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Anti Hyperglycemic and Anti Hyperlipidemic Activity of *Linum usitatissimum* and *Glycyrrhiza glabra* Extracts in Streptozotocin-Induced Diabetic Rats

Jabbar Ahmed Qureshi[1*], Zahida Memon[1], Kausar Moin Mirza[1], Shanza Agha[2], Fizza Saher[3] and Nisa Fatima Sunderjee[4]

[1]Department of Pharmacology, Ziauddin Medical College, Ziauddin University, Karachi, Pakistan.
[2]Department of Biochemistry, Liauqat National University and Hospital, Karachi, Pakistan.
[3]Department of Oral Biology, Ziauddin Dental College, Ziauddin University, Karachi, Pakistan.
[4]Ziauddin Medical College, Ziauddin University, Karachi, Pakistan.

Authors' contributions

This work was carried out in collaboration between all authors. Author JAQ designed the study, performed the statistical analysis, author ZM wrote the protocol, author KMM wrote the first draft of the manuscript. Authors SA and FS managed the analyses of the study and author NFS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The purpose of the present experimental study was to assess the antihyperglycemic activity of ethanolic extract of *Linum usitatissimum* seeds and *Glycyrrhiza glabra* roots with standard drugs metformin and glimepiride in streptozotocin-induced diabetic rats model.
Materials and Methods: Total 42 Wistar albino rats were utilized in this study which separated into seven groups with six animals in each group. Negative controls for normal base line reading and was not induced with diabetes were administered 0.9% sodium chloride (NaCl). and positive control i.e. in which diabetes was induced with streptozotocin were administered 0.9% sodium chloride

**Corresponding author: E-mail: drjabbar.qureshi@hotmail.com;*

(NaCl). Other three groups were given metformin 10 mg/kg body weight (bw), glimepride 0.1 mg/kg bw and rosuvastatin 10 mg/kg/day bw as standard treatment , whereas in last two groups, ethanolic extract of *L. usitatissimum* seeds and *G. glabra* roots in combinations of 200 mg/kg and 400mg/kg were given respectively as experimental drugs. These drugs were given to all groups except negative control for 28 days after induction of diabetes with streptozotcin. At the end of the study, FBS, lipid concentration, insulin, HbA1c and serum amylase levels were evaluated.

Results: Both doses of *L. usitatissimum* and *G. glabra* extracts showed significant (p<0.001) decrease in FBS of diabetic rats. Especially *L. usitatissimum* and *G. glabra* in the combination dose of 400 mg/kg b.w showed more potency in decreasing blood glucose levels in comparison with anti-diabetic drug glimepride at the end of the experiment. All extracts showed a noteworthy decrease in total cholesterol, triglycerides, LDL-C, VLDL-C, HbA1c, insulin and serum amylase levels with improved HDL-C levels in diabetic rats compared with positive control.

Conclusions: This study indicates that the combination of both *L. usitatissimum* and *G. glabra* extracts have antihyperglycemic and anti hyperlipidemic effects in diabetic rats which might be useful for the search of dietary supplements inefficient management of Type-2 diabetes mellitus.

Keywords: Diabetes mellitus; hyperglycemia; hyperlipidemia; flax seeds; licorice.

1. INTRODUCTION

Diabetes mellitus is a metabolic disease, described by hyperglycemia along with weakened metabolism of carbohydrate and other vitality yielding fuels, for example, lipids and proteins" [1]. Recently, the International Diabetes Federation (IDF) reported that around 415 million was having diabetes mellitus in 2015 and this figure is assumed to rise to 642 million by 2040 throughout the world [2]. This alarming illness is mainly of two types, i.e. type1 which is caused by insulin deficiency while type II mainly attributed to insulin resistance [3]. Along with other co-morbidities, dyslipidemia is also associated with poorly controlled diabetes mellitus which can lead to multiple micro and macro vasculopathies including coronary heart disease and stroke that in turn are the real reasons for dreariness and mortality in diabetic patients. Moreover, enhance lipid per oxidation associated with this fearsome illness, leads to increased oxidative stress which sequentially responsible for retinopathy, neuropathy and nephropathy in long-term uncontrolled diabetes [1].

The oral treatment regimen for diabetes mellitus is classified into insulin sensitizers, insulin secretagogues and miscellaneous which includes sulfonylureas, biguanides, α-glucosidase inhibitors, Dipeptidyl peptidase 4 inhibitor and glinides [4]. These agents are generally used in combination with each other to get better effects. The management and control of diabetes by these synthetic drugs with no symptoms is a great challenge because mostly all these oral anti hyperglycemic medications have various genuine antagonistic impacts and distressing complications with the development of resistance on enduring exploitation [5,6].

Furthermore conventionally used oral hypoglycemic drugs have no effects on hyperlipidemia which is the main consequence of poorly controlled diabetes. Herbal drugs remain the focus of attraction not only in ancient times but still today a large number of entire world's population is solely dependent on herbal medications for the treatment of various diseases [7]. According to historical literature, there are about 45000 plants which possess different medicinal properties including antihyperglycemic activity, *Linum usitatissimum* and *Glycyrrhiza glabra* are two very regularly used herbs both for culinary and ayurvedic purposes in South Asia since a long time [8-11].

Linum usitatissimum commonly known as Flax seeds or Alsi is one of the commonly used items in Asian kitchen since centuries, but in the last two decades, it has been focused by the researchers due to its various biologically active components [12] which exert potential health benefits. It has multiple advantages, which makes it very popular in modern alternative medicine like it exerts anti-inflammatory and antioxidant activity, which help in decreasing many cardiovascular ailments, decrease the risk of prostate, colon and breast cancer, gainful in creating positive diuretic impact and lessening of post-menopausal symptoms and osteoporotic movement in women [13]. *L. usitatissimum* is rich in omega 3 fatty acid i.e. alpha-linolenic acid [14] and lignans (phytoestrogens) [12]. Extended use of *L. usitatissimum* has also been reported to decrease the likelihood of obesity-related

diseased conditions. It increases the duration of gastric emptying and thus immersion of useful nutrients from the lesser bowel [12].

G. glabra is the root of *Glycyrrhiza inflate* Batalin (Fabaceae), commonly known as sweet wood, licorice or mulathi. According to current literature, *G. glabra* is medically used for multiple purposes such as antioxidant, antidote for peptic ulcers and gastritis and prevention or remedy of common cold [15] Furthermore, It has potent antitussive activity, muscle relieving property, weight reduction potential, immune boosting action via increasing WBC counts and anti-diuretic and anti-inflammatory effects [16]. The naturally dynamic segments of *G. glabra* are liquiritins, liquiritigenin, glycyrrhizic acids and flavones. Glycyrrhizin is the major saponin in licorice root, and glycyrrhetinic acid is its predominant metabolite and a pharmacologically active form of glycyrrhizin [17]. Together these flavonoids confirm significant anticancer, antioxidative, antimicrobial, and antiviral effects [18]. As indicated by Chen et al. in 2014, licorice can decrease liver injury by enhancing antioxidant and anti-inflammatory capacity which makes it a potent hepatoprotective agent [19]. *G. glabra* has demonstrated evident hair development, growth and it tends to be securely utilised in herbal definitions in the treatment of different kinds of Alopecia [20].

Keeping in view the multiple beneficial effects of these herbs, the present study aims to assess the antihyperglycemic activity of ethanolic extract of *L. usitatissimum* seeds and *G. glabra* root combination and their effects on body weight and lipid profile of diabetic rats model.

2. MATERIALS AND METHODS

The study was conducted from May 2018 till September 2018.

2.1 Collection of Plant Material and Preparation of Ethanolic Extract

L. usitatissimum and *G. glabra* root were obtained from the local market of Karachi. The plants were authenticated and identified from the botany department of Karachi University, and taxonomy number was obtained. (Taxonomic number of *Glycyrrhiza glabra* is: 17234 and Taxonomic number of *Linum usitatissimum* is 29226).

The seeds of *L. usitatissimum* and *G. glabra* roots were washed and dried separately in open air for 48 hours. The seeds and roots were then minced into powder using a mechanical grinder. The powder was mixed and macerated with absolute ethanol at a 1:10 ratio (100 gram in 1 L solvent) for 7 days in separate jars. The mixtures were filtered through a Whatman No 1 filter paper followed by rotary evaporation of fíltrate with the help of rotary evaporator for the removal of ethanol after which concentrated extracts of both herbs were obtained. The crude extracts were reconstituted in freshly prepared 2.5% dimethyl sulfoxide DMSO and kept in separate jars for evaluation of antihyperglycemic and antihyperlipidemic property in diabetic rats.

2.2 Experimental Animals

42 adult male and female Wistar albino rats (aged 7-8 weeks, weighing 180–240 grams) were acquired from the Animal house of Aga Khan University. Animals were held under standard laboratory conditions (25 ± 3ºC, 12 h light/dark cycle) They had free access to standard diet and clean tap water for 30 days of the experimental period [21]. The study was conducted at the animal house of Jinnah postgraduate medical centre (JPMC).

2.3 Induction of Diabetes in Rats

With the exception of the negative control group, diabetes was induced to all animals by injecting a freshly arranged solution of Streptozotocin (STZ) by dissolving dry powder of STZ in 0.1 M citrate buffer of pH 4.5. and was used after filtration [22]. It was injected via intraperitoneal route (i.p) at a dose of 55 mg/kg, as a single dose to overnight fastening rats. On 3rd day 10 ml of blood was taken from tail for FBS. The rats who developed hyperglycemia (i.e., blood glucose concentration >250mgdL−1) was selected for the subsequent experiment and considered as diabetic rats [23]. Herbal extract and standard treatment were given to all the rats except positive control through metallic feeding syringe orally for 28 days in the following manner:

2.4 Grouping of Animals

Total 42 animals divided into 6 groups: **Group-I** negative control non-diabetic rats were treated with 0.9%sodium chloride (NaCl) **Group II** positive control diabetic rats were treated with 0.9% NaCl. **Group-III** diabetic rats were treated with glimepride at 0.1 mg/kg bw. **Group-IV** diabetic rats were treated with metformin at 10 mg/kg bw. **Group V** diabetic rats were treated

with rosuvastatin 10 mg/kg/day bw. **Group- VI** diabetic rats were treated with Ethanolic Extract of a combination of *L. usitatissimum* and *G. glabra* at a dose of 200 mg/kg. **Group- VII** diabetic rats were treated with Ethanolic Extract of a combination of *L. usitatissimum* and *G. glabra* at a dose of 400 mg/kg for both extract.

2.5 Physical and Biochemical Parameters Estimation

Toward the beginning and end of the treatment, the body weight of the rats was recorded with the help of electronic balance [24]. After completion of treatment course, on 29th day, following overnight food deprivation, rats were deeply anesthetized by ether exposure and all the animals were sacrificed as per *Institutional Animal Ethics Committee* (***IAEC***) guidelines.10 ml of blood samples were collected by cardiac puncture and was transferred into vacuum tubes which were then centrifuged at 3000 rpm for 10 minutes. After centrifugation, sera were separated for different biochemical assays [25].

2.6 Biochemical Assays

Blood Glucose level, serum insulin, HbA1c level, serum amylase levels, lipid profile (Total Cholesterol , HDL, VLDL, LDL) were estimated in serum samples by standard enzymatic methods using commercially available kits (Bartham, Trinder, Richmond and Schettler) according to manufacturer advice respectively.

2.7 Data Analysis

The data were analysed using SPSS software 20. The fasting blood glucose and lipid levels were expressed as mean ± Standard Error of Mean (SEM). The mean and SEM of the treatment groups were generated by use of the analysis of variance (ANOVA) test. The significant difference between and within the treatment groups was considered significant at set p value < 0.05.

3. RESULTS AND DISCUSSION

3.1 Body Weight of Control and Diabetic Rats Treated with *L. usitatissimum* and *G. glabra* Extracts

Table 1 shows that mean body weight of all treated groups before and after the intervention. The weight gain in *L. usitatissimum* and *G. glabra* 400 mg/kg b.w. group (277.78 ± 17.86) is more or less equal to the negative control group (275.43 ± 20.3) on the 29th day. In one previous prospective study, 26 human volunteers were included who were given 60g of flaxseed powder or rice powder as a dietary supplement for 42 days. The results of this study showed significant weight loss and reduction of waist circumference in flax seed, i.e. *L. usitatissimum* group [26] which is in concordant with our results. The observed weight loss is may be due to the presence of omega−3 and α-linolenic acid present in *L. usitatissimum* extract. [27] and may be due to the presence of glabridin, a phytochemical component in *G. glabra* extract [28]. Moreover, Flavonoids present in *G. glabra* can alone hold back central abdominal fat growth in fatty hyperglycemic mice [29].

According to a study, *L. usitatissimum* along with quercetin significantly reduced adiponectin and leptons levels, the proteins mainly associated with obesity and metabolic syndrome in rats with high fructose diet [30].

3.2 Effects of *L. usitatissimum* and *G. glabra* Extracts on Fasting Blood Glucose Levels

First of all, before inducing diabetes we took a baseline fasting blood sample of all the rats to make sure that there is no difference in the blood glucose level between the rats. After 3 days of inducing diabetes, the FBS values raised significantly as seen in table 2 and compared with other studies [31]. The optimal blood sugar level must be below 140 mg/dl, and this sugar level was achieved more or less in all groups after giving standard drugs and the combination of herbs for 29 days [32] with significant p-value (p<0.001). Only *L. usitatissimum* and *G. glabra* at the dose of 400 mg/kg b.w showed comparable effect with glimepride (0.1 mg/kg b.w.) which is probably the optimum dose, though *L. usitatissimum* and *G. glabra* at the dose of 200 mg/kg b.w. also significantly (p<0.001) decreased FBS. In previous studies the role of *L. usitatissimum* and *G. glabra* has been documented individually [33,30] but this is the first study which demonstrates the combined effects of both these herbs. The underlying mechanism for reducing hyperglycemia may be due to the presence of lignan containing omega-3 and Secoisolariciresionol diglucoside (SDG) in *L. usitatissimum* is the underlying reason for reducing hyperglycemia [34]. Their effects are exerted by suppressing the expression of the phosphoenolpyruvate carboxykinase. This gene codes for an integral enzyme involved in the

process of glucose synthesis in the liver (13) In addition *G. glabra non*-hydrophilic flavonoids can reduce abdominal fat as well to exert their anti-hyperglycemic effects. This is carried out by the activation of peroxisome proliferator-activated receptor-γ (PPAR-γ). [35]. As per the study carried out by Kelley et al. when rats received dietary addition of 0.5% conjugated linoleic acid along with 0.5% flax oil for 4 weeks, blood sugar levels were seen to reduce by 20% [36] Moreover *L. usitatissimum* is a innate alpha-amylase α-amylase and α- glucosidase inhibitors in contrast to acarbose which is second line synthetic euglycemic agent used for the treatment of type II diabetes. The involved mechanism is the reduction of glucose discharge from polysaccharides taken in diet, owing to which these agents practically decrease postprandial hyperglycemia mellitus and associated obesity. So our combination herbs possess both innate alpha-amylase inhibiting property along with natural PPAR-gamma inhibitory potential and modify two different pathways of glucose metabolism which otherwise will be provided by two different classes of OHG agents.

The glucose-lowering potential of these herbs is moreover confirmed by obvious reduction of HbA1c levels which reached to the height in positive control group i.e. 14.78 ± 1.69 as compared to the negative control rats with a value of 4.60 ± .44. However, the HbA1c levels of the *L. usitatissimum* and *G. glabra* 400 group were significantly decreased and were comparable to the glimepride group. In a randomized controlled study done by Pan et al. in 2007, it was evident that the Flaxseed lignan capsules (360mg/day) intake improve insulin sensitivity and decreases HbA1c levels and when compared with placebo group [37].

3.3 Effects of *L. usitatissimum* and *G. glabra* extracts on Serum Lipid Concentration

Diabetes is a metabolic syndrome, i.e. it is a collection of numerous metabolic and cardiovascular risk factors including central obesity, dyslipidemia and hypertension [38]. Particularly dyslipidemia which is a single key cause of nearly all consequent co-morbidities of diabetes [39]. Like rosuvastatin group, lipid concentration in diabetic rats treated with standard and research herbs *L. usitatissimum* and *G. glabra* extract showed significant improvement in all parameters at the end of study as compared to positive control group (p<0.001) seen in table 3. The presence of phytosterols and saponins in *G. glabra* and omega -3 fatty acids in *L. usitatissimum* could be an important factor in cholesterol elimination [40] [41]. Phytosterols are reported to displace intestinal cholesterol and reduce cholesterol absorption from the intestine [42] Our results overlap with those of Abeulgassim who reported that *L. usitatissimum* has a significant effect on serum TG and TC concentrations. [43] The NHLBI Family Heart Study showed that dietary Alpha-linolenic acid (ALA) present in *L. usitatissimum* decrease fat accumulation in the liver because the acid stimulates the β-oxidation of fatty acids and inhibits their synthesis. [44] Furthermore, Secoisolariciresionol diglucoside (SDG) is plant lignin present in *L. usitatissimum* is associated with lower plasma TG concentrations [41]. Furthermore, Serum HDL levels also showed significant improvement in the *L. usitatissimum* and *G. glabra* group at both 200 and 400mg/ kg which is in contrast with the study done by Abuelgassim who reported no change in the HDL levels in *L. usitatissimum* group [43]. The reason may be that they used the only single herb, whereas in this study the combination of *G. glabra* with *L. usitatissimum* was given. Similarly, in a study done by Shalaby et al. demonstrated that ethanolic extract of licorice root decreased TC and TG levels with no changes in LDL, HDL and VLDL-c in male rats [45]. This is again in contrast with our study which showed improvement in all parameters of cholesterol.

The increase in HDL-C and reduction in TC observed in our study, might be due to stimulation of pre-β HDL-C and reverse cholesterol transport as demonstrated by Rodriguez et al. [46] and maybe because of suppression of hydroxymethylglutaryl-CoA synthase activity by Glycyrrhizin, the active component of *G. glabra*.

Hassan et al. reported that when 15 % flaxseed meal enriched biscuits were fed to hypercholesterolemic rats for 8 weeks, resulted in reduced TC, TG, LDLD and VLDL levels with a significant increase in HDL levels [47]. Regardless of mechanisms involved, the present study illustrated that herbs combination reduce bad and improve good cholesterol levels in diabetic rats which could be due to synergistic effects of both SDG in *L. usitatissimum* seeds and phytosterol and saponin content of *G. glabra* root.

Table 1. Body weight of control and diabetic rats treated with standard drugs, *L. usitatissimum* seed and *G. glabra* root extract

	Negative control	Positive control	Glimepride	Metformin	Rosuvastatin	LU+GG 200	LU+GG 400
Body wt. initial (gms)	180.59 ± 0.4	188.33 ± 6.6 *	185.7 ± 4.70 *	199.26 ± 9.73 *	189.18 ± 4.22 *	189.09 ±6.23 *	193.64 ± 8.85 *
Body wt. final (gms)	275.43 ± 20.3	369.03 ± 20.07*	340.70 ± 0.98*	359.18 ±7.54 *	325.88 ± 12.86*	311.83 ±28.44*	277.78 ± 17.86*

** shows p-value highly significant as compared to the control group.*
LU: L. usitatissimum, GG: G. glabra

Table 2. Effect of Fasting blood sugar, HbA1C and Insulin Serum concentration in comparison with negative and positive control group

	FBS DAY 3 mg/dl	FBS DAY 29 mg/dl	HbA1C m.mol/L	Insulin Serum ug/L
Negative control	99.72 ± 3.44	103.94 ± 2.15	4.60 ± .44	3.34 ± .47
Positive control	492.16 ± 36.71*	613.33 ± 34.18*	14.78 ± 1.69*	7.76 ± .65*
Glimepride	458.33 ± 7.36*	94.68 ± 3.05*	9.64 ± .294*	5.03 ± .16*
Metformin	494.16 ± 10.12*	116.63 ± 9.03*	9.29 ± .28*	5.38 ± .10*
LU+GG 200	448.50 ± 13.32*	168.66 ± 6.99 *	12.57 ± .38 *	6.42 ± .11*
LU+GG 400	460.16 ± 14.87*	133.04 ± 11.41*	10.69 ± .28*	5.83 ± .13*

LU: L. usitatissimum, GG: G. glabra, FBS: Fasting blood sugar

Table 3. Total cholesterol TC, TG, HDL, VLDL, LDL, in control and diabetic rats treated with Rosuvastatin, *L. usitatissimum* and *G. glabra*

	TC mg/dl	TG mg/dl	HDL mg/dl	VLDL mg/dl	LDL mg/dl
Negative control	81.62 ± 1.78	39.43 ± 1.71	42.13 ± 1.59	7.79 ± 0.14	35.88 ± 1.17
Posositive control	264.88 ± 4.30*	211.78 ± 13.41*	32.79 ± 1.60*	54.54 ± 3.49*	124.45 ± 2.82*
Rosuvastatin	83.37 ± 1.80*	60.88 ± 0.96*	49.54 ± 0.50*	11.90 ± 0.51*	37.28 ± 0.60*
LU+GG 200	90.52 ± 1.45*	71.18 ± 1.02*	52.61 ± 1.56*	18.12 ± 0.97*	41.64 ± 0.53*
LU+GG 400	88.59 ± 0.65*	68.37 ± 0.65*	51.06 ± 1.00*	16.83 ± 0.82 *	39.90 ± 0.58*

LU : L. usitatissimum, GG : G. glabra, TC: Triglycerides, TC: Total cholesterol, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein, LDL: Low-density lipoprotein

Table 4. Serum amylase level when compared with *L. usitatissimum* and *G. glabra.*

	Serum Amylase (m.mol/L)
Negative control	1142.83 ± 5.41
Positive control	1941.00 ± 139.41*
LU+GG 200	1411.25 ± 16.74*
LU+GG 400	1392.00 ± 16.54*

LU: L. usitatissimum, GG: G. glabra

3.4 Effects of *L. usitatissimum* and *G. glabra* Extracts on Serum Amylase Levels

As pancreatitis is the common complication of few oral hypoglycemic drugs [48] which is usually encountered clinically as raised serum amylase levels [19]. Therefore, we aimed to evaluate the effects of our herbs on pancreatic enzymes and we found no aberration in this enzyme. The serum amylase levels were significantly increased in the diabetic rats when compared to normal rats and these levels reduced back to near normal after 28 days of treatment with *L. usitatissimum and G. glabra* as seen in table 4. Data is scared regarding the effects of these two plants on pancreatic physiology but according [49] *G. glabra* did Improve the activities of serum alanine aminotransferase (ALT) and aspartate transaminase (AST) in a cadmium-induced hepatotoxicity of animal model and significantly revert the inflammatory changes of the liver. This improvement might be owing to antioxidative, anti-inflammatory and anti lipid peroxidation potential of this herbs. Similarly, the study was done by Ghule et al. in 2012 showed significant reduction of Pancreatic malondialdehyde (MDA) and ROS by mononuclear cells and preservation of antioxidant enzymes activity of pancreatic tissue in alloxan induced diabetic rats [50].

4. CONCLUSION

Herbal born food sources show a striking therapeutic advancement in the treatment of diabetes either alone or in combination. The current study concludes that the combination of both *L. usitatissimum* and *G. glabra* extracts have significant glucose lowering effects with a protective role against well-recognized diabetes complications dyslipidemia and obesity. As an alternative, these two herbs combination therapy can be timely utilized for the management of diabetes and associated weight gain and dyslipidemia without any obvious irregularity of pancreatic functions.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard was written ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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