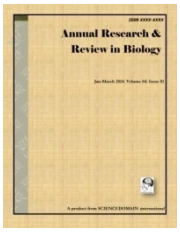

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Effect of Mexican Oregano (*Lippia graveolens* Kunth) on Streptozotocin Induced Diabetic Mice and its Role in Regulating Carbohydrate Metabolic Enzymes and Their inhibitory Effect on the Formation of Advanced Glycation end Products

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Author's contribution

This whole work was carried out by author RMPG.

Original Research Article

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ABSTRACT

Aim of the Study: The aim was to evaluate the effect of extracts of the leaves of *Lippia graveolens* in streptozotocin-induced mildly diabetic (MD) and severely diabetic (SD) miceand on the formation of advanced glycation end products (AGEs).
Study Design: Diabetes has emerged as a major global challenge in healthcare delivery, particularly in recent times, with the global incidence reaching epidemic proportions. Because of this there is an increasing demand to research for natural products with antidiabetic activity.
Place and Duration of Study: Laboratory of Natural Products Research. School of chemical engineering, National Polytechnic Institute between August 2013 and April 2014.
Methodology: Extracts were orally administered to MD and SD rats for 30 days, and a set of biochemical parameters were studied: glucose, cholesterol, triglycerides, lipid peroxidation, glycogen, SOD, CAT, GSH, GPX, glucose-6-phosphatase, glucokinase, hexokinase activities, SGOT, SGPT, ALP and insulin level. In this study, we investigate the

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inhibitory effects *In vitro* on the formation of specific AGE representatives including AGEs-BSA formation, Amadorin activity, methylglyoxal (MGO). *In vivo* was to investigate the effect of extracts on oxidative stress, glycation of hemoglobin and MGO, glycolaldehyde (GA) levels, TBA-reactive substance level in kidney mitochondrial instreptozotocin-induced diabetic mice.
Results: Methanol extract (LG-M) reduced the intake of food, water and body weight loss as well as levels of blood glucose, serum cholesterol, triglyceride, lipoprotein and increase HDL-cholesterol, antioxidant enzymes, improves TBARS–reactive substance, marker enzymes of hepatic function. These results, support that improves glucose metabolism by reducing insulin resistance. LG-M is an inhibitor of fluorescent AGE, methylglyoxal and the glycation of haemoglobin.
Conclusions: This study demonstrated that *Lippia graveolens* possesses considerable anti-AGE and hepatoprotective role, inhibits hyperglycemic, hyperlipidemic and oxidative stress indicating that these effects may be mediated by interacting with multiple target operating in diabetes mellitus.

Keywords: Antidiabetic; hyperlipidemia; glycation; lippie graveolens; antiglycation.

1. INTRODUCTION

Diabetes is a serious metabolic disorder with micro and macro vascular complications resulting in significant morbidity and mortality. Diabetes mellitus is principally characterized by insulin resistance (IR) and elevated blood glucose levels [1]. Prolonged hyperglycemia contributes importantly to the pathogenesis of diabetic complications by increasing protein glycation, leading to the gradual buildup of advanced glycation end products (AGEs) in body tissue [2]. The complex, fluorescent AGE molecules formed during Maillard reaction can lead to protein cross-linking, which contributes to the development and progression of various diabetic complications [3].

Diabetes is characterized by abnormal lipid and protein metabolism, along with specific long-term complications affecting the retina, the kidney and the nervous system mainly [4]. Oxidative stress and advanced glycation endproducts (AGEs) formation induced by hyperglycemia are known to influence diabetic renal changes and nephropathy [5]. The number of people with diabetes is increasing worldwide due to population growth, aging, urbanization, increasing prevalence of obesity, calorie rich diet and physical inactivity. Current treatments, although provide a good glycemic control do little preventing complications [6]. Besides, most of the prescribed hypoglycemic drugs or insulin are associated with unwanted side effects. Herbal medicines are an option because of their comparably therapeutic effects and nontoxic side effects [7].

*Lippia graveolens*is, commonly known as “Mexican oregano”, distributed widely in México, Central and South America. It is widely used in Mexico as food seasoning and a folk remedy.Aerial part ofthis species is used as an antiseptic, antipyretic, analgesic, abortive, antispasmodic, anti-inflammatory agent and for the treatment of menstrual disorders and diabetes [8]. *L. graveolens*is used in Central America for the treatment of gastrointestinal and respiratory diseases, and as antipyretic, digestive, anti-inflammatory and antifertility drug [9]. The essential oil presented antifungal activity which can be attributed to the presence of carvacrol, α-terpinyl acetate, cymene, thymol, pinene and linalool, which are already known to exhibit antimicrobial activity [10]. Most of the studies on the chemical composition of

L. graveolens have mainly focused on the terpene compounds contained in the essential oil as thujene, β-pinene, m-cymene, 1,8-cineole, α-terpinyl acetate, linalool, camphor, terpinen-4-ol, bornyl acetate, carvacrol, thymol, cadina-4(5),10(14)-diene, guaia-1(10),11(12)-diene, isocaryophyllene, humulene, aristol-1(10)-ene, α-bisabolol, β-bisabolene, β-candinene, himachalene and cedrene [10]. *In vitro* studies have shown that essential oil from methanolic extract have antioxidant and antimutagenic properties [11]. *L. graveolens* has been reported that essential oils with a highcarvacrol content displayed antimicrobial [12] and antiviral effects [13]. An investigation of the leaves of *L.graveolens* from Guatemala provided iridoid and secoiridoid glucosidesas loganin, secologanin, secoxyloganin, dimethyl secologanoside, loganic acid, 8-epi-loganic acid, caryoptosidic acid, caryoptoside, lippiosidel and II [14]. In several phytochemical studies on this plant led to the isolation of thirty-oneflavonoids [15]. Furthermore, the aqueous extract of leaves inhibited some trophozoites [16]. In addition, flavanone(-)(2S)-5,6,7,3',5'-pentahydroxyf1avanone-7-O-β-D-glucopyranoside shown anti-inflammatory and cytotoxic activities [17]. Although oregano is popular around the world its beneficial and/or adverse effects on human health have not been scientifically determined yet. No previous study has, for instance, so far reported on the systematic investigation and evaluation of the antidiabetic and antiglycation activities of oregano. To the authors' knowledge, the present work is the first attempt to investigate the protective effects of oregano on diabetes and its complications on the functions of the liver, kidney, and pancreas.

2. MATERIALS AND METHODS

2.1 Plant Material

Lippia graveolens Kunth belong to the Verbenaceae family, leave were collected in Morelos state and were taxonomically authenticated in the Herbarium of Escuela Nacional de Ciencias Biologicas, Instituto Politécnico Nacional. A voucher specimen of the plant is stored for reference (No. 9289). Leaves of *L graveolens* was air dried and the ground (2kg) was extractedtwice with hexane, chloroform and methanol each for 3h. The extracts were evaporated invacuum to generate a residue (28.5, 36.2, 23.4 g residue respectively).

2.2 Experimental Animals

2.2.1 Experimental animals

The study was conducted in CD1 male mice, weighing about 25-30g. Before and during the experiment, animals were fed a standard laboratory diet (Mouse Chow 5015, Purina) with free access to water. The experiments reported in this study were carried following the guidelines stated in Principles of Laboratory Animal Care (National Institute of Health publication (NIH) 85-23, revised 1985 and the Mexican Official Normativity (NOM-062-Z00-1999).All experiments and protocols described in present study were approved by the Institutional Animal Ethical Committee of Escuela Nacional de Ciencias Biologicas-IPN (Regd. No. 735).

2.3 Induction of Severe Diabetes

Severe diabetes was induced in overnight fasted male mice by a single intraperitoneal injection of streptozotocin (Sigma Chemical Company, St. Louis, MO, USA), at a dose of 50 mg/kg body weight dissolved in cold citrate buffer (pH 4.5) [18]. Hyperglucemia was

confirmed by measuring glucose 72h after the streptozotocin shot and 7 days after injection, confirming a high glucose level. Rats with permanent high fasting blood glucose level >300mg/dl were included for the experiments.

2.4 Induction of Mildly Diabetes

Mild diabetes was induced in overnight fasted mice by administering a single dose of freshly prepared solution of streptozotocin (STZ), 45mg/kg *b.w.* i.p) in 0.1mol/l cold citrate buffer (pH 4.5), 15 min after the intraperitoneal administration of 120mg/kg nicotinamide (Sigma Chemical Company, St. Louis, MO, USA). The STZ treated animals were allowed to drink 5% glucose solution over night to overcome drug induced hypoglycemia. After 10 days of development of diabetes, mice with moderate diabetes having persistent glycosuria and hyperglycaemia (blood glucose>250mg/dl) were used for further experimentation [19].

2.5 Experimental Design in Diabetes Mice

2.6 Effect of Single Oral Administration of Extracts of *L. Graveolens*in Glucose Level in Normal, Severe and Mild Diabetic Mice

After the mice had been denied access to food/water overnight, they were randomly divided into twelve groups (six rats per group) matched for body weight. The test groups were orally administered 100, 200 and 400mg/kg body weight (b.w.) of extracts of hexane (LG-H), chloroform (LG-C) and methanol (LG-M) suspended in Tween 80, 1% via gavage). Glibenclamide (GB) at the dose of 5mg/kg b. w as standard drug. Blood samples were collected from the tail vein at 0, 2, 4, 6, 8 and 12h after the administration. The plasma glucose concentration was determined by an enzymatic colorimetric method using a commercial kit (Sigma Aldrich, USA).

2.7 Antidiabetic Test in Chronicsevere and Mild Streptozotocine-Induced Diabetic Mice

In a parallel study seven groups (n=10) of diabetic mice were used to determine the chronic effect of LG-H,LG-C and LG-M extracts. Each group was submitted to a specific treatment, as follows. Normal control and severe and mild diabetic rat, groups, were fed with normal diet and drinking water *ad libitum,* and were given saline by gastric gavage. Severe and mild diabetic mice that received extracts by gastric gavage (400mg per kg of body weight) every day were designated as SD + LG-C and MD + LG-C. Two groups with severe (SD +GB) and mild diabetes (MD +GB) mice were administered with glibenclamide (GB) 5mg/kg as positive control.

2.8 Determination of Body and Food Intake

Body weights of mice and the intake of food and water and were taken prior to the induction of hyperglycemia, at day 0of treatment, and on a daily basis thereafter, for 4 weeks.

2.9 Oral Glucose Tolerance Test (OGTT) In Normal Mice

Oral glucose tolerance test was performed in overnight (16h) starved normal mice. The mice were randomly divided into three groups (n = 6). Glucose 2g/kg was fed 30 min after the

administration of 400mg/kg of extract and glibenclamide. Blood was withdrawn from the tail vein 0, 30, 60, 90 and 120 min, blood glucose level were appraised by commercial kit (Sigma Aldrich, USA).

2.10 Oral Glucose Tolerance Test (OGTT) In Diabetic Mice

Animals of each group were orally administered LG-C extract at doses of 400mg/kg body weight on a daily basis for 30 days. At the end of the experiment, an oral glucose tolerance test (OGTT) was performed to assess the animals' sensitivity to a high glucose load. Overnight-fasted rat were fed orally 2g glucose/kg b. w. Blood samples were collected from the caudal vein from a small incision at the end of the tail at 0 min (immediately after glucose load), 30, 60, 90 and 120 min after glucose administration.

2.11 Collection of Organ Tissues

At the end of chronic diabetes experiments all mice were anesthetized with 1.0% pentobarbital sodium and blood was obtained from the retro-orbital plexus of each animal following the injection of heparin (100 IU kg^{-1}body weight) into a tail vein for 10 min. The liver and kidney were removed according to defined anatomical landmarks.

2.12 Plasma Biochemical Analysis

Blood samples were collected from tail vein of the mice into micro centrifuge tubes containing heparin (10µl, 1000 IU ml^{-1}). The blood samples were then centrifuged at 1600 x *g* for 15 min at 4ºC for the preparation of plasma. Concentrations of plasma glucose, total cholesterol (TC), triglycerides (TG) and HDL-cholesterol, were measured with enzymatic assay kit (Genzyme Diagnostics), LDL-cholesterol was calculated as the remaining difference of total cholesterol and HDL. Blood glucose levels were measured employing the glucose oxidase-peroxidase (GOD-POD) method. Lipid peroxidation (LPO), that is, thiobarbituric acid reactive substances (TBARS) was estimated by the method of Fraga et al. [20] and expressed as µM/g of liver and kidney tissue. Serum glutamate oxaloacetate ransaminase (SGOT), glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP) and total protein, using a commercial Diagnostic Kit Biocompare**,** BioVision, Biocompare and Thermo scientific respectively. Malondialdehyde (MDA) as thiobarbituric acid reactive substances was measured at 532 nm spectrophotometrically.

2.13 Antioxidant Parameters Levels in Serum, Liver, Pancreas and Kidney

Activity of serum superoxide dismutase (SOD) was measured by the xanthine oxidase method using commercial kits with the absorbance measured using spectrophotometer at 550 nm. Serum catalase (CAT) and glutathione peroxidase (CSH-Px) activities were measured by the colorimetric method measuring absorbances at 405 nm and 412nm respectively. Glutathione reductase (GSH**)** by measuring the rate of NADPH oxidation at 340nm. All the assay kits were purchased from Cayman Chemical (Michigan, USA), and the procedures were according to the kits instructions. In the pancreas the protein concentration was determined by the Bradford method as described in the Bio-Rad protein assay kit.

2.14 Estimation Al Glucose Metabolic Enzymes Activities in Liver Tissues

When the mice were sacrificed, the liver tissues were removed and immediately frozen by liquid nitrogen and stored at -80ºC for further study. The activity of glucokinase and glucose-6-phosphatasewas assayed by the color change of TMB substrate using commercial EUS Akits purchased from R & O system (USA), and the color change was measured spectrophotometrically at the wavelength of 450nm. Protein concentration and liver tissue glycogen were estimated using commercial kits purchased from Cayman Chemical (Michigan, USA), all the tests were carried out according to the kit instructions, respectively.

2.15 Determination of Serum Insulin Level, Pancreatic Insulin Content

Serum insulin and pancreatic insulin content were measured by enzyme linked inmunosorbent assay (ELISA) using the kit (Boehringer Mannheim Diagnostic, Mannheim, Germany). The level of insulin was expressed in µIU/ml.

2.16 *In vitro* Glycation of Proteins

2.17 Bovine Serum Albumin (BSA)-Glucose Assay

The methodology was based on that of Brownlee et al [21]BSA (l0mg/ml) was incubated with glucose (500mM) in phosphate buffered-saline (PBS) (5 ml total volume, pH 7.4) and extract containing 0.02% sodium azide at 37ºC with a final concentrations of BSA (2mg/ml), glucose (40mM), sample (0.1 to 0.5mg/ml). All the reagent and samples were sterilized by filtration through 0.2µm membrane filters. The protein, the sugar and the prospective inhibitor were included in the mixture simultaneously. Aminoguanidine was used as an inhibitor positive control. Reactions without any inhibitor were also set up. Each solution was kept in the dark in a capped tube. After 15 days of incubation, fluorescence intensity (excitation wavelength of 370nm and emission wave-length of 440nm) was measured for the test solutions.

2.18 Bsa-Methylglyoxal Assay

This assay was modified based on a published method [22].The assay evaluates the middle stage of protein glycation. BSA and methylglyoxal were dissolved in phosphate buffer (100 mM, pH 7.4) to a concentration of 20mg/ml and 60mM, respectively. Isolated were dissolved in the same phosphate buffer. One milliliter of the BSA solution was mixed with 1 ml of methylglyoxal solution and 1 of ml compounds 1 and 2. The mixture was incubated at 37ºC. Sodium azide (0.2g/l) was used as an aseptic agent. Phosphate buffer was used as a blank. Aminoguanidine and phloroglucinol were used as positive controls. After seven days of incubation, fluorescence of the samples was measured using an excitation of 340 nm and an emission of 420nm, respectively.

2.19 Amadorin Activity

Amadorin activity was determined using a post-Amadori screening assay [23]. Lysozyme (10mg/ml) was incubated with 0.5 M ribose in 0.1 M sodium phosphate buffer containing 3 mM sodium azide, pH 7.4 at 37°C for 24h. Unbound ribose was removed by dialysis against 4 1 of 0.1M sodium phosphate buffer, pH 7.4 at 4ºC for 48h with 5-6 changes. Following dialysis, the protein concentration was determined using the Bio-Rad standard protein assay kit based on the Bradford dye-binding procedure [24]. Dialysedribated lysozyme (10mg/ml)

was reincubated with 10 mg/ml of 1-8 and aminoguanidine in 0.1M sodium phosphate buffer containing 3mM sodium azide, pH 7.4 at 37ºC for 15 days.

2.20 *In vivo* Glycation of Proteins

2.20.1 Glycosylated haemoglobin

(HbA_{1c}) was estimated using a commercial diagnostic kit from Sigma-Aldrich (Human haemolysate [glycated haemoglobin (HbA_{1c})] Kit).

2.21 Glucose and Age Level in Kidney

The renal glucose level was determined by the method of Momose et al. [25]. In brief, frozen kidney tissue was homogenized with ice-cold physiological saline and, after being deproteinized, the glucose content was determined using the Wako kit described above. The renal AGE level was determined by the method of Nakagawa [26]. In brief, minced kidney tissue was dilapidated with chloroform and methanol (2: 1, v/v) overnight. After washing, the tissue was homogenized in 0.1 N NaOH, followed by centrifugation at 8000x*g* for 15 min at 4°C. The amounts of AGEs in these alkali-soluble samples were determined by measuring the fluorescence at an emission wavelength of 440 nm and an excitation wave length of 370 nm. A native BSA preparation (l mg/mL of 0.1 N NaOH) was used as a standard, and its fluorescence intensity was defined as one unit of fluorescence. The fluorescence values of the samples were measured at a protein concentration of 1mg/mL and expressed in arbitrary units (AU).

2.22 Mitochondrial TBA-Reactive Substance Level in Kidney

Mitochondria were prepared from kidney homogenate by differential centrifugation (800x*g* and 12000x*g*, respectively) at 4ºC according to the methods of Jung & Pergande [27], with minor modifications. Each pellet was resuspended in preparation medium and the concentration of TBA-reactive substances was determined by the method of Uchiyama & Mihara [28].

2.23 Methylglyoxal (MG) and Glycolaldehyde (GA) Levels In Kidney

The frozen kidney tissue was homogenized with ice-cold physiological saline and MG and GA values in the homogenates were evaluated. Determination of MG was performed by applying the principle that MG is quantitatively converted with glutathione (GSH) and glyoxalase 1 to s-lactoyl-GSH, which can be directly measured by the change in extinction (Δ_{240}) [29]. The renal GA value was determined by applying the principle that the GA formed is reduced by NADH, with the reduction being catalysed by alcohol dehydrogenase (ADH), and measured by the change in extinction (Δ_{340}) caused by addition of ADH [30].

2.24 Data Analysis

Data are expressed as mean ± S. E. M. of multiple experiments. Paired Student's *t-tests* were used to compare two groups or ANOVA with Tukey for multiple comparisons using PRISM software (Graph Pad, San Diego, eA, USA). Values of $P<0.05$ were considered statistically significant.

3. RESULTS

The effect of LG-C and LG-M in lowering of blood sugar levels on normal, severe and mild diabetic mice are summarized in Table 1 and2. Treatment with hexane extracts did not significantly inhibit the rise inblood glucose levels in normal, diabetic mice. Chloroform extract (LG-C) produced a significant reduction in blood glucose levels at three doses 100, 200 and 400 mg/kg of 16%, 18% and 26% respectively (Table 1). The oral administration of LG-C at dose of 100, 200mg/kg and 400 mg/kg produced a significant hypoglycaemic effect after 4h. The most pronounced effect ofAL-M was observed after 8h. However, methanol extract produced an important effect on fasting blood glucose levels (FBG) of normal, diabetic, and treated mice are summarized in Table 2. Administration of ALG-M at doses of200mg/kg b. w. produced significant (0.01) and dose-dependent fall in blood glucose levels when compared with the STZ control group. FBG-reducing effect by LG-M at a dose of 400mg/kg b. w. was found to be more potent than of the reference drug GB, for this reason it was decided to study only methanol extract.

During the study period of 4 weeks, body weight, food and intake of each mice were Recorded daily but data is presented only at day 0 and the end of the experimentation period (Table 3). Thebody weight, liver and kidney weights of mice from STZ control group (after 15 days) were significantly *(P*<0.001) decreased when compared with normal control group. The extract at dose of 400mg/kgb. w. significantly (P<0.001) maintained the body weight, liver and kidney weights toward normal as compared with STZ control.

In the oral glucose tolerance test, the blood glucose levels of glucose treated diabetic mice were increased markedly at 30 min. EP at dose of 400 mg/kg inhibited the increasing blood sugar level significantly *(p*<0.05) at the 60 min and 120 min when compared with disease control (Table 4). Effect of EP on oral glucose tolerance test in normal mice is shownin Table 7. The different doses (100, 200 and 400 mg/kg) produced a significant reduction in blood glucose level at 120 min when compared to the vehicle control (Table 5).

Table 4 shows the effect of LG-M on plasma glucose after 30-day oral administration. Diabetic mice showed increased level of plasma glucose. Oral administration of LG-M at a dose of 400mg/kg b. w. significantly decreased the plasma glucose level.

Biochemical parameters like SGOT, SGPT, SALP, and proteins in the STZ control group were significantly (P<0.001) elevated as compared with the normal control group. Treatment with ALG-M at the dose of 400 mg/kg b.w. significantly (P<0.001) brought the SGOT, SGPT, SALP, and serum protein toward the normal values (Table 6). Table 6 shows the effect of the methanol extract on G6Pase, GK, HK activity and glycogen content of liver and skeletal muscle. Administration of LG-Mat 400mg/kg body weight, increased the content of hepatic glycogen, GK and HK in diabetic mice while G6Pase decreased. Our results showed that the hexokinase activity tended to be reversed to normal values, while normal mice did not exhibit any significant alteration.

There was a significant elevation in serum triglycerides, total cholesterol and TBARS levels in the liver and kidney of diabetic mice while LDL-cholesterol decreased (Table 7). Daily administration of *L. graveolens* extract at a dose of 400 mg/kg to diabetic mice for 30 days significantly reduced in severe and mildly diabetes total cholesterol and triglycerides. Lipid peroxidation (LPO) was measured by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA) a product formed due to the peroxidation of membrane lipids. TBARS level in diabetic mice also decreased after treatment with LG-M extract, kidney.

LDL-cholesterol on the other hand upon treatment also got a decrease. The results also demonstrate a significant control of serum lipid profiles in the LG-C extract treated diabetic mice, with responses comparable with those of the standard drug. Treatment of LG-M led to significant reductions of renal glucose, triglyceride, and total cholesterol contents in diabetic mice (Table 7), which suggested that extract prevented the excessive glucose supply and abnormal lipid accumulation in the kidney.

The antioxidant effect of the LG-M over tissue oxidative markers was studied here (Table 8). Diabetic mice showed a significant reduction in SOD, CAT, GSH and GPx in hepatic and renal tissues. Low levels of SOD, CAT, GSH and GPx in diabetic mice was reverted to near normal values after treatment with methanol extract. The readings obtained from the treated groups were comparable to that of the standard drug glibenclamide. Administration of *L. graveolens*to diabetic mice showed restoring of liver and kidney activities as reflected by these parameters. Hyperglycemia induced oxidative stress may also cause liver cell damage. Lower activity of antioxidant enzymes such as SOD, GSH, GPx, CAT and increased rate of glycation oxidation leads to diabetes complications.

There was a significant *(P<0.05)* decrease in the level plasma insulin in untreated diabetic mice compared to normal control mice. Oral administration of LG-M (400mg/kg) daily for a period of 30 days to diabetic mice significantly *(P<0.05)* increased the level of serum insulin level and pancreatic insulin content compared to diabetic mice (Table 9).

Table 9 shows the levels of HbAlc of normal and diabetic mice. Diabetic mice showed a significant *(P<0.05)* elevation in the level of HbAlc compared to normal mice. Treatment with LG-M and glibenc1amide to diabetic mice significantly *(P<0.05)* decreased the level of HbAlc.

Table 10 shows the effect of LG-M on HbAlc level. Significant increase in HbAlc level was observed in STZ diabetic mice when compared to normal control mice. Oral administration of LG-M at doses of 400mg/kg, for 4 weeks decreased the HbAlc level significantly (P<0.001) a dose dependent manner. There wasa significant change in HbAlc level of LG-M treated rats when compared normal control mice.

In order to determine the inhibitory effect of the methanol extract from *L. graveolens*on the formation of AGEs, several assay methods have been proposed, including assays based on the inhibition of specific fluorescence generated during the course of glycation and AGE formation, and assays based on the inhibition of AGE-protein cross-linking. Table 10 displays the inhibitory effects of LG-M on AGE formation in BSA-glucose and BSA-methylglyoxal models. LG-M, phloglucinol and aminoguanidine exhibited higher inhibitory activity against AGE formation after the incubation at 37ºC for 15 days, with IC_{50} values of 879µM, 712µMand 965µM, respectively. Methylglyoxal-mediated protein glycation inhibition was evaluated for LG-M, which exhibited substantial activity compared with methylglyoxal; This has received considerable attention as a mediator of advanced glycation end-product formation and are known to react with lysine, arginine and cysteine residues in proteins to form glycosylamine protein crosslinks [31].

Table 1. Acute effect of chloroform extract of *Lippia graveolens*on fasting blood glucose level of normal and diabetic mice Corrected

Groups/dose (mg/kg)	mg/dL blood glucose					
	0 h	**2h**	**4h**	**6h**	**8h**	**12h**
Normal	106.2±3.47	104.4±4.32	104.5±3.49	103.8±1.51	104.3±2.40	103.5±1.81
Normal + LG-C 100	104.3±2.81	99.8±2.73 (4.31)	94.7±2.40[a] (9.20)	93.1±2.48 (10.74)	91.2±2.30 [a](12.56)	101.3±2.23 (2.88)
Normal + LG-C 200	105.2±3.23	96.3±2.54 [a](8.46)	90.2±2.40[a] (14.26)	87.5±1.81 (16.83)	86.4±2.07 [a](17.87)	96.6±3.11[a] (8.17)
Normal + LG-C 400	103.8±2.20	92.2±1.81 [a](11.18)	83.4±2.70[a] (19.65)	78.8±1.51 (24.08)	76.3±1.14 [a] (26.49)	86.8±3.28[a] (16.38)
MD Control	202.6±2.17	201.8±1.64	202.4±2.54	201.0±2.0	201.4±2.07	200.8±1.92
MD + LG-C 100	278.8±1.54	265.2±1.78 (4.88)	253.7±1.67[a] (9.00)	234.8±2.77 (15.78)	216.4±1.78 [a] (22.38)	231.6±1.48[a] (16.93)
MD + LG-C 200	276.8±1.67	269.8±1.78(2.53)	261.5±1.78[a] (5.53)	227.4±1.14 (17.85)	200.2±1.30 [a] (27.67)	236.2±1.78[a] (14.67)
MD + LG-C 400	279.3±2.06	253.2±2.58[a] (9.34)	230.2±1.92[a] (17.58)	206.6±2.70 (26.03)	183.1±1.87 [a] (34.44)	197.6±1.14[a] (29.25)
SD Control	278.3±2.32	275.9±2.86	276.2±2.07	280.6±2.68	279.5±2.28	278.7±1.64
SD + LG-C 100	201.9±1.78	196.5±3.34 (2.67)	191.8±2.16 (5.00)	182.0±2.44 (9.86)	171.8±1.78 [a] (14.91)	179.6±1.51[a] (11.05)
SD + LG-C 200	200.6±1.57	193.9±2.54 (3.34)	181.2±1.67[a] (9.67)	167.4±2.88 (16.55)	158.3±1.92 [a] (21.09)	174.2±1.48[a] (13.16)
SD + LG-C 400	202.1±2.52	189.8±3.19[a] (6.09)	164.8±2.58[a] (18.46)	145.3±1.92 (28.10)	142.5±1.81 [a] (29.49)	146.8±2.28[a] (27.36)
SD + GB	201.5±0.99	186.3±3.11[a] (7.54)	221.4±3.38[a] (20.30)	139.3±1.78 (30.86)	143.8±2.30 [a] (28.64)	156.2±1.92[a] (22.48)
MD + GB	277.8±1.97	250.8±2.86[a] (9.71)	152.0±2.23[a] (24.57)	185.3±2.88 (33.30)	191.2±1.64 [a] (31.17)	210.6±1.51[a] (24.19)

Each values represent Mean ± SD (n=6). [a]p<0.05 compared to normal group (ANOVA) followed by Dunnett´s test. [b]p<0.05 compared to diabetic group (ANOVA) followed by Dunnett´s test. Gibenclamide (GB)

Table 2. Acute effect of methanol extract of *Lippiagraveolens* (LG-M) on fasting blood glucose level of normal and diabetic mice Corrected

Groups/dose (mg/kg)	mg/dL blood glucose					
	0 h	**2h**	**4h**	**6h**	**8h**	**12h**
Normal	106.2±3.47	104.4±4.32	104.5±3.49	103.8±1.51	104.3±2.40	103.5±1.81
Normal + LG-M 100	102.9±4.23	97.4±4.87 (5.34)	92.1±3.90[a](10.49)	90.1±7.21 (12.43)	88.2±6.31[a](14.25)	99.0±3.87 (3.79)
Normal + LG-M 200	103.6±6.19	93.5±5.87[a] (9.74)	87.1±6.32[a] (15.92)	84.7±6.49 (18.24)	80.2±3.89[a] (22.58)	89.3±6.45[a] (13.80)
Normal + LG-M 400	105.7±2.67	89.4±8.19[a] (15.42)	82.0 ±6.72[a] (22.42)	77.3±5.87 (26.86)	70.7±6.12[a](33.11)	80.2±5.98[a] (24.12)
MD Control	202.6±2.17	201.8±1.64	202.4±2.54	201.0±2.0	201.4±2.07	200.8±1.92
MD + LG-M 100	265.1±4.64	253.1±3.46 (4.52)	239.5±4.28[a] (9.65)	228.7±7.23 (13.73)	196.3±4.80[a] (25.95)	205.3±3.90[a](22.55)
MD + LG-M 200	282.6±5.29	261.5±4.39 (7.46)	252.6±3.57[a] (10.61)	234.7±6.39 (16.9)	210.4±6.34[a] (25.4)	224.1±7.2[a] (20.70)
MD + LG-M 400	279.8±6.21	244.1±5.76[a] (12.75)	208.6± 6.32[a] (25.44)	181.4±7.54 (35.16)	162.9±8.43[a] (41.7)	193.4±6.43[a] (30.87)
SD Control	278.3±2.32	275.9±2.86	276.2±2.07	280.6±2.68	279.5±2.28	278.7±1.64
SD + LG-M 100	329.6±10.4	316.5±5.72 (3.97)	301.9±4.62 (8.40)	289.8±5.67 (12)	254.5±6.32[a] (22.7)	275.2±7.75[a] (16.50)
SD + LG-M 200	310.5±8.37	290.6±6.48 (6.40)	282.3±4.56[a] (9.08)	263.6±7.65 (15.10)	236.3±5.65[a] (23.89)	253.0±5.84 [a] (18.45)
SD + LG-M 400	342.0±9.56	310.3±5.44[a] (9.26)	276.3±7.59[a] (19.21)	239.5±8.43 (30.0)	222.6±8.43[a] (34.91)	254.5±4.95[a] (25.58)
SD + GB	201.5±0.99	186.3±3.11[a] (7.54)	221.4±3.38[a] (20.30)	139.3±1.78 (30.86)	143.8±2.30[a] (28.64)	156.2±1.92[a] (22.48)
MD + GB	277.8±1.97	250.8±2.86[a] (9.71)	152.0±2.23[a] (24.57)	185.3±2.88 (33.30)	191.2±1.64[a] (31.17)	210.6±1.51[a] (24.19)

Each values represent Mean ± SD (n=6). [a]p<0.05 compared to normal group (ANOVA) followed by Dunnett´s test. [b]p<0.05 compared to diabetic group (ANOVA) followed by Dunnett´s test. Gibenclamide (GB)

Table 3. Effect of methanol extract of *Lippiagraveolens*on the body weight, organ weight, food and water intake Corrected

Group (mg/kg)	Body weight (g)			Intake (g/d)		Final (g)	
	Initial	Final	Gain	Food	Water	Liver weight	Kidney weight
Normal control	27.4±5.1	34.2±6.2[a]	6.8±1.9[a] (25)	29.3±3.2[a]	34.1±6.3[a]	6.76±2.7[a]	1.31±0.5[a]
SD	28.6±4.7	30.8±6.1[c]	2.2±0.8[c] (8)	39.2±5.8[c]	189.5±9.6[c]	3.48±1.3[c]	0.81±0.05[c]
MD	26.2±5.3	29.0±7.1[c]	2.4±0.5[c] (9)	37.6±6.1[c]	180.7±10.4[c]	3.40±1.5[c]	0.82±0.04[c]
SD + LG-M	28.6±6.6	33.9±5.4[b]	5.3 ±0.6[b] (18)	34.4±8.1[b]	137.4±8.1[b]	5.21±1.8[b]	1.0±0.04[b]
MD + LG-M	27.8±8.2	33.4±7.4[c]	5.6±1.4[b] (20)	32.9±6.5[b]	122.0±7.8[b]	5.91±1.7[b]	1.23±0.08[b]
SD + GB	24.5±6.2	29.3±5.8[b]	4.8±1.7[b] (20)	31.2±9.5[b]	113.3±6.6[b]	6.02±2.4[b]	1.26±0.7[b]
MD + GB	25.9±7.6	31.9±6.3[b]	6.0 ±1.3[b] (23)	30.2±9.5[b]	101.2±8.0[b]	6.37±2.1[b]	1.29±0.5[b]

Each value represents mean ± S. E. M. (n=10), ANOVA followed by multiple two-tail "t" test. In each vertical column, mean with different superscripts (a, b, c) differ from "t" each other significantly, p<0.05. () indicates %. All values are expressed as Mean ± SD, n=6 Values

Table 4. Effect of methanol extract of *L. graveolens*on blood glucose level after 30 day treatment and on postprandial blood glucose level of normal and diabetic mice Corrected

Group (mg/kg)	Fasting blood glucose level (mg/dl) Blood glucose levels (mg/dl)									
	Week of supplements					Minutes				
	0	1	2	3	4	0	30	60	90	120
No-diabetic control	106.2±3.47	106.9±1.34[a]	10.7.2±1.87[a]	108.6 ±1.67[a]	109.3±1.37[a]	109.3±1.37[a]	177.8 ±168	166.5±1.21	123.6±2.00[a]	102.2±1.30[a]
SD control	378.3±2.32	383.6±1.15[b]	375.6±1.67[b]	383.7±1.48[b]	381.6±1.18[b]	372.8±6.91	423.6±6.98[a]	430.5±4.87[a]	397.6±7.49[a]	372.6±5.89[a]
MD control	202.2±1.68	210.1±1.18[b]	232.4±2.01[b]	243.9±1.89[b]	250.3±2.48[b]	223.3±5.87	276.3±8.95[a]	265.3±7.84[a]	219.2± 5.65[a]	159.2±10.55[a]
SD + LG-M	379.3±2.06	347.7±2.15[c](8.33)	334.5±1.47[c](11.81)	317.3±1.33[c](16.34)	299.8±2.22[c](20.95)	329.88±9.43	386.5±9.64[a]	432.9±6.88[c]	422.8± 8.57[c]	375.6±10.94[c]
MD + LG-M	202.1±2.52	181.8 ±1.69[c](10.04)	158.9 ±1.34[c](21.3)	132.8±1.84[c](34,28)	116.6±2.64[c](42.3)	246.6±7.23	272.5±8.72[a]	284.0±5.25[c]	218.2± 6.88[c]	115.6±9.51[c]
SD + GB	377.8±1.97	360.4±1.48[c]	333.1±1.11[c]	300.9±2.09[c]	288.6±2.02[c]	368.6±4.39	339.1±3.88[a]	398.3 ±7.38[c]	387.6 ± 9.04[c]	364.8±4.96[c]
MD + GB	212.5±0.99	192.8±1.15[c]	177.4±1.67[c]	158.6±1.43[c]	136.3±2.48[c]	246.3±3.98	282.3±7.07[a]	158.1 ±6.97[c]	116.5 ± 8.11	107.9±6.23[c]

Each value represents mean ± S.E.M. (n=10), ANOVA followed by multiple two-tail "t" test. In each vertical column, mean with different superscripts (a, b, c) differ from "t" each other significantly, <0.05.Glibenclamide (GB) at doses 5 mg/kg. () % inhibition

Table 5.Effect of methanol extract of *L. graveolens*on oral glucose tolerance test in normal mice Corrected

Groups	Time (min)				
	0	30	60	90	120
Glucose control	94.2±5.31	126.6±4.80	121.3±7.39	100.2±5.49	96.5±7.43
Glucose +LG-M (100 mg/kg)	90.5±2.98	112.7±6.87[a]	106.4±11.21[a]	97.3±12.45[b]	88.1±10.31[c]
Glucose + LG-M (200 mg/kg)	91.8±6.17	104.2± 9.41[a]	99.3±11.25[b]	89.2±9.31[c]	84.2±7.12[c]
Glucose + LG-M (400 mg/kg)	85.1±4.38	100.7±8.35[b]	95.30± 9.54[c]	85.3±8.15[c]	74.6±8.29[c]
Glucose +GB (5mg/kg)	89.0±7.59	99.2± 6.87[b]	85.4±5.88[c]	80.6±12.64[c]	72.6± 8.74[c]

All values represent mean ± [a]p<0.05; [b]p<0.01; [c]p<0.001; ANOVA, followed by Dunnett´s multiple comparison test

Table 6. Effect of methanol extract of *L.graveolens*on hepatic glucose regulation enzyme activities and of normal and diabetic mice Corrected

Group	G6Pase activity (mU)	GK activity (mU)	Glycogen (mg/g)		HK activity (IU/l)	SGOT (U/mg protein)	SGPT(U/mg protein)	ALP(U/mg protein)	Total protein (g/dl)
			Liver	skeletal muscle					
Normal control	0.40±0.006	3.38±0.05	19.64 ±2. 45	11.74±4.39	1.69±0.07	20.9±3.2	24.3 ± 5.8	170.3±10.4	7.9 ± 2.1
SD Control	0.71±0.005[a]	1.36±0.06[a]	9.67 ±3. 12[a]	4.89±1.34[a]	1.31±0.03[a]	39.2±8.9[a]	42.3±7[a]	245.1±11.6[a]	4.3±1.5[a]
MD Control	0.69±0.002[a]	1.27±0.08[a]	10.23±4.35[a]	5.27 ±1.56[a]	1.29±0.05[a]	38.7±7.7[a]	42.9±8.6[a]	239.8±9.7[a]	4.0±1.2[a]
SD+ LG-M	0.53±0.008[ab]	2.8±0.04[ab]	[d]17.53±5.18[b]	[d]9.78 ±1.54[b]	[d]1.53±0.09[ab]	27.3±6.2[b]	34.6±5.5[b]	209.2 ±10.7[b]	6.5±1.4[b]
MD + LG-M	0.46±0.007[ab]	3.09±0.03[ab]	[d]18.13±5.80[b]	[d]10.89±2.41[b]	[d]1.59±0.05[ab]	24.0±4.8[b]	28.6±6.3[b]	195.8 ±9.1[b]	6.7±2.4[b]
GB (5 mg/kg)	0.39±0.004[b]	3.11±0.04[b]	17.78±2.30[b]	11.01±1.96[b]	1.60±0.03[ab]	23.1±8.0[b]	25.1±5.7[b]	192.1±11.8[b]	7.2±3.8 [b]

Each values represent Mean ± SD, (n=10); ANOVA followed by multiple two tail "t" test. In each vertical column, mean with different superscripts (a,b) differ from each other. Significant difference of diabetic control from normal control [a]P<0.001. Significant difference of treated groups from diabetic control [b]P<0.01, [c]P<0.05, [d]P<0.01 when compared with glibenclamide5mg/kg treated group

Table 7. Effect of methanol extract of *L. graveolens*on lipid profile, malondialdehyde concentration and on glucose, triglyceride, total cholesterol contents in the kidney of normal and diabetic mice Corrected

Group (mg/kg)	Mean Concentration (mg/g) ± SEM								
	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	TBARS (µM/g)		Kidney (mg/tissue)		
					Liver	Kidney	Glucose	Triglyceride	Total cholesterol
Normal control	102.23±5.65	132.37±6.98	71.53±5.52	45.29±7.21	0.99±0.002	1.8±0.04	1.34±0.56	2.71±0.49	2.23±0.16
SD control	193.18±7.31[a]	279.46±9.41[a]	37.29±8.17[a]	78.13±8.49[a]	1.64±0.05[a]	2.7±0.02[a]	4.42±1.17	4.68±0.76	4.21±0.44
MD control	180.12±7.14[a]	253.21±8.01[a]	41.36±7.19[a]	70.65±10.32[a]	1.60±0.03[a]	2.5±0.08[a]	3.50±0.85	3.42±0.81	3.11±0.56
SD + LG-M	116.10±8.17[b]	163.20±10.28[b]	53.62±9.29[ab]	54.36±9.82[a]	1.10±0.08	2.0 ±0.03[b]	3.34±0.38	3.29±0.35	3.26±0.87
MD + LG-M	94.32±5.80[b]	140.11±8.57[b]	62.33±8.27[ab]	49.40±7.73[b]	1.00±0.08[b]	1.8±0.06[b]	2.08±0.71	2.70±0.54	2.30±0.53
SD +GB	96.70±9.32[b]	131.17±6.88[b]	54.39±9.75[ab]	46.94±4.32[b]	1.05±0.03[b]	1.9±0.04[b]	3.57±0.58	3.41±0.39	3.75±0.73
MD+GB	94.29±2.48[b]	129.89±7.58[b]	66.23±6.42[ab]	42.29±6.22[b]	0.93±0.001[b]	1.8±0.05[b]	2.17±0.67	2.79±0.60	2.42±0.59

All values are expressed as Mean ± SEM, n=10. [a]P<0.05 when compared to normal control group, [b]P<0.01 when compared to diabetic control group, where the significance was performed by Oneway ANOVA followed by post hoc Dunnett´s test.. Plasma insulin values at 0 h before drug administration are significantly different compared to respective days 30 after drug treatment. Significant difference of diabetic control from normal control [a]P<0.001. Significant difference of treated groups from diabetic control [b]P<0.01, [c]P<0.05. [d]P<0.01 when compared with glibenclamide5 mg/kg treated group

Table 8. Effect of methanol extract of *L. graveolens*on antioxidant enzyme activities of normal and diabetic mice Corrected

Parameters	Normal control	Control SD	control MD	SD+LG-M	MD+LGM	SD+G
SOD-Liver	7.54 ±2.19	3.79 ±0.82[a]	4.05±1.38[b]	5.87±0.89[b]	6.01±1.05[b]	6.80±0.41[c]
SOD-Kidney	13.82±3.51	7.35 ±1.37[a]	8.36±2.53[b]	10.24±2.63[b]	11.47±3.41[b]	13.025 ±1.57[b]
SOD-pancreas	54.52±5.21	36.47 ±3.28[a]	35.99±5.42[b]	43.88±5.39[b]	46.90±5.48[b]	50.71±4.36[b]
CAT-Liver	82.36±6.17	44.53±3.13[a]	47.32±7.14[b]	64.56±6.38[b]	67.12±6.74[b]	70.26±2.16[b]
CAT-Kidney	35.61±2.33	20.76±1.65[a]	22.01±9.53[c]	29.56±2.81[c]	32.07±2.53[c]	34.17±1.79[b]
CAT- pancreas	59.32±5.38	25.56±4.19[a]	23.19±5.46[a]	40.24±3.67[a]	43.79±5.18[a]	47.83±4.36[c]
GSH –Liver	47.68±7.12	21.80±1.97[a]	29.15±6.36[b]	36.52±4.86[b]	39.48±4.36[b]	42.38±2.28[b]
GSH –Kidney	24.45±3.71	5.79±2.06[a]	7.21±3.53[b]	15.29±3.83[b]	17.20±3.53[b]	19.27±3.58[b]
GSH- pancreas	12.48±3.39	5.19±0.94[a]	7.42±2.56[b]	6.91±1.80[b]	8.47±1.56[b]	10.79±1.92[b]
GPx –Liver	7.26±1.59	4.31±1.30[a]	5.39±2.01[b]	5.35±1.83[b]	5.64±1.87[b]	5.92±1.26[b]
GPx –Kidney	5.92±1.42	3.53±0.92[a]	3.40 ±1.43[b]	4.53 ±1.34[b]	4.60 ±1.73[b]	4.78±0.94[b]
GPx- pancreas	4.64±1.19	2.32±0.36[a]	3.42±1.26[b]	3.31±0.61[b]	3.70±1.26[b]	3.89±0.27[b]

All values are expressed as Mean ± SEM, n=6 Values. [a]P<0.01 when compared to normal control group; [b]P<0.01, [c]P<0.05 compared to diabetic control group; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett´s test. glibenclamide (GB). Control (C), the values are given in U/mg of protein

Table 9. Effect of methanol extract of *L. graveolens* on serum and pancreatic insulin concentration and glycosylated haemoglobin of normal and diabetic mice Corrected

Groups	Before administration (0 h)	Plasma insulin (μIU/ml)	Pancreatic insulin (μIU/ml)	Glycosylated hemoglobin (mg/g of Hb)
Normal control	3.60±0.52	3.60±0.15	25.79± 5.42	0.89±0.017
SD control	0.73±0.064	1.50±0.37[a]	15.98± 7.83[a]	1.16±0.034[a]
MD control	0.78±0.041	[d]1.68±0.51[b]	17.52 ± 9.20[bc]	1.20±0.045[a]
SD + LG-M	0.74±0.028	[d]2.87±0.80[b]	19.29 ± 7.47[bc]	1.02±0.072[a]
MD + LG-M	0.79±0.042	[d]1.99±0.61[b]	22.88 ± 9.46[bc]	0.96±0.054[a]
SD +GB	0.76±0.065	3.03±0.28[b]	18.79 ± 6.59[bc]	1.02±0.029[a]
MD +GB	0.796±0.034	2.10±0.57[b]	21.68 ± 8.38[bc]	0.96±0.052[a]

All values are expressed as Mean ± SD, n=6 Values. Plasma insulin values at 0 h before drug administration are significantly different compared to respective days 30 after drug treatment. Significant difference of diabetic control from normal control [a]P<0.001. Significant difference of treated groups from diabetic control [b]P<0.01, [c]P<0.05.[d]P<0.01 when compared with glibenclamide 5 mg/kg treated group

Table 10. The inhibitory effects of methanol extract from *L. graveolens*and aminoguanidine on the formation of advanced glycation end products (AGEs), *in vitro* induced by glucose and methylglioxal Corrected

Inducer	Treatment	AGEs IC_{50} (μM)
Glucose	Extract (LG-M)	879 ±15.86
	Aminoguanidine	965±13.56
	Phloroglucinol	712± 22.19
Methylglyoxal	LG-M	325±10.56
	Aminoguanidine	331±16.80
	Phloroglucinol	205±9.70

Data are mean ± standard deviation of triplicate tests

4. DISCUSSION

The present work was aimed to study the hypoglycemic and anti-AGEs activities of ALG-M in STZ-induced diabetic mice. The STZ is a broad-spectrum antibiotic; high dose of STZ has been selectively destroys insulin-producing β-cells of the pancreas by inducting high levels of DNA strand breaks in these cells, causing activation of ADP-ribose polymerase, resulting in reduction of cellular NAD^+, which results in cell death producing type 1diabetes[32]. The experimental diabetic model used low dose of STZ (45 mg/kg b.w) destroyed partial some population of pancreatic β-cells [33]. There were residual β-cells which secreted insufficient insulin causing type 2 diabetic models [34], which lead to impaired glucose uptake by peripheral cells and impaired insulin secretion and action and contributes a number of features similar with human diabetes. Diabetes is characterized by severe weight loss, which was observed in the present study. The reduction in body weight may be attributed to insulin depletion provoking a loss of adipose tissues. The weight loss in diabetic mice might also be the result of degradation of structural proteins due to unavailability of carbohydrate as energy source [35]. In the present study, oral administration of LG-M or glibenclamide to diabetic mice caused significant improvement in the body weight. This may be due to the

antihyperglycemic effect of LG-M thereby enhancing glucose metabolism. STZ-diabetic mice showed significantly decreased plasma glucose level on treatment with LG-M which might bring about glucose lowering action by stimulating the surviving β-cells of islets of Langerhans to release more insulin.

Chronic hyperglycemia and dyslipidemia are associated with a variety of metabolic disorders in human and animal diabetic patients, causing oxidative stress, depleting the activity of the antioxidative defense system, and resulting in elevated levels of ROS [36].Drugs with antioxidant properties may supply endogenous defense systems and reduce both initiation and propagation of reactive oxygen species. Results of the present study clearly showed that LPO level was decreased in LG-M treated diabetic mice. These results suggest that LG-M administration in diabetic mice reduce LPO possibly by decreasing free radical formation, increasing antioxidant enzymes CAT, SOD, GSH GPx in liver, kidney and pancreas. In addition, showed a significant increase in the concentration of MDA, a secondary product of LPO, in STZ treated animals. LPO induced by STZ was associated with the decreased enzymatic activities. Since an oxidative stress affects the cellular integrity only when antioxidant mechanisms are no longer able to cope with the free radical generation, supplementation of an antioxidant could gear up the detoxification machinery.

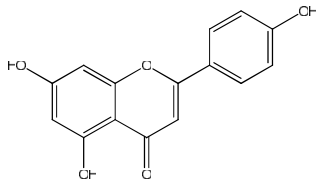
Oxidative environments might cause the damage of cells and tissues in the liver and kidney, which is observed in the increased levels of SGOT, SGPT and ALP activities (indices o fliver dysfunction). The decreased total protein content in STZ-induced animals also substantiated the hepatic damage by STZ. In increase in activities of these enzymes might be mainly due to the leakage from the liver cytosol into blood stream which gives an indication of the hepatotoxic effect of STZ [37]. Reductions in the activity of these enzymes in LG-M treated diabetic mice indicated the hepatoprotective role in preventing diabetic complications. In addition, LG-M decreased G6Pase activity and increased GK activity in liver, which indicates this can be an increase in hepatic glucose uptake and decrease in hepatic glucose release. One of the key enzymes in the catabolism of glucose is hexokinase, which phosphorylates glucose and converts it into glucose-6-phosphate. The increased activity of hexokinase can promote glycolysis and increase utilization of glucose for energy production [38]. Also hepatic glycogen was found to be increased in both liver and skeletal muscle in diabetic rats suggested also a reduction in glycogenolysis and an increase in glycogenesis. It is well known that in uncontrolled diabetes mellitus, there is an increase in total cholesterol in blood, which may contribute to coronary artery diseases [39].

Repeated administration of LG-M resulted in hypoglycemia with reduced plasma insulin levels. These results indicate that LG-M improves hyperinsulinemia in type 2 diabetes (MD). Insulin resistance in peripheral tissues is known to be one of the major pathogenic factors in type 2 diabetes.

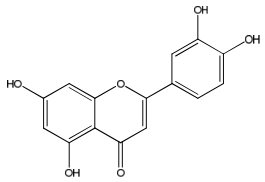
LG-M extract was evaluated in the fluorescence-based assay of AGE formation revealing a log-correlation between inhibition of fluorescent AGE crosslink formation and the concentration of the extract. Increased glycation during hyperglycaemia can cause intra or inter molecular crosslinking of proteins as they accumulate advanced glycation endproducts. Numerous studies have shown that buildup of crosslinked advanced glycation endproducts on long-lived proteins may underlie the development of complications affecting diabetes and ageing. Furthermore, the levels of serum advanced glycation endproducts reflect the severity of these complications whereas therapeutic interventions aimed at reducing advanced glycation endproducts can inhibit or delay their progression [22]. In this study we found that

LG-M inhibited formation of methylglyoxal derived advanced glycation end-products in a bovine serum-albumin-methylglyoxal system, and may also act by blocking conversion of dicarbonyl intermediates to advanced glycation endproducts. Furthermore, ours results show that extract could react with carbonyl groups from reducing sugars, Amadori adducts and dicarbonyl intermediates therefore blocking their conversion to advanced glycation endproducts. Dicarbonyl intermediates such as methylglyoxal have received considerable attention as mediators of advanced glycation endproduct formation and are known to react with lysine, arginine and cysteine residues in proteins to form glycosylamine protein crosslinks[40]. LG-M polyphenols may, therefore, prevent the damage and death of pancreatic β-cells, and/or stimulate the regeneration of this type of cells in diabetic mice. The administration of polyphenols, such as quercetin and epicatechin, to surviving diabetic rats protects the architecture of pancreatic β-cells, preserves the secretion of insulin, and stimulates the regeneration of this type of cells [41]. The ability of LG-M to reduce the blood glucose level could also be attributed to its ability to modulate the immune system [42]. Leading to the decrease of β-cell damageswhich could presumably be attributed to the largeamounts of polyphenols and flavonoids.

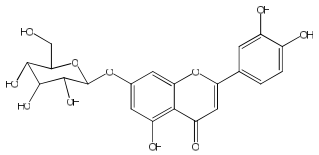
As discussed earlier in the introduction section, the leaves and stem of *L. graveolens* contains the flavonoids naringenin, kaempferol, quercetin, apigenin, luteolin, sakuranetin, eriodictyol, taxifolin and phloridzin (Fig. 1) which are proven to be potent hypoglycaemic agents, and their hypoglycaemic activity. Naringeninprovided a significant amelioration of hypoglycaemic andantioxidant activity in STZ-induced diabetic rats [43]. Kaempferol, may be a naturally occurring anti-diabetic compound by protecting pancreatic β-cell survival and function in a hostile environment that would otherwise lead to type 2 diabetes [44]. Quercetin and naringenin might possibly be able to protect β-cells from cytokines toxicity by enhancing cell survival through PI3-kinase pathway, independent of p-p38 MAPK or iNOS [45]. Apigenin,and luteolin are potential SGLT2 inhibitor for diabetic neuropathy [46]. In addition, luteolin ameliorated inflammation related endothelial insulin resistance in an IKKβ/IRS-1/Akt/eNOS-dependent pathway [47]. Treatment with apigenin, significantly lowered the blood glucose levels of diabetic animals and protected the liver and kidneys against STZ-induced damage in rats [48]. Sakuranetin shown antidiabetic properties [49]. Eriodictyol can increase glucose uptake and improve insulin resistance, suggesting that it may possess antidiabetic properties [50]. Finally, Phloridzin, a known antidiabetic agent, potentially interacts with proteins central to T2DM mechanisms which ispotentially involved in making critical interactions with MAPK1 [51]. Therefore, it can be postulated that the presence of flavonoidsin the extract might be the reason of the antihyperglycemicaction shown by LG-M. However, the leaves contain loganinis an iridoidpossessinga protective effect against hepatic oxidative stress under type 2 diabetes through regulations of protein expressions related to oxidative stress, inflammation, and apoptosis [52].



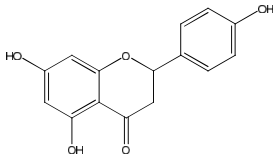
Apigenin



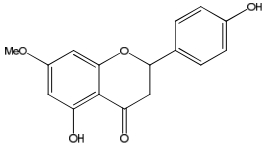
Luteolin



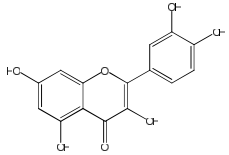
Luteolin 7-O-glucoside



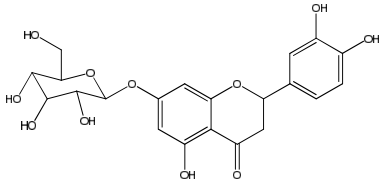
Quercetin



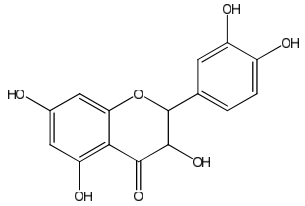
Naringenin



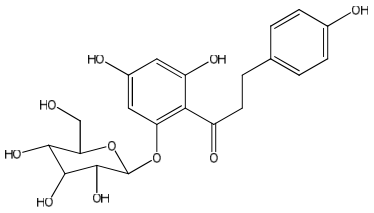
Sakuranetin



Eriodictyol 7-O-glucoside



Taxifolin



Phloridzin

Fig. 1. Structures of the flavonoids isolated from Mexican oregano [15]

5. CONCLUSIONS

In conclusion, our study showed that *Lippia graveolens* was effective in inhibiting the formation of AGEs. The antiglycation activities of *L. graveolens* were attributed in part to their antioxidant activity and its abilities to scavenge reactive carbonyls. Furthermore, LG-M alleviated oxidative stress under diabetic conditions through the inhibition of lipid peroxidation, prevent and/or delay the onset renal and hepatic damage. In addition improved glucose tolerance suggesting a decrease in insulin resistance and helping to maintain blood glucose levels steady which may indicate certain induction of peripheral utilization of glucose.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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