls.

	Hb (swL)	*RBC (x1012/L)*	*WBC (x109/L)*	*PLATELET x109/L)*	*N(%)*	*L*	*E*	*M*
GROUP1NC	13.65 ± 1.12	6.19 ± 0.17	8.22 ± 0.17	191 ± 46.16	22 ± 2	64.4 ± 4.56	3.8 ± 1.30	8 ± 1.22
GROUP 2PC	7.48 ± 0.58	3.55 ± 0.79	12.12 ± 1.5	187.39 ± 45.87	19.2 ± 1.48	65.8 ± 3.83	1.2 ± 0.44	2.6 ± 0.54
GROUP 4NDETL400mg	12.21 ± 0.83	5.17 ± 0.48	8.94 ± 0.40	180.2 ± 26.99	20.4 ± 3.84	58.4 ± 7.05	3.2 ± 0.83	7.4 ± 1.81
GROUP 7DETL400mg	14.44 ± 0.57	5.46 ± 0.81	8.78 ± 1.64	236.8 ± 22.43	18 ± 2	57.2 ± 4.32	2.2 ± 1.30	8.2 ± 0.84
GROUP 8DETL200mg/kg	14.28 ± 0.95	5.66 ± 0.36	10.21 ± 1.13	267.6 ± 13.81	19 ±2	58.2 ± 5,07	2.2 ± 0.83	8.2 ± 1.30
GROUP 9DMF100mg/kg	12.92 ± 1.03	5.63 ± 0.42	7.11 ± 0.89	227.8 ± 30.31	20.8± 1.48	61.4 ± 6.22	1.6 ± 0.89	4 ± 1.41
p-values	0.0001	0.0001	0.0001	0.0002	0.1156	0.0799	0.0014	0.0001
F-values	10.35	13.46	12.38	5.433	1.996	2.27	4.917	19.1
Tukey's Multiple Comparison Test	Summary	Summary	Summary	Summary	Summary	Summary	Summary	Summary
Group 1 vs Group 2	Ns	***	***	Ns	Ns	Ns	**	***
Group 1 vs Group 4	Ns	Ns	Ns	Ns	Ns	Ns	ns	ns
Group 1 vs Group 7	**	Ns	Ns	Ns	Ns	Ns	ns	ns
Group 1 vs Group 8	Ns	Ns	Ns	*	Ns	Ns	ns	ns
Group 1 vs Group 9	**	Ns	Ns	Ns	Ns	Ns	*	***
Group 2 vs Group 4	Ns	**	**	Ns	Ns	Ns	*	***
Group 2 vs Group 7	**	***	***	Ns	Ns	Ns	ns	***
Group 2 vs Group 8	Ns	***	Ns	**	Ns	Ns	ns	***
Group 2 vs Group 9	**	***	***	Ns	Ns	Ns	ns	ns
Group 4 vs Group 7	*	Ns	Ns	Ns	Ns	Ns	ns	ns
Group 4 vs Group 8	Ns	Ns	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	ns	ns
Group 7 vs Group 9	Ns	Ns	Ns	Ns	Ns	Ns	ns	***
Group 8 vs Group 9	***	Ns	**	Ns	Ns	Ns	ns	***

Note:NC-Negative Control, PC-Positive Control, NDET400mg-Non-Diabetic fed orally with ethanolic extracts of MMLS., DET400mg-Diabtic 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 results revealed on Table 4.2 indicated a significant increase in HB concentration, RBC, WBC, platelet, N, L, decreased E, and increased M counts of the diabetic male rats treated with 400mg/kg of aqueous leaves extracts of *MMLS* when compared with the decrease in concentration of HB, and counts of RBC, WBC, platelet, N, L, E, M counts (Table 4.2) of the diabetic male rats treated orally for 30 days could be as a result of the presence of the phytochemical constituents of flavonoids (Sumati & Kapoor, 1986) the dose of 400mg/kg of aqueous leaves may have as against the decreased levels of HB concentration, RBC, WBC, Pletelets, L, N, E and M counts observed in the diabetic male rats treated with 200mg/kg of aqueous leaves extracts of *MMLS*, revealing that the high dosage of aqueous extracts of *Morus mesozygia Linn. S.*,of these hematological parameters may have stimulatory effects on serious health implications on a diabetic individual such as in leukocytosis., and reduced iron carrying deficiency produced from hemoglobin such as in anemia (Schroeder & Kreamer, 1974) thus this dosage may improve the immune related cause of this disease. This finding was in consonance with that of (Auwal *et al*., 2013) that observed the hematological parameters of the RBC, WBC counts and HB concentrations of orally fed wistar albino rats with 400mg/kg of aqueous mesocarp from Hyphaene the baica extracts for four weeks and reported a significant increase of the RBC, WBC counts and HB concentrations.

Table 4.50 revealed a non- significant increase in HB, RBCs, monocytes levels in Group 7 of the diabetic rats treated orally for 30 days compared to a decrease in HB, RBCs, monocytes, concentration of the diabetic rats in Group 8 treated orally with 200mg/kg of ethanolic leaves extracts probably because the 400mg/kg dose of the ethanolic leaves had a high presence of Saponins. The saponin present is a phenolic glycoside and a triterpene that could have acted on a mechanism of ensuring that the increased levels of hemoglobin as a protein in the red blood cells assisted to store and transport oxygen that supports the diabetic individual against peripheral vascular derangements as well as other higher forms of macrovascualar damages that can lead to cardiovascular events. Reports by (Javed *et al*., 2015) stated that a shortage or decrease in hemoglobin which is a great carrierof oxygen rich blood progresses the effect of diabetic peripheral neuropathy and likewise impairs the ability of the RBCs to transform their shapes in other to adjust themselves to the tissue micro-environment. This finding was not in agreement with the work done by (Allison *et al*., 2015) as they observed that reduced doses of 200mg/kg of ethanolic extracts from the leaves of *Morusalba Linn*. (Moraceae) did not show any activity on increased hemoglobin as well as leukocytes and monocytes.

Table 4.6 indicated a significant increase in correlations between the HB, RBCs and WBCs count which revealed anti-inflammatory and immunological responses to infections of the cells of the diabetic rats treated with 400mg/kg of aqueous leaves extracts compared to the decreased HB, RBCs and WBCs of the diabetic rats treated with 200mg/kg leaves extracts of *MMLS*. This anti-inflammatory effects exhibited by this dosage may be due to the presence of flavonoids. This was similar with the work done by Auwal *et al*., (2013) who observed significant increase in HB, RBCs, WBCs and non-significant differences in lymphocytes, monocytes count against infections with the treatment on diabetic rats using400mg/kg body weight of *Hyphaene thebaica*.

Table 4.8 indicated a significant increase in HB, RBCs, platelets, L and M and a decrease in N, E, of the diabetic rats treated with 200mg/k of ethanolic leaves extracts revealing an increase in the defence mechanism of the white blood cells against cell infections when compared with that of the diabetic rats treated with 400mg/kg of ethanolic leaves of *MMLS.* This immunological protective mechanism of the 200mg/kg dosage may be due to the presence of tannins. This was similar with the report of Fahad *et al*., (2017) as it has been reported that the ethanolic leaves of *Morus alba* pointedly reduced neutropenia and increased phagocytic index in carbon clearance assay and likewise increased hemoglobin volume in mice.

There was a significant increase in HB, WBC, Platelets, L, M, in the diabetic rats treated for 30 days with 200mg/kg of methanolic leaves extracts when compared with the decreased HB,WBC,L,M and a non-significant difference in RBC,E, in the diabetic rats treated with 400mg/kg. There were no siginificant differences in the hematological parameters observed with the treatment of 200mg/kg of methanolic leaves when compared with that of 400mg/kg for 60 days respectively.

CONCLUSION

The decreased TNF-α, exhibited by the 400mg/kg compared with that of the 200mg/kg dosages and those of the orthodox metformin standard drug as being antihyperglycemic, anti-inflammatory, anti-antidiabetic, more than the ethanolic and aqueous leaves could be due to its inhibitory effect in suppressing TNF-α against insulin insensitivity of the islets of the pancreas in mopping in glucose into the cell membranes., likewise, these polyphenolic potentials in diabetic treatment of the methanolic extract may be due to the polar index capability in its extraction and revelation of the highest pharmacological components when compared with those of ethanol and aqueous extracts. 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.

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