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Molecular and Biochemical Studies on Some Natural Compounds and Their Effect on the Streptozotocin-induced Diabetic Rats and Their Role in Treatment

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Abstract

Background: The number of individuals with diabetes mellitus (DM) worldwide has more than doubled over the past three decades, and it has been predicted that the number of diabetic patients would increase to 439 million by 2030, so many efforts are being made to find a new and effective treatment for diabetes mellitus.

Objective: This work aims to study the biochemical and molecular effect of the Moringa oleifera ***MO*** and Ficus sycomorus ***FLE*** in streptozotocin-induced diabetic rats and compare them with the effect of metformin, by estimation of the expression of β-actin and glucose transporter GLUT2, GLUT4, and Insulin Receptors genes in studied groups, Determination of fasting blood glucose and insulin levels before and after induction of (STZ), Quantitative estimation serum cholesterol, TG levels, HDL, LDL, Some antioxidant enzyme activity Glutathione peroxidase, catalase and lipid peroxidation in plasma. **Results**: ***MO*** and ***FLE*** showed promising anti-diabetic potential in diabetic-bearing albino mice which can be attributed to its anti-inflammatory effect. This could serve as a stepping stone towards the discovery of newer safe and effective anti-diabetic treatment.

Keywords: Streptozotocin, anti-diabetic, metformin, Gene expression studies

1. Introduction

Diabetes mellitus is a group of syndromes characterized by chronic hyperglycemia resulting from impaired insulin action/secretion, altered metabolism of lipids, carbohydrates, and protein, and an increased risk of complication of vascular diseases and is classified into two major categories, type 1 and type 2 (**Parillo and Riccardi 2004, American Diabetes Association 2012, Priyadarshani et al., 2013**).

The number of individuals with diabetes mellitus (DM) worldwide has more than doubled over the past three decades, and it has been predicted that the number of diabetic patients would increase to 439 million by 2030 (**Chen et al., 2011**).

Effective control of hyperglycemia in diabetic patients is critical for reducing the risk of micro-and macrovascular disease (**Ismail-Beigi et al., 2010, Li et al., 2012).**

Streptozotocin (STZ) is often used to induce

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diabetes mellitus in experimental animals through its toxic effects on pancreatic Beta-cells. STZ-induced diabetes mellitus is associated with the generation of reactive species causing oxidative damage (**Szkudelski, 2001**). Diabetics and experimental models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of the antioxidative defense system and thus promotes de novo free radicals generation (**Baynes et al., 1997**). Over the past few decades, there has been increasing scientific and public interest in the so-called antioxidant hypothesis. Therefore, in addition to control of blood glucose levels, control of oxidative stress offers another avenue for the treatment of the disease. Chemicals with antioxidant properties and free radical

scavengers may help in the regeneration of Beta-cells and protect pancreatic islets against the cytotoxic effects of STZ (**Coskun et al., 2005).**

rats afflicted with diabetes chemically by Streptozotocin (Elias **et al., 1994**). Using 60mg/kg Streptozotocin dose can begin an autoimmune process that results in the destruction of the Langerhans islets beta cells and the 60mg/kg Streptozotocine dose results in the toxicity of beta cells with the emergence of clinical diabetes within 2-4 days.

For transplantation of Langerhans islets of healthy rats under the testis subcutaneous of diabetic rats, we had to induce experimental diabetes to study the effect of grafting the Langerhans islets in diabetic rats. Therefore, the study made us, first, induce experimental diabetes mellitus to study the effect of transplantation of the Langerhans islets in diabetic rats with Streptozotocin to be able to study the clinical parameters before and after the pancreas islet cells transplantation (**Weiss et al., 1982**).

Considerable progress has been made in orthodox anti-diabetic drugs. However, new remedies are still in great demand because of the limited efficacy and undesirable side effects of current orthodox drugs. Nature is an extraordinary source of antidiabetic medicines. To date, more than 1200 flowering plants have been claimed to have anti-diabetic properties (**Chang et al., 2013**).

The use of natural products is very common among non-industrialized societies because these remedies are more accessible and affordable than modern pharmaceuticals. In developed countries, the use of herbal products has recently increased as scientific evidence about their effectiveness has become broadly available (**Salimifar et al., 2013**).

Moringa oleifera (M. oleifera) belongs to the monogeneric family Moringaceae. Different parts of this plant are used in the indigenous systems of human medicine for the treatment of a variety of human ailments. Ethanolic leaves extract of Moringa oleifera used as hypotensive (**Akhtar and Ahmad 1995**).

The leaves of Moringa oleifera are reported to be used as a hypocholesterolemic agent, and hypoglycemic agent (**Dangi et al., 2002**).

Hamza, (2010) suggested that Moringa seed extract can act against carbon tetrachloride (CCl(4))-induced liver injury and fibrosis in rats by a mechanism related to its antioxidant properties, anti-inflammatory effect, and its ability to attenuate the hepatic stellate cells activation.

Alhakmani et al., (2013) suggested that flowers of M. oleifera possess potent anti-inflammatory activity and are also a good source of natural antioxidants.

Ficus sycomorus Linn (F. sycomorous) belongs to Moraceae, a family that is reputable for its medicinal values and consists of about 40 genera and over 1,400 species of trees, shrubs, vine, and herbs, often with milky latex juices (**Zerega et al., 2005**). They are usually found near streams in the savannah area. F.syocomorus is a tree attaining a height of 20 m with widely spreading branches and a massive crown. F. sycomorus have been suspected to possess antidiarrhoeal (**Ahmadu et al., 2007**) and anticonvulsant activities (**Sandabe et al., 2003**). The plant has also been reported to be a potent antimicrobial agent against ciprofloxacin-resistant Salmonella typhi (**Adeshina et al., 2010, Adoum et**

al., 2012.).

Adoum et al., (2012) suggested that methanol extract of stem-bark of F. sycomorous at the dose of 250 mg/kg had significant hypoglycaemic activity. Further work is recommended to evaluate its effects on serum lipids, kidney functions, etc.

Mohamed El-Sayed et al. (2010) suggested that leaves of F. sycomorous are a good source of natural antioxidants.

2. Materials and methods

2.1. Chemicals

2.1.1 - STZ obtained from (Amresco, Solon, Ohio, USA)

2.1.2- Metformin obtained from (250 mg/kg/d; Sigma-Aldrich, St. Louis, MO, USA)

2.2. Animals

Fifty male albino rats (110-120 g) were obtained from the Egyptian Organization for Biological Product and Vaccines at Giza, Egypt which were used throughout the present experiments. Animals were housed in cages under good ventilation and illumination conditions; they had access to unlimited water and standard rodent chow. Animal maintenance and treatments were conducted following the National Institute of Health Guide for Animal, as approved by Institutional Animal Care and Use Committee (IACUC).

2.3. Experimental Design

Experimental animals were randomly divided into 5 groups each of 10 rats as follows:

1. Group I: Negative control (Normal mice).

Control group matched in age, sex, and weight receiving normal saline in a volume of 1ml/kg.

2. Group II:

Diabetic group (induced by streptozotocin (STZ) (60 mg/ kg, 15 min after the intraperitoneal (i.p) during 3 weeks (**Yu et al., 2016**).

3. Group III:

MO treated diabetic rats receiving 200 ml/kg and streptozotocin (STZ) (60 mg/ kg, 15 min after the intraperitoneal (i.p) for 3 weeks.

4. Group IV :

FLE treated diabetic rats receiving 200 ml/kg and streptozotocin (STZ) (60 mg/ kg, 15 min after the intraperitoneal (i.p) for 3 weeks.

5. Group V:

Metformin-treated diabetic rats receiving 250 ml/kg and streptozotocin (STZ) (60 mg/ kg, 15 min after the intraperitoneal (i.p) during 3 weeks.

2.4. Biochemical analysis

2.4.1- Determination of blood glucose level in the serum was determined according to a method of (**Trinder 1969**). using kit purchased from Spinreact Co., Egypt.

2.4.2- Quantitative estimation of Insulin level in the serum was carried out according to the method of (**Tanigaki et al., 2016**) Crystal Chem's Ultra Sensitive Mouse insulin.

2.4.3- Determination of plasma total cholesterol was determined according to the method described by **Richmond (1974).**

2.4.4- Determination of Plasma triacylglycerol was determined by an enzymatic method which was described by (**Schettler and Nussel 1975**).

2.4.5-Determination of HDL-cholesterol was determined according to the method of **Allain et al., (1974).**

2.4.6-Determination of LDL-cholesterol was calculated according to the method described by **Frohlich (2001).**

2.4.7-Determination of malonaldehyde (MDA) was determined in plasma as TBARs substances. It was determined according to the method of (**Padurariu et al., 2010).**

2.4.8-Determination of glutathione (GSH) was determined in blood samples according to (**Chanarin 1989**).

2.4.9-Determination of Catalase (CAT) activity was determined in serum samples according to (**Johansson 1988**). using OxiSelect™ Catalase Activity Assay Kit, Colorimetric.

2.5. Gene expression studies

After the completion of treatment, animals were sacrificed and processed for the gene expression studies:-

2.5.1- RT-PCR analysis of β-actin and glucose transporter GLUT2 genes expression (**Ha-il Kim et al., 2000**).

2.5.2- RT-PCR analysis of β-actin and glucose transporter GLUT4 genes expression (**Jung et al., 2012**).

2.5.3- RT-PCR analysis of β-actin and glucose transporter Insulin receptor genes expression (**Eswar et al., 2020**).

2.6. Statistical Analyses.

Data are presented as mean values+ S.E.M. and were tested for statistical significance with Student's t-test. Linear regression analysis was performed by the method of least-squares. r2 was > 0.9 with a statistical significance of $P < 0.05$ for the quantification of RT-PCR experiments..

3. Results

3.1. Biochemical results

3.1.1- ***Determination of blood glucose and serum insulin level in the serum:-***

Rats injected with STZ exhibited a significant increase *(P<0.05)* in serum glucose and decreasing *(P<0.05)* in serum insulin when compared with the control normal group.

Treatment of diabetic rats with *MO or FLE* exhibited a significant decrease *(P<0.05)* in glucose level and increasing *(P<0.05)* in insulin level when compared with a diabetic control group.

Diabetic rats treated with metformin showed a significant decrease *(P<0.05)* in the glucose level and no significant change *(P>0.05)* in the insulin level when compared with *MO or FLE* alone but a significant decrease *(P<0.05)* when compared with a diabetic group as shown in table (1).

Table (1):- effect of MO and FLE treatment on insulin level and glucose in diabetes mellitus induced experimentally in male albino rats after 3 weeks treatment compared with metformin treatment

Groups	Insulin (µIU/ml)	Glucose (mg/dl)
Negative Control group	4.09 ± 0.07	85.40 ± 4.07
Diabetic control group	0.85 ± 0.01[a]	408.35 ± 50.01[a]
STZ + *MO* group(200 mg/kg b. wt)	1.59 ± 0.03[b]	159.75 ± 0.03[b]
STZ + *FLE* group(200 mg/kg b.wt)	1.09± 0.02[b]	189.54± 0.02[b]
STZ + Metformin group	1.35 ±0.06[c]	115.50 ±6.06[c]

Data are expressed as means ± standard error (SE) for 10 animals/group.

Means within the same column carrying different superscript letters are significantly different (P<0.05)

3.1.2- Serum level of lipid:-

Rats injected with STZ exhibited a significant increase (P<0.05) in serum cholesterol, triacylglycerol, and LDL but no significant change in HDL (P>0.05) hen compared with the control normal group.

Treatment of diabetic rats with MO or FLE exhibited a significant decrease (P<0.05) in cholesterol level, triacylglycerol, and LDL but no significant change in HDL (P>0.05) when compared with the diabetic control group.

Diabetic rats treated with metformin showed no significant change (P>0.05) in the cholesterol level, triacylglycerol, LDL, and HDL when compared with MO or FLE alone. But significant decrease (P<0.05) when compared with the diabetic group table (2).

Table (2):-effect of MO and FLE treatment on cholesterol, triacylglycerol, HDL and LDL-cholesterol level in diabetes mellitus induced experimentally in male albino rats after 3 weeks treatment comparing with metformin treatment

Groups	Cholesterol (ml/dl)	triacylglycerol (ml/dl)	HDLcholesterol (ml/dl)	LDL-cholesterol (ml/dl)
Negative Control group	54.09 ± 5.07	84.09 ± 5.07	81.09 ± 5.07	91.09 ± 5.07
Diabetic control group	100.85 ± 9.01[a]	200.85 ± 9.01[a]	70.85 ± 9.01[a]	170.85 ± 9.01[a]
STZ + *MO* group(200 mg/kg b.wt)	71.59 ± 2.03[b]	91.59 ± 2.03[b]	91.59 ± 2.03[a]	121.59 ± 2.03[b]
STZ + *FLE* group(200 mg/kg b.wt)	78.09± 3.02[b]	88.09± 3.02[b]	88.09± 3.02[a]	138.09± 3.02[b]
STZ + Metformin group	75.35 ±1.06[c]	85.35 ±1.06[c]	85.35 ±1.06[a]	115.35 ±1.06[c]

Data are expressed as means ± standard error (SE) for 10 animals/group.
Means within the same column carrying different superscript letters are significantly different (P<0.05).

3.1.3-Determination of anti-oxidants:-

Rats injected with STZ exhibited a significant increase (P<0.05) in serum MDA but showed a significant decrease (P<0.05) in GSH and CAT when compared with the control normal group. Treatment of diabetic rats with MO or FLE exhibited a significant decrease (P<0.05) in MDA and an increase (P<0.05) in GSH and CAT levels when compared with the diabetic control group. Diabetic rats treated with metformin showed non-significant change (P>0.05) in the MDA, GSH, and CAT levels when compared with MO or FLE alone. But significant change (P<0.05) when compared with the diabetic group table (3).

Table (3):- Effect of MO and FLE treatment on MDA, GSH, and CAT level in diabetes mellitus induced experimentally in male albino rats after 3 weeks treatment compared with metformin treatment.

Groups	malonaldehyde (MDA) (nM/g)	glutathione (GSH) (IU/g)	Catalase (CAT) (IU/g)
Negative Control group	28.09 ± 1.07	32.09 ± 1.07	7.09 ± 0.07
Diabetic control group	64.85 ± 2.01[a]	11.85 ± 2.01[a]	1.85 ± 0.01[a]
STZ + *MO* group(200 mg/kg b.wt)	38.59 ± 2.03[b]	28.59 ± 2.03[b]	4.59 ± 0.03[b]
STZ + *FLE* group(200 mg/kg b.wt)	48.09± 3.02[b]	18.09± 3.02[b]	3.09± 0.02[b]
STZ + Metformin group	35.35 ±1.06[b]	25.35 ±1.06[b]	3.35 ±0.06[b]

Data are expressed as means ± standard error (SE) for 10 animals/group.
Means within the same column carrying different superscript letters are significantly different (P<0.05).

3.2. *Gene expression studies results*

3.2.1- RT-PCR analysis of β-actin and glucose transporter GLUT2 genes expression

Rats injected with STZ exhibited a significant down-regulation (P<0.05) in the expression level of the GLUT2 gene in the pancreas when compared with the control normal group.

Treatment of diabetic rats with MO or FLE exhibited a significant up-regulation (P<0.05) in the expression level of the GLUT2 gene in the pancreas when compared with the diabetic control group.

Diabetic rats treated with metformin showed significant up-regulation (P<0.05) in the expression level of the GLUT2 gene in the pancreas when compared with MO or FLE alone fig (1).

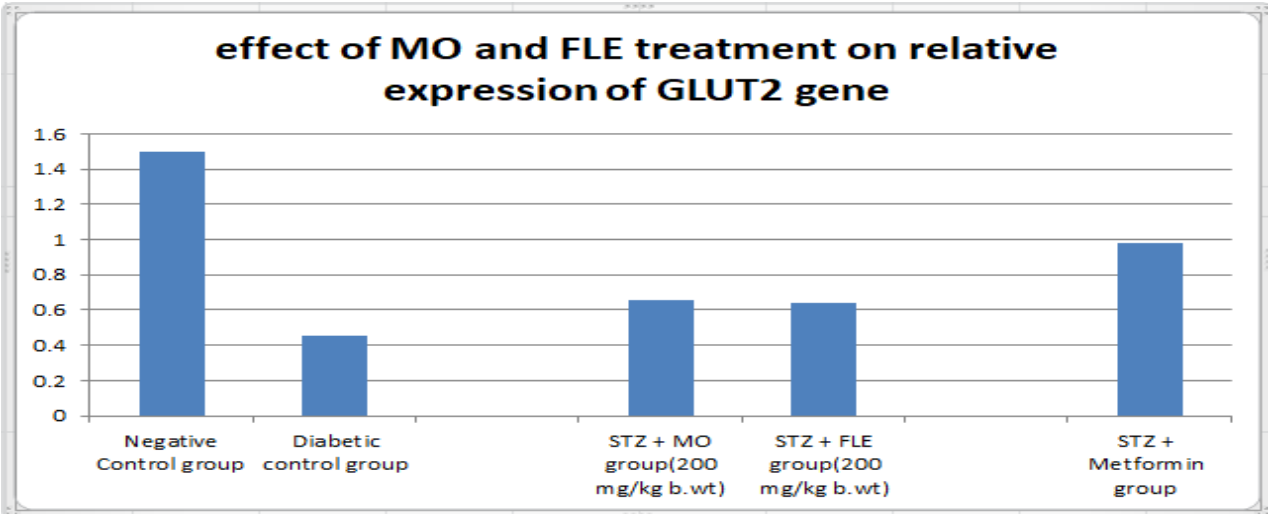

Fig (1):- effect of MO and FLE treatment on the relative expression of GLUT2 gene in the pancreas in diabetes mellitus induced experimentally in male albino rats after 3 weeks treatment compared with metformin treatment

3.2.2- RT-PCR analysis of β-actin and glucose transporter GLUT4 genes expression.

Rats injected with STZ exhibited a significant down-regulation (P<0.05) in the expression level of the GLUT4 gene in the pancreas when compared with the control normal group.

Treatment of diabetic rats with *MO or FLE* exhibited a significant up-regulation (P<0.05) in the expression level of the GLUT4 gene in the pancreas when compared with a diabetic control group.

Diabetic rats treated with metformin showed significant up-regulation (P<0.05) in the expression level of GLUT4 gene in the pancreas when compared with *MO or FLE* alone Fig (2).

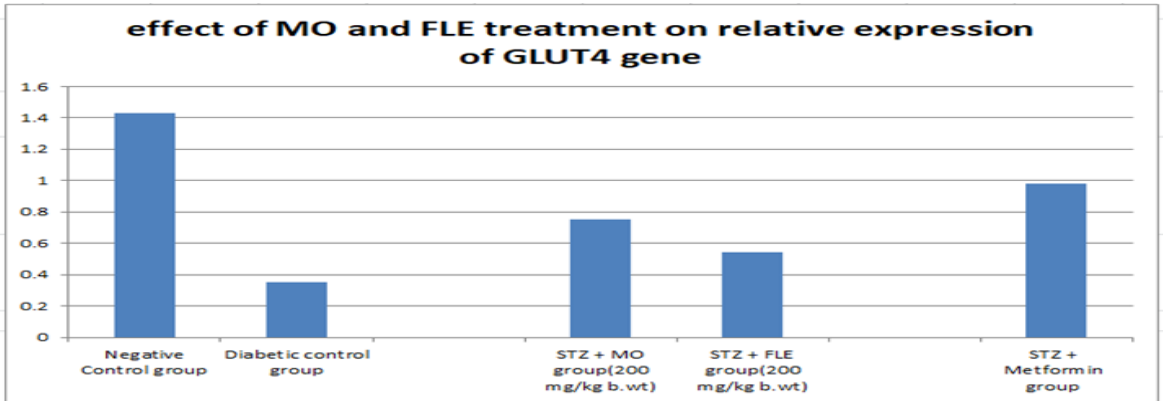

Fig (2):- effect of MO and FLE treatment on the relative expression of GLUT4 gene in the pancreas in diabetes mellitus induced experimentally in male albino rats after 3 weeks treatment compared with metformin treatment**.**

3.2.3- RT-PCR analysis of β-actin and glucose transporter Insulin receptor genes expression.

Rats injected with STZ exhibited a significant down-regulation (P<0.05) in the expression level of the IR gene in the pancreas when compared with the control normal group.

Treatment of diabetic rats with MO or FLE exhibited a significant up-regulation (P<0.05) in the expression level of IR gene in the pancreas when compared with the diabetic control group.

Diabetic rats treated with metformin showed significant up-regulation (P<0.05) in the expression level of IR gene in the pancreas when compared with MO or FLE alone fig (3).

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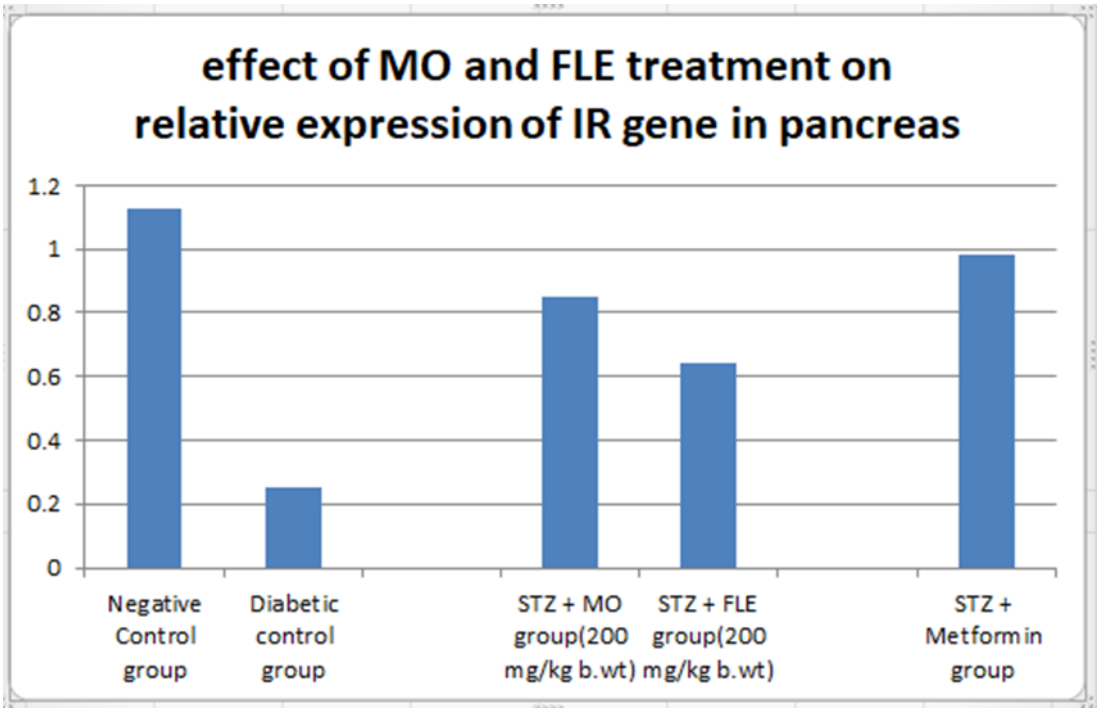

Fig (3):- effect of MO and FLE treatment on the relative expression of IR gene in the pancreas in diabetes mellitus induced experimentally in male albino rats after 3 weeks treatment compared with metformin treatment

4. Discussion

Diabetes is a chronic metabolic progressive disease with hyperglycemia or insulin resistance or both (**Putta et al., 2018**). People at age of 20 to 79 years were prone to diabetes and it was reported that 382 million diabetics were reported among 219 countries in the world and was estimated to increase up to 592 million in 2035 (**Guariguata et al., 2014).** The uncontrolled hyperglycemia that tends to the development of microvascular and macrovascular complications might be due to the oxidative stress, polyol pathway, hexosamine pathway, formation of advanced glycation end products, and also due to incretin effect (**Marcovecchio et al., 2011**). The insulin secretion was stimulated by the incretin hormones, which are produced from the gastrointestinal tract in response to nutrient entry (**MacDonald et al., 2002**).

Plants are important medicinal sources in different countries and therefore are used as potent and efficacious drugs. Plants have been used traditionally as medicine for several years in folk medicine (**Sathyaprabha et al., 2010**). Moringa oleifera (family Moringaceae) popularly called Horseradish tree, Drumstick tree or ben oil tree in English, Zogalle by the Hausas, Ikwe Oyibo by the Igbos, Eweile by the Yorubas, and Gawara by the Fulanis, all of Nigeria (**Paul et al., 2012**) is a fast-growing evergreen deciduous, perennial tree which grows to a height of 10-12 meters with a trunk which may reach 45cm. The plant is slender with drooping and brittle branches. The leaves are feathery, pale green, compound tripinnate, and 30-60cm, with many small leaflets. Flowers are white or creamy with a flagrant smell and are bisexual (**Paul et al., 2012**). The plant is reported to be used in phytomedicine as antioxidant, antimicrobial, anti-inflammatory, antipyretic, antiulcer, antidiabetic, antitumor, and as a hypocholesterolemic agent (**Ijioma et al., 2014)**.

Because of the presence of particular bioactive compounds. Furthermore, our focus is to chart a new way for the plant-based drug which will be docked and modeled so that it could be used to control metabolic and other diseases.

Lipid-lowering drugs work in several ways including decreasing cholesterol production, decreasing cholesterol absorption from the intestine, and removing cholesterol from the bloodstream (**Aurigemma et al., 2004**). Drugs that act directly to decrease cholesterol levels also have the beneficial effect of further lowering cholesterol levels by stimulating the production of additional LDL receptors (**Harey et al., 2005**). There are currently five major types of medications available for treating hypercholesterolemia. HMG-CoA reductase inhibitor (Statins), bile acid-binding resins, cholesterol absorption inhibitor agents, niacin and its congeners, and the fibrates (**Wu et al.,2005**).

The protective effect of bioactive compounds in Moringa oleifera and Ficus sycomorus against cytokine-mediated inhibition of insulin secretion is accompanied by a decrease of NO level in beta cells (**Kim et al., 2007)**. Similarly, EGCG and quercetin potentiate glucose-induced insulin secretion concomitant with a decrease in NO production (**Dai et al., 2013)**.

As shown in table (1) Rats injected with STZ exhibited a significant increase in serum glucose when compared with the control normal group.

Treatment of diabetic rats with MO or FLE exhibited a significant decrease in glucose level when compared with the diabetic control group.

Diabetic rats treated with metformin showed a significant decrease in the glucose level when compared with MO or FLE alone.

The STZ alkylates the pancreatic DNA; thereby it produces reduced insulin levels, altered glucose metabolism, and utilization. The STZ also causes stimulation of nitric oxide free radicals and initiates DNA strand break of β cells of the pancreas (**Rodrí guez et al., 1997).**

It indicates that protective activity of MO or FLE from hyperglycemia might be the reason for the improvement in insulin secretion as shown in table (1).

The results in table (2) showed that Rats injected with STZ exhibited a significant increase in serum cholesterol when compared with the control normal group.

Treatment of diabetic rats with *MO or FLE* exhibited a significant decrease in cholesterol level when compared with the diabetic control group.

Diabetic rats treated with metformin showed no significant change in the cholesterol level when compared with *MO or FLE* alone.

And table (2) showed that Rats injected with STZ exhibited a significant increase in serum triacylglycerol when compared with the control normal group.

Treatment of diabetic rats with *MO or FLE* exhibited a significant decrease in triacylglycerol level when compared with the diabetic control group.

Diabetic rats treated with metformin showed no significant change in the triacylglycerol level when compared with *MO or FLE* alone.

In table (2) Rats injected with STZ exhibited no significant change in serum HDL-cholesterol when compared with the control normal group.

Treatment of diabetic rats with *MO, FLE, or* metformin exhibited also no significant change in HDL-cholesterol level when compared with the diabetic control group.

But in the table (2) showed that Rats injected with STZ exhibited a significant increase in serum LDL-cholesterol when compared with the control normal group.

Treatment of diabetic rats with *MO or FLE* exhibited a significant decrease in LDL-cholesterol level when compared with the diabetic control group.

Diabetic rats treated with metformin showed a non-significant change in the LDL-cholesterol level when compared with *MO or FLE* alone. Diabetes was accompanied by an increase in TC, LDL-C, and TG and a reduction in HDL-C in STZ-induced diabetic rats. Treatment of STZ-induced diabetic rats with *MO or FLE* could profoundly improve lipid disturbances. Treatment of diabetic rats with A. esculentus notably

reduced serum TG and TC levels while HDL-C levels remained unchanged. The liver plays a pivotal role in lipid metabolism. CHOL is essential in Vitamin D synthesis and hormone metabolism. The normal range is beneficial but when exceeded it becomes harmful to cellular integrity. Excess CHOL forms plaque in artery walls, narrowing it and reducing the rate of blood circulation leading to a condition known as arteriosclerosis.

Our results are in agreement with (**Chumark et al., 2008**) who reported hypolipidemic activity of MO leaves extracts, indicating that MO significantly lowered CHOL level. (**Manohar et al., 2012**) also reported similar results in which MO caused a reduction in serum CHOL level.

Lipoproteins help transfer lipids around the body in the extracellular fluid (**Baynes et al., 2014**).

LDL is synthesized in the liver by the action of lipolytic enzymes with increased concentrations leading to CHOL build-up in the arteries (**Ganji et al., 2003).** A high level of LDL was observed in diabetic rats when compared to nondiabetic rats. Interestingly, after treatment with MO, LDL levels of diabetic rats and nondiabetic rats reduced significantly when compared to the control. Many other studies have alluded to the fact that MO has hypoglycemic and hypolipidemic effects which are in tandem with findings from this study (**Ezuruike et al., 2014).**

HDL mediates the reverse transport of CHOL from the extrahepatic tissues to the liver where the CHOL is converted to bile acid and excreted (**Khovidhunkit et al., 2004).**

Through enhancing insulin sensitivity and decreasing fat digestion and/or absorption, fibers, and polyphenols in F. sycomorus leaves may exert inhibitory actions on fat accumulation in liver tissues leading to a marked decrease in lipid profile (**Dukehart et al., 1989 & Meydani et al., 2010).**

Moringa oleifera is a rich source of antioxidants (**Singh et al.,2009**) such as quercetin and kaempferol (major bioactive compounds of phenolics) and are responsible for antioxidant activity (**Siddhuraju et al.,2003).**

Flavonoids can exert their antioxidant activity by various mechanisms, e.g., by scavenging or quenching free radicals, by chelating metal ions, or by inhibiting enzymatic systems responsible for a free radical generation.56 The antioxidant property also can be due to the presence of carotenoids, alkaloids, proanthocyanidins in this plant 56 or to the high content of flavonoids such as kaempferol, presence of other polyphenols, carotenoids, and cinnamic acid derivatives (**Bajpai et al., 2005).**

Furthermore, the malondialdehyde (MDA) concentration showed decreased in the treated groups when compared to the untreated diabetic control group (table 3). This is associated with increased activity of GSH and CAT respectively. This means that the M. oleifera leave and its methanol extract can reduce reactive oxygen free radicals and improve the activities of the antioxidant enzymes.

The antiradical activity of Ficus sycomorus could be due to the high content of tannins and flavonoids in the leaves. Flavonoids have important antioxidant and antiradical activities. Their protective effects in biological systems are linked to their ability to transfer electrons to free radicals, chelate metals, activate antioxidant enzymes, reduce radicals of alpha-tocopherol, or inhibit oxidases (**Bruneton, 2009**). Also, the effects of hypoxia are partially mitigated in response to treatment with antioxidants (**Carriere et al., 2004**).

Bouchet et al. (1998) showed that hydrolyzable or condensed tannins have antiradical and antioxidant properties expressed by their inhibiting effect on lipid peroxidation (induced by Fe2+) and radical-scavenging ability on DPPH radical. In addition to the molecular abnormality that governs the synthesis of abnormal S hemoglobin, sickle cell anemia is one of the conditions in which the production of free radicals results in a state of oxidative stress. It was also showed that the excessive production of superoxide anions and hydroxyl radicals in erythrocytes, resulting from the instability of S hemoglobin, can be the initiating factor of hemolysis, which releases in the plasma ionized iron and heme molecules generating in turn, free radicals (**Deby et al., 1986**).

Our results show that Ficus sycomorus could be of interest in finding new molecules with antioxidant activity because of their high content in phenolic compounds.

Our results in fig. (1) showed that Rats injected with STZ exhibited a significant down-regulation in the expression level of the GLUT2 gene in the pancreas when compared with the control normal group.

Treatment of diabetic rats with *MO or FLE* exhibited a significant up-regulation in the expression level of the GLUT2 gene in the pancreas when compared with the diabetic control group.

Diabetic rats treated with metformin showed significant up-regulation in the expression level of the GLUT2 gene in the pancreas when compared with *MO or FLE* alone.

The preferential uptake of STZ by kidney and liver among extrapancreatic mammalian tissues may be explained by the fact that these tissues are among the few that express GLUT2 as their major glucose transporter isoform (**Thorens et al., 1988**). It is well recognized that multiple low-dose injections of STZ induce a syndrome of insulitis and p-cell destruction that resembles insulin-dependent diabetes mellitus (IDDM) (**Like et al., 1976**). Furthermore, insulitis and progression to diabetes can be blocked in nonobese diabetic (NOD) mice by injection of high doses of nicotinamide, an agent that suppresses poly (ADP-ribose) synthetase and restores cellular levels of NAD (**Yamada et al., 1982**). In light of these data, it has been suggested that diabetes induced by STZ and other chemically related nitroso compounds, such as the rodenticide Vacor (**Karam et al., 1980**) and AT-nitroso compounds in smoked meats (**Helgason et al., 1982**), may induce β-cell destruction by related mechanisms. The demonstration in this study that GLUT2 can transport glucose molecules modified by a JV-nitroso group suggests that β-cell cytotoxicity in IDDM might be induced or accelerated by the accumulation of similarly modified glucose derivatives or more distantly related compounds under conditions of T-cell infiltration and insulitis. Indeed, islet-infiltrating monocytes have been reported to produce reactive oxygen and nitrogen-free radicals (**Nathan et al., 1987**) that may be directly cytotoxic or that could react with other molecules, such as glucose, to produce a new class of β-cell cytotoxins. If so, GLUT2-expressing neuroendocrine cell lines, such as those used in this study, may represent a good model for probing mechanisms of cytotoxicity that may be relevant to β-cell destruction in IDDM

Our results showed in fig 2 that Rats injected with STZ exhibited a significant down-regulation in the expression level of the GLUT4 gene in the pancreas when compared with the control normal group.

Treatment of diabetic rats with *MO or FLE* exhibited a significant up-regulation in the expression level of the GLUT4 gene in the pancreas when compared with a diabetic control group.

Diabetic rats treated with metformin showed significant up-regulation in the expression level of the GLUT4 gene in the pancreas when compared with *MO or FLE* alone.

The major route for disposal of glucose load following a meal is the insulin-stimulated glucose transport into the skeletal muscle and adipose tissue. The principal glucose transporter protein that mediates this uptake is the glucose transporter 4 (GLUT4) which is a key regulator of whole-body glucose homeostasis (**Huang et al., 2007).**

The GLUT4 remains sequestered intracellularly in the absence of insulin but it quickly translocates to the plasma membrane in presence of insulin (**Shepherd et al., 1999).** In the early stages of the development of type 2 diabetes, impaired glycogen synthesis in muscle is the primary defect responsible for insulin resistance (**DeFronzo et al., 1997).**

Later, it has been shown that the impairment of insulin-stimulated glucose transport is responsible for resistance to insulin-stimulated glycogen synthesis in muscle in subjects with type 2 diabetes (**Cline et al., 1999).** So, impaired glucose transport plays a major role in the pathogenesis of type 2 diabetes

Our results in fig.3 showed that Rats injected with STZ exhibited a significant down-regulation in the expression level of the IR gene in the pancreas when compared with the control normal group.

Treatment of diabetic rats with *MO or FLE* exhibited a significant up-regulation in the expression level of IR gene in the pancreas when compared with the diabetic control group. Diabetic rats treated with metformin showed significant up-regulation in the expression level of IR gene in the pancreas when compared with *MO or FLE* alone.

Recent studies revealed a cross-talk between the cAMP/PKA and extracellular signal-regulated kinase (ERK) 1/2 signaling pathways in beta cells and compounds that activate cAMP/PKA cascade (e.g., insulin receptor gene) amplify both glucose-stimulated insulin release and ERK1/2 phosphorylation (**Costes et al., 2006)**. ERK1/2 kinases, in turn, control the protein levels and phosphorylation of cAMP-responsive element-binding protein and therefore play a key role in the regulation of beta cells functions and survival (**Costes et al., 2006)**.

5. Conclusion

Therefore, from the present study, it can be concluded that MO or FLE showed promising antidiabetic potential in diabetic-bearing albino mice which can be attributed to its anti-inflammatory content. This could serve as a stepping stone towards the discovery of newer safe and effective anti-diabetic. So we recommend eating MO or FLE to protect the body from many diseases and effects caused by diabetes.

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