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SYNERGISTIC EFFECT OF EXTRACTS OF GINKGO BILOBA LEAF AND PANAX GINSENG ROOT ON CARBOHYDRATE AND LIPID METABOLISM GENE EXPRESSION IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The present study aimed to evaluate the anti-diabetic properties of *Ginkgo biloba* leaves extract (GBE) and *Panax ginseng* roots extract (PGE) in different combinations. A total of 40 rats were fed on high-fat-diet for 14-days, then divided into five groups (N=8). Non diabetic group (NDG), Diabetic-group (DG), Mixed-group-1 (MG1), Mixed-group-2 (MG2), Mixed-Group-3 (MG3). Alloxan monohydrate (120-130 mg/Kg BW) was used as a diabetogenic agent. The data of blood glucose and body weight (BW) were monitored regularly weekly. Basal blood was collected from the heart for biochemical analyses. Skeletal muscle, hepatic, and adipose tissue were obtained for mRNA expression of genes. A Significant decrease in BW was found in all treated groups. A significant reduction in fasting serum glucose, AST, ALT, and creatinine were recorded in dose dependent-manner. The treatments showed up-regulation of GLUT-4 in the muscle (all treated groups) and hepatic tissues (MG3); IR in the muscle (MG3) and adipose tissue (MG3), and IRS-1 in hepatic (MG3) and adipose tissue (MG3). Our results showed that these herbs improve dyslipidemia and have strong antioxidant activities. We found significant down-regulation for SREBP-1c in dose-dependent manner in the liver and significant up-regulation for FAS (MG2 & MG3) in the liver. Significant up-regulation was found for PPAR-α in muscles and PPAR-γ in adipose tissues in all treated groups. Significantly down-regulation for TNF-α seemed in all studied organs. In conclusion, GBE and PGE showed strong anti-diabetic, anti-hypercholesterolemia and anti-oxidative effects in combination by regulating the genes involved in carbohydrate and lipids metabolism.

Key words: Alloxan, Diabetes, *Ginkgo biloba* leaf extract, Metabolism, *Panax ginseng* root extract, Rats.

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INTRODUCTION

Diabetes mellitus (DM) becoming a leading health problem in the last few decades and characterized by chronic hyperglycemia; associated with metabolic disturbance of carbohydrate, protein and fats; caused due to deregulation in the mechanism of insulin actions and its secretion, or even both (Naseem *et al.,* 2016). Oxidative stress is a common problem in diabetes which may lead to neuropathy, retinopathy, nephropathy, cardiovascular disease and other major health complications (Krishnamurthy *et al.,* 2011). There is strong evidence to support the fact that hyperglycemia elevated reactive oxygen species (ROS) generation (Lin *et al.,* 2008; El-Karim *et al.,* 2017). Enhanced ROS results in an imbalance between free radicals generations and anti-oxidative defense systems of the body, thus leading to the development of diabetic-associated complications (Kumar, 2012). Interest in the plants derived anti-hyperglycemic agents are gradually increased in the last few years due to its less adverse effects (Lee *et al.,* 2010).

Ginkgo biloba (GB) is a well-known oldest tree species that still exists on the earth with certain health benefits (Naseem *et al.,* 2016). Terpenoids (6-7%) and flavonoids (34-26%) are the active ingredients of ginkgo (Smith and Luo, 2003). Ginkgo is a dioecious tree belongs to *Ginkgoaceae* family, and is traditional Chinese herbal medicines used to treat diabetes, cancer, bronchitis, sexual dysfunction, chilblains, aging, Alzheimer's disorder, cerebral, peripheral and neuronal diseases (Unger, 2013; Kamel and Abd-Elrhman, 2018).

Panax ginseng is a perennial herb having fleshy root, belongs to *Araliaceae* family. Ginsenosides is the active component of this herb (Kim *et al.,* 2014). Ginseng is another traditional Chinese herb has strong anti-inflammatory, anti-fatigue, anti-aging, anti-oxidant and anti-apoptotic properties. Also use to treat diabetes, Central nervous system disorders, cardiac stroke, weak immunity and sexual dysfunction (Amin *et al.,* 2011; Kim *et al.,* 2014; Vuksan *et al.,* 2019).

To the best of our knowledge, no published data is available on the anti-diabetic properties of GBE and PGE in combination. The objective of the study was to analyze the anti-diabetic activities of GBE and PGE the molecular mechanisms of carbohydrates and lipid metabolism in diabetic rats. Furthermore, the positive role of these two natural remedies on hyperlipidemia, hypercholesterolemia and oxidative stress related to diabetes was also assessed.

MATERIALS AND METHODS

Experimental animals: Forty adult Wistar male rats (150-200 g); with two rats per cage were confined in a controlled environment "24 ± 5 °C; 12 h light/dark cycle" with free water and food access in the animal facility of University of Veterinary and Animal Sciences, Lahore (UVAS), Pakistan, prior to the experiment. Body weight (BW) was recorded weekly during the study of 14 weeks. The experiment was performed according to guidance and recommendations of the institutional ethical committee for the care of Laboratory Animals (UVAS).

Experimental Design and Animal Grouping: Rats were fed on a high fat diet "HFD: 12.7% maize starch, 6% cellulose, 6.5% dextrose, 31% beef tallow, 28.6% casein, 3.9% sun flower oil, 9.7% minerals and 1.3% vitamins by weight" for 14 days, and randomly divided into following five groups (N=8):

- **Non diabetic group (NDG):** This group included non-diabetic rats (negative control), feed on the standard diet without herbal extract treatment.
- **Diabetic-group (DG):** This group included diabetic rats (positive control) and feed on the standard diet without herbal extract treatment.
- **Mixed-group-1 (MG1):** The diabetic rats were treated with the combination of GBE (50 mg/ kg/ day) and PGE (150 mg/ kg/ day) mixed in standard chow.
- **Mixed-group-2 (MG2):** The diabetic rats were treated with the combination of GBE (100 mg/ kg/ day) and PGE (300 mg/ kg/ day of PGE) mixed in standard chow.
- **Mixed-group-3 (MG3):** The diabetic rats were treated with the combination of GBE (150 mg /kg/ day) and PGE (450 mg/ kg/ day) mixed in standard chow.

After 14 days, alloxan monohydrate (Sigma, USA) dissolved in 0.5ml saline and injected intra-peritoneal as a single dose (120-130 mg/kg BW) in overnight fasting rats. Hypoglycemia was prevented by giving 20% (for 6h) and 5% (for next 24h) glucose solutions (Ebuehi *et al.,* 2010). After 72h from alloxan dosing, fasting blood glucose level was measured by tail puncture; rats having blood glucose level higher than 250 mg/dl were selected for the experiment (Cheng *et al.,* 2012; Naseem *et al.,* 2016). Supplementation of the standardized GBE (terpenoids 6-7% flavonoids 24-27%) and PGE (ginsenosides 4%) (Hunan Nutramax Inc, China) was started. Dosing was done by mixing both herbal extracts in different combinations in the animal diet for 14-weeks.

Blood collection and Serum biochemical analysis: After 14 weeks rats were kept in fasting state for 24 hours and then blood samples were taken directly from heart puncture. Serum was separated from blood by the method described by (Kumar, 2012) and kept at -20˚C for further analysis. The biochemical analyses of fasting serum glucose (FSG), malondialdehyde (MDA), catalase (CAT), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and creatinine were performed by commercially available kits (Randox, UK). Total serum cholesterol (TC) and triglycerides (TG) were done by enzymatic kits (Bio-Merieux, France). Fast Protein Liquid Chromatography (FPLC USA) was used for lipo-protein profile (VLDL-c, LDL-c, HDL-c).

Skeletal muscle, hepatic and adipose tissues collection and their mRNA expression of genes determination: Rats were killed skeletal muscle, adipose and hepatic tissues were collected, washed with normal saline and kept at -80°C.The total RNA extraction from the frozen tissues was done by using trizol reagent (Ambion, USA) as per manufacturer's instructions. The quantification of total RNA was done by nanodrop spectrophotometer (absorbance at 260nm). Thereafter, about 1µg of total RNA was reverse-transcribed into complementary DNA (cDNA) using Super-Script-III reverse Transcriptase (Invitrogen, France) in a 20 µl reaction volume. The denaturation step was carried out for 5 min at 70°C, followed by an elongation for 45 min at 55°C. Quantitative PCR was performed on a MyiQ2 Real-Time-PCR (Bio Rad, Marnes-la-coquette, France) using SYBR Green (Bio Rad) supermix according to the manufacturer's instructions. The PCR was carried out for 45 cycles of 95 °C for 30 s and 60 °C for 30 s and 72°C for 30 s. The fluorescence was read during the reaction, allowing continuous monitoring of amount of PCR product. Primers were designed through primer3 website. The GADPH was used as a house-keeping gene. The $2^{\Lambda}\Delta\Delta CT$ method was used for relative quantification. Primers used for qRT-PCR are given in Table 1.

Statistical analysis: Results represented as mean ± SEM. We used one way ANOVA followed by PLSD Fisher's test (Statview software; SAS Institute Inc, USA) to measure physiological and biochemical parameters while the data of mRNA genes expression were analyzed by Kruskal Wallis test, followed by Fisher's test. The $p<0.05$ was considered as significant.

RESULTS AND DISCUSSION

The prevalence of diabetes increasing globally at an alarming rate is attributed to a mordent lifestyle, high cholesterol diet and lack of physical activities. Pharmacological drugs for the treatment of diabetes cause a high risk of secondary health issues (Xie *et al.,* 2002). Thus due to this risk, the therapeutic use of herbal remedies has been significantly increased worldwide. Since *G. biloba* and *P. ginseng* are widely used for the treatment of many disorders including diabetes, but we used both these natural remedies in combination. In the present study, we evaluated the synergistic effects of these two herbs on the metabolic disturbance associated with alloxan induced diabetic rat model. To the best of our knowledge, no data is available as an anti-diabetic effect of GBE and PGE in combination, so we discussed all the parameters separately for GBE and PGE throughout the text.

Body weight: The BW was measured weekly; however, data of the 1st and 14th week is presented here (Table 2). Significant reduction ($p<0.0001$) in BW from week-1 to week-14 was found in diabetic rats, however, all treated groups showed suppression in BW reduction. The results for alloxan-induced diabetic rats are according to other authors (Lee *et al.,* 2012; Cheng *et al.,* 2012; Naseem *et al.,* 2016). In diabetes, decrease in BW might be due to improper protein, lipids or glucose metabolisms (Baynes *et al.,* 1991), tissue proteins degradation (Swanston-Flatt *et al.,* 1990), adipocytes degeneration, excessive conversion of glycogen to glucose by muscle and frequent micturition (Ramadan *et al.,* 2009).

Blood glucose concentration and mRNA gene expression of carbohydrate metabolism: Impaired glucose metabolism is a characteristic feature of diabetes. We evaluated that GBE and PGE contain hypoglycemic properties, since, all treated groups in this study indicated a significant ($p<0.0001$) decrease in blood glucose level (Table 2). Furthermore, fasting serum glucose (FSG) levels were also measured at the end of the trial. Significant ($p<0.0001$) increase in FSG concentration was noted for DG however; significant ($p<0.0001$) reduction was found in all the treated groups (Table 3). Previously, many researchers studied the anti-hyperglycemic effects of GBE and PGE separately. Impaired glucose tolerance is a characteristic feature of diabetes. Untreated prolonged hyperglycemia may cause β-cells toxicity which leads to enhanced blood glycation thus damages various body tissues/organs (Alam *et al.,* 2014).

To know the underlying mechanism, how GBE and PGE reduce blood glucose concentration we also performed mRNA gene expression involved in carbohydrate metabolism (GLUT-4, IR and IRS-1). In muscles, significant up-regulation was observed for GLUT-4 ($p<0.0001$) in all treated groups (Figure 1) and MG3 ($p<0.05$) in the liver (Figure 2), which previously found to be down-regulated in DG. No significant results were found for GLUT-4 in adipose tissues (Figure 3). Results for ginkgo (Naseem *et al.,* 2016) and ginseng (Jeon *et al.,* 2013; Cheon *et al.,* 2015) are in agreement with others. Glucose utilization by skeletal muscles is attributed to GLUT-4 which itself regulated by blood insulin concentration (Leto and Saltiel, 2012). The primary storage site for both glycogen and glucose in skeletal muscles and also oxidized these molecules to release an adequate amount of energy. Glucose metabolism is regulated by removing excess of glucose from the circulatory blood via various glucose transport channels/mechanisms like GLUT-4 (Huang and Czech, 2007).

We found significant up-regulation for IR in MG3 both in skeletal muscles ($p<0.0001$) (Figure 1) and adipose tissues ($p<0.05$) (Figure 3), however, no significant data was found for hepatic tissue (Figure 2). Insulin is the primary factor to regulate glucose uptake by body tissues via a complex cascade signaling pathway. Insulin binds with IR to carry out its various cellular activities to regulate glucose metabolism. Thus any impairment in IR might lead to the disturbance of insulin efficacy and develops insulin resistance (Calle *et al.,* 2008). For IRS-1 significant down regulation was found in the diabetic group. However, significant up-regulation was noted in MG3 both for the liver ($p<0.05$) (Figure 2) and adipose tissues ($p<0.0001$) (Figure 3). IRS-1 plays a vital role in transduction intracellular insulin signals. Thus, if intracellular insulin concentration is enhanced IRS-1 can up-regulate its expression (Zhou *et al.,* 2011). Since hepatic insulin resistance is a key factor for diabetes development. Hepatic insulin resistance linked with the down-regulation of IRS-1 and IRS-2 (Taniguchi *et al.,* 2005). Thus regulation of GLUT-4, IR and IRS-1 in our study indicated that GBE and PGE help to improve insulin cell sensitivity and thus regulate glucose utilization by tissues/muscles.

Serum lipid Profile and mRNA gene expression of lipid/fat metabolism: Dyslipidemia and hyper-triglyceridemia are important factors of cardiovascular disorders in diabetes. In diabetes increased lipolysis, dysfunction in cholesterol packing and elevated plasma free fatty acid level is the primary reasons for hyper-cholesterolemia and hyper-triglyceridemia which leads to the development of cardiovascular disorders (Cho *et al.,* 2006).

The results showed that these herbs in combination improve dyslipidemia and hyper-triglyceridemia. A significant decrease for VLDL-c was found in MG1 ($p<0.05$), ($p<0.0001$) MG2 and ($p<0.0001$) MG3, a significant ($p<0.0001$) decrease for LDL-c serum level was also recorded in all treated groups. However,

for HDL-c significant increase was recorded in MG1 ($p<0.001$), both for MG2 and MG3 ($p<0.0001$). A significant decrease for TG was recorded in all the three treated groups, however, we found a significant reduction for TC only in MG3 ($p<0.001$) (Table 3). Other researchers also found a significant increase in TC and TG blood levels in alloxan-induced diabetic rats (Ebuehi *et al.,* 2010; Naseem *et al.,* 2016).

We also try to evaluate how GBE and PGE regulate dyslipidemia in association with lipid metabolic genes (SREBP-1c, FAS, PPAR-α, and PPAR-γ). SREBP -1c was done only in hepatic tissues and we observed down-regulation in MG2 ($p<0.05$) and MG3 ($p<0.001$), which showed up regulation in diabetic rats (Figure 2). The liver is the main organ for fat and carbohydrate metabolism. Insulin regulates SREBP-1c; a lipid metabolic gene (Yuan *et al.,* 2011). SREPB-1c regulates several genes that are involved in free fatty acid, phospholipids, TG and TC synthesis (Horton *et al.,* 2002). The findings for ginkgo (Zhou *et al.,* 2011) and ginseng (Lee *et al.,* 2010) are in agreement with other researchers. In hepatic tissues, significant up-regulation was noted for FAS in MG2 and MG3 ($p<0.05$), which previously showed down-regulation in DG (Figure 2), we observed no significant change for FAS in any of the treated group in adipose tissues (Figure 3). In liver; FAS catalyzed the biosynthesis of fatty acid (Menendez *et al.,* 2009). However, diabetes markedly suppressed its expression in the liver (Naseem *et al.,* 2016), as also found in our study.

In muscles, PPAR-α was found to be down-regulated in diabetic condition and we measured significant up-regulated ($p<0.001$) of this gene for all treated groups (Figure 1). No significant change was found for PPAR-α in hepatic tissues (Figure 2). PPAR-α regulates genes involved in lipoproteins and lipids metabolism binding with PPAR response elements. The PPAR-α along with apo AI and AII enhanced the HDL blood concentration. Thus dysfunctioning in the expression of PPAR-α distorts the fatty acid and lipoprotein metabolisms; in turn leads to the abnormal levels of blood TC and TG (Yoon *et al.,* 2003).

The PPAR-γ was done only in adipose tissues and we found significant up-regulation in MG1 and MG2 ($p<0.001$) and MG3 ($p<0.0001$), which down-regulated in DG (Figure 3). PPAR-γ is widely distributed in the adipocytes to help to enhance insulin sensitivity (Kahn *et al.,* 2010) and have a vital function in reducing fatty acid blood concentration; thus regulate lipid metabolism (Lee *et al.,* 2010). This study showed that GBE and PGE in combination have synergistic effects to improve dyslipidemia and hyper-cholesterolemia by regulating the expression of SREBP-1c and FAS (liver), PPAR-α (muscle) and PPAR-γ (adipose tissues) of diabetic rats.

Determination of anti-oxidative activities: A significant increase for MDA ($p<0.0001$) and a significant decrease for CAT ($p<0.0001$) serum concentrations were found in diabetic rats. However, we recorded a significant decrease for MDA both in MG1, MG2 ($p<0.001$), and MG3 ($p<0.0001$) and a significant increase for serum CAT ($p<0.05$) in all the three treated groups (Table 3). Scientific evidence showed that oxidative stress caused chronic hyper-glycemia in diabetes thus reducing anti-oxidant defense properties and if remain untreated might promote the generation of free radicals and cause various health problems (Jung *et al.,* 2005). Our data showed that GBE and PGE overcome oxidative stress and have strong anti-oxidative properties. The anti-oxidative activities of ginkgo are attributed to flavonoids (Maltas and Yaldiz, 2012) and that of ginseng is ginsenosides (Murphy and Lee, 2002).

Down-regulation of TNF- α by GBE and PGE: Down regulation of MG2 ($p< 0.001$) and MG3 ($p<0.0001$) in muscles (Figure 1), of MG3 ($p<0.05$) in hepatic tissues (Figure 2) and down-regulation ($p<0.0001$) in all treated groups in adipose tissues (Figure 3) was observed, which previously found to be up-regulated in diabetic rats. Results are consistent with (Naseem *et al.,* 2016) for ginkgo and (Jeon *et al.,* 2013) for ginseng on mRNA expression of TNF- α. TNF-α; plays a vital role in the inflammatory response. In obesity; the up-regulation of adipocyte TNF-α increases the chance of diabetes by increasing β-cells apoptosis (Badawi *et al.,* 2010).

Determination of Biochemical Parameters: Serum AST, ALT, and Creatinine concentrations are given in Table 3. A significant decrease for serum AST was found in MG2 ($p<0.05$), and MG3 ($p<0.0001$), whereas, we recorded a significant reduction for ALT only for MG3 ($p<0.05$). Furthermore, a significant decrease was found for MG1 ($p<0.05$) and MG3 ($p<0.05$) for serum creatinine levels. The stability of AST, ALT, and creatinine showed protecting the effect of GBE and PGE against toxic properties of alloxan.

Liver enzymes (AST and ALT) are important bio-marker to identify liver diseases; and literature supports the arguments that these said enzymes are linked to the development of diabetic complications (Kunutsor *et al.,* 2013). High creatinine concentration in blood is an indicator of renal dysfunction (Salih, 2012).

Table 1. Genes and the primer sequences used in this study.

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
GAPDH	"TCCCATTCTTCCACCTTTGATGCT"	"ACCCTGTTGCTGTAGCCATATTCAT"
GLUT4	"GCTTCTGTTGCCCTTCTGTC"	"TGGACGCTCTCTTTCCAACT"
IR	"GTGCTGCTCATGTCCTAAGA"	"AATGGTCTGTGCTCTTCGTG"
IRS-1	"GCCAATCTTCATCCAGTTGC"	"CATCGTGAAGAAGGCATAGG"
SREBP-1c	"GGCATGAAACCTGAAGTGGT"	"TGGGCTTTCACCTGGTTATC"
FAS	"CGCCGTGGTGCTGGAGATTG"	"CTTGCCGAGGTTGGTGAGGAAG"
PPAR-α	"GAGACCCTCGGGGATCTTAG"	"CGTCTTGTGTCCTGAGCTTG"
PPAR-γ	"CTGACCCAATGGTTGCTGATTAC"	"GGACGCAGGCTCTACTTTGATC"
TNF-α	"GCAGAGCCTTCCAAGCCTACC"	"GTTACCCAGCCCACCTCCTTTG"

Abbreviations: Glyceraldehyde 3 Phosphate Dehydrogenase (GAPDH), Glucose Transporter- 4 (GLUT-4), Insulin Receptor (IR), Insulin Reseptor Substrate-1 (IRS-1), Sterol Regulatory Element Binding Protein-1c (SREBP-1c), Fatty Acid Synthase (FAS), Peroxisome Proliferator-Activated Receptor- α (PPAR- α), Proliferator-Activated Receptor- γ (PPAR- γ), Tumor Necrosis Factor- α (TNF- α)

Table 2. Synergistic effects of *Ginkgo biloba* leaf extract (GBE) and *Panax ginseng* root extract (PGE) on body weight and blood glucose concentration at week-1 and week-14 in alloxan induced diabetic rats (N=8)..

Parameters	NDG		DG		MG 1		MG 2		MG 3	
	Wk-1	Wk- 14	Wk-1	Wk-14	Wk-1	Wk-14	Wk-1	Wk-14	Wk-1	Wk-14
Body weight (g)	165.36 ±1.51	206.20 ±2.36^{a}	165.93 ±1.14	159.08 ±2.53^{c}	165.98 ±1.37	159.08 ±2.53^{b}	166.91 ±1.53	163.93 ±3.14^{b}	166.76 ±1.16	160.08 ±1.94^{b}
Blood Glucose (mg/dl)	83.43 ±1.28^{b}	83.68 ±1.35^{c}	417.07 ±25.43^{a}	485.58 ±25.52^{a}	412.81 ±15.28^{a}	178.05 ±1.63^{b}	404.96 ±7.75^{a}	178.87 ±1.22^{b}	388.86 ±7.05^{a}	151.37 ±3.05^{b}

Data represented as Mean ± SEM. Superscripts^{a-e} represent significant variation between groups in rows ($p<0.05$).

Table 3. Synergistic effects of GBE and PGE on Bio-chemical and lipid profile fasting in alloxan induced diabetic rats (N=8).

Parameters	NDG	DG	MG1	MG2	MG3
FSG (mg/dl)	83.68±1.35^{c}	485.58±25.52^{a}	178.05±1.63^{b}	178.87±1.22^{b}	162.55±0.91^{b}
TC (g/L)	0.82±0.01^{c}	1.34±0.01^{a}	1.29±0.01^{ab}	1.32±0.01^{a}	1.25±0.02^{b}
VLDL-c (g/L)	0.07±0.005^{e}	0.28±0.005^{a}	0.26±0.01^{b}	0.22±0.002^{c}	0.18±0.003^{d}
LDL-c (g/L)	0.06±0.001^{d}	0.55±0.01^{a}	0.43±0.004^{b}	0.43±0.005^{b}	0.30±0.005^{c}
HDL-c (g/L)	0.68±0.01^{b}	0.49±0.009^{e}	0.54±0.01^{d}	0.647±0.007^{c}	0.76±0.01^{a}
TG (g/L)	1.25±0.007^{c}	2.12±0.02^{a}	1.38±0.03^{b}	1.28±0.01^{c}	1.23±0.01^{c}
CAT (KU/L)	20.83±0.26^{a}	18.60±0.65^{b}	19.55±0.13^{b}	19.50±0.12^{b}	19.49±0.16^{b}
MDA (mmol/L)	6.54±0.23^{b}	7.55±0.28^{a}	6.35±0.21^{b}	6.34±0.11^{b}	6.32±0.11^{b}
AST (µ/L)	76.88±1.43^{c}	210.06±3.08^{a}	199.66±0.36^{ab}	195.28±1.15^{ab}	194.3±12.23^{b}
ALT (µ/L)	36.23±0.72^{c}	40.9±1.68^{a}	39.48±0.64^{ab}	39.96±0.47^{ab}	37.58±0.61^{bc}
Creatinine (mg/dl)	1.63±0.12^{b}	2.01±0.03^{a}	1.75±0.06^{b}	1.82±0.04^{ab}	1.7±0.03^{b}

Data represented as Mean ± SEM. Superscripts^{a-e} represent significant variation between groups in rows ($p<0.05$)
Abbreviations: FSG (Fasting Serum Glucose), TC (Total Cholesterol), VLDL-C (Very Low Density Lipoprotein-Cholesterol), LDL-C (Low Density Lipoprotein-Cholesterol), HDL-C (High Density Lipoprotein-Cholesterol), TG (Total Triglyceride), CAT (Catalase), MDA (Malondialdehyde), AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase)

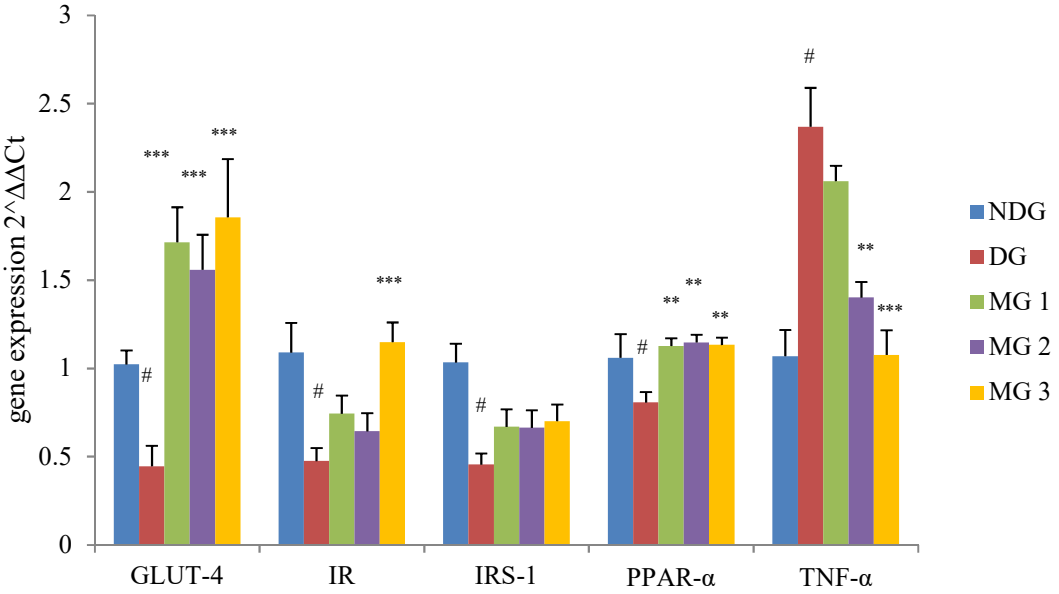

Figure 1. Synergistic effects of GBE and PGE on mRNA expression of GLUT-4, IR, IRS-1, PPAR-α and TNF-α genes in skeletal muscles of non-diabetic (NDG), diabetic (DG), mixed group-1 (MG1), mixed group-2 (MG2) and mixed group-3 (MG3) in alloxan-induced diabetic rats (N=8). Data represented as Mean ± SEM. #indicates comparison among DG and NDG, *indicates comparison among treatment groups and DG. * stands for P<0.05, ** P<0.001 and ***P<0.0001.

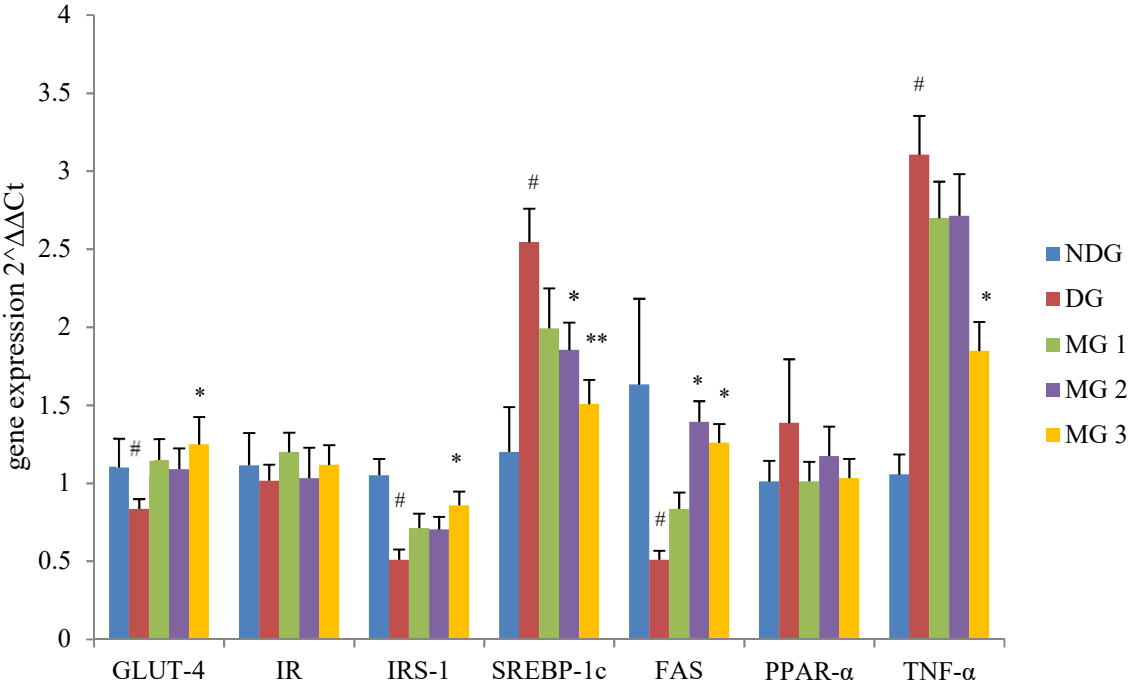

Figure 2. Synergistic effects of GBE and PGE on mRNA expression of GLUT-4, IR, IRS-1, SREBP-1c, FAS, PPAR-α and TNF-α genes in hepatic tissues of non-diabetic (NDG), diabetic (DG), mixed group-1 (MG1), mixed group-2 (MG2) and mixed group-3 (MG3) in alloxan-induced diabetic rats (N=8). Data represented as Mean ± SEM. #indicates comparison among DG and NDG, *indicates comparison among treatment groups and DG. * stands for p<0.05 and ** p<0.001

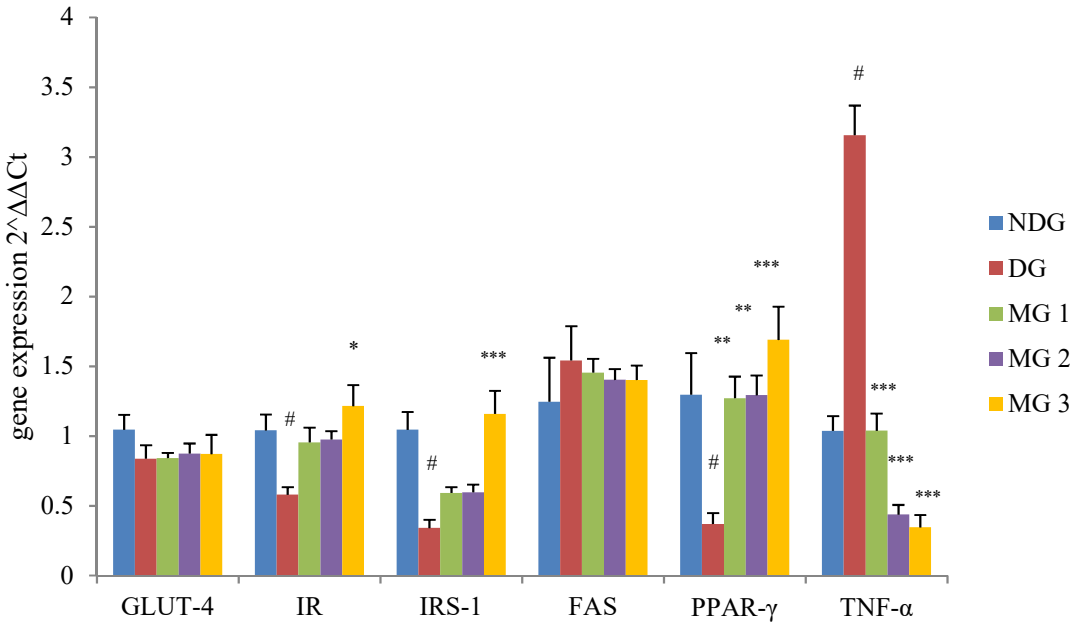

Figure 3. Synergistic effects of GBE and PGE on mRNA expression of GLUT-4, IR, IRS-1, FAS, PPAR-γ and TNF-α genes in adipose tissues of non-diabetic (NDG), diabetic (DG), mixed group-1 (MG1), mixed group-2 (MG2) and mixed group-3 (MG3) in alloxan-induced diabetic rats (N=8). Data represented as Mean ± SEM. #indicates comparison among DG and NDG, *indicates comparison among treatment groups and DG. *stands for p<0.05, ** p<0.001 and ***p<0.0001.

Conclusion: In conclusion, we found, that GBE and PGE in combination showed synergistic effects depending upon doses. The combination of both herbal extracts showed effective anti-hyperglycemic effects by up-regulated GLUT-4 and IRS-1 in hepatic tissues; GLUT-4 and IR in skeletal muscle; IR and IRS-1 in adipose tissue, anti- hypercholesterolemia by up-regulating the expression of FAS in hepatic tissues, anti-hypertriglyceridemic by up-regulation SREPBP-1c in hepatic tissues; PPAR-α in skeletal muscle and PPAR-γ in adipose tissue, anti-oxidative by decreasing MDA and increasing CAT serum level and in addition to these effects down-regulated the expression of TNF-α in studied organs in alloxan-induced diabetic rats.

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