ORIGINAL ARTICLE

# ANTI HYPERGLYCEMIC AND ANTI DYSLIPIDEMIC EFFECTS OF FLAX SEEDS (LINUM USITATISSIMUM) EXTRACT IN DIABETIC RATS MODEL

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**ABSTRACT**

**Background:** Herbal medications, due to their various biologically active components and less toxic profile, have been popular amongst researchers since decades. One good example is Linum usitatissimum (Lu) commonly known as Flaxseeds. The aim of present study was to assess the anti hyperglycemic and anti dyslipidemic activities of ethanolic extract of flax seeds (*Linum usitatissimum*) in streptozotocin induced diabetic rat model.

**Methods:** The ethanolic extract of flax seeds (*Linum usitatissimum*) at a dose of 200 mg/kg and 400mg/kg were given to the streptozotocin-induced diabetic rats for the period of 28 days. FBS, insulin, HbA1c, lipid profile and serum amylase were evaluated and were compared with positive and negative controls and standard drugs like Glimepiride 0.1 mg/kg b.w., Metformin 10mg/kg b.w. and Rosuvastatin 10mg/kg/day b.w.

**Results:** Both doses of flax seeds 200 mg/kg and 400 mg/kg (*Linum usitatissimum*) extracts demonstrated significant ($p<0.001$) decrease in FBS of diabetic rats. Mainly Linum usitatissimum at the dosage of 400 mg/kg b.w. showed good efficacy in declining fasting blood glucose levels which was comparable with standard anti hyperglycemic drugs. Both doses of herbal extracts also showed significant decline in triglycerides, total cholesterol, and low-density lipoprotein (LDL-C), very low-density lipoproteins (VLDL-C), and serum amylase levels with prominent improvement in HDL-C levels in diabetic rats compared to positive controls.

**Conclusion:** This study reveals that the flax seeds (Lu) at the dose of both 200mg/kg and 400mg/kg have noteworthy potential to reduce hyperglycemia and dyslipidemia associated with diabetes, therefore may be it is useful in the management of Type-2 diabetes mellitus.

**Keywords:** Diabetes Mellitus; Dyslipidemia; Hyperglycemia; Flax Seeds.

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## INTRODUCTION

Diabetes mellitus is one of the largest epidemics of the world that is increasing rampantly due to urbanization, dietary changes and lifestyle modifications in both developed and developing nations[1]. Currently, the International Diabetes Federation declared that around 415 million with diabetes mellitus in 2015 and approximately 629 million people will be suffering with diabetes in 2045[2]. Two types of diabetes mellitus are common, type 1 and type 2. The type 2 diabetes mellitus (T2DM) caused by relative insulin resistance, incidence is expected to double (350 million) by the end of 2030[3].

Dyslipidemia is widely regarded as one of the adverse complication of diabetes[4]. A strong correlation has been shown in several studies between coronary heart disease with serum cholesterol and triglyceride level in patients with both type 1 and type 2 diabetes[5,6]. Evidence based researches propose an important role of insulin resistance in the development of dyslipidemia[7]. Despite traditional pharmacological treatment of diabetes with drugs which demonstrate good efficacy such as suphonylureas, biguanides, meglitinides, thiazoli-

dinedione, alpha glucosidase inhibitors, and gliptins, increasing side effects of these pharmacological interventions has led to uncertainty in their usage [8] for longer durations.

On the other hand, herbal medicines have started gaining popularity amongst researchers for the past 2 decades, due to their various biologically active components and less toxic profile. One good example is Linum usitatissimum (Lu) commonly known as Flaxseeds or Alsi[9]. In a study conducted in India, flax seeds were therapeutically beneficial to T2DM patients since they considerably reduced fasting blood glucose, glycated hemoglobin and other important parameters of lipidemia[10]. Extended use of LU has also been reported to decrease the likelihood of obesity-related diseases. It increases the duration of gastric emptying and thus immersion of useful nutrients from the lesser bowel[9].

Due to the increased burden of diabetes worldwide and lack of affordable treatment especially in the developing nations[11] it is crucial to find a sustainable treatment for the diabetes mellitus and its most common associated complication, dyslipidemia. Hence, this in vivo study was conducted in Karachi Pakistan, to find the outcome of antihyperglycemic effects of LU on streptozotocin induced diabetes model of rats. Furthermore, we also aimed to identify the effect of different doses of LU on body weight and lipid profile in the rat models.

## METHODS

This study included 42 adult male and female Wistar albino rats, (aged 7-8 weeks, weighing 180-240 grams) purchased from the Animal house of Aga Khan University. The study was conducted from May to September 2018 under standard laboratory conditions (25 ± 3°C, 12 h light/dark cycle) where they had free access to standard diet and clean tap water in their 30-days study duration and was conducted in the animal house of Jinnah postgraduate medical centre (JPMC).

With the exception of the negative control group, diabetes was induced to all animals by injecting a freshly produced solution of Streptozotocin (STZ) by dissolving dry powder of STZ in 0.1 M citrate buffer of pH 4.5 that was used after filtration. It was injected via intraperitoneal route at a dose of 55 mg/kg, as a single dose to overnight fasting rats. On 3rd day 1 ml of blood was taken from tail for fasting blood glucose (FBS). The rats who developed hyperglycemia (i.e., blood glucose concentration >250mgdl−1) were selected for the subsequent experiment and considered as diabetic rats.

Herbal extract consisted of L. usitatissimum seeds obtained from the local market of Karachi. The plants identified and legitimized from Karachi University botany department provided with a taxonomy number (Taxonomic number Linum usitatissimum is 29226).

The extract of L. usitatissimum seeds was obtained by washing and mincing the dried seeds with a grinder, then mixed and softened by using absolute alcohol at 1:10 ratio (100 grams in 1 L solvent) for 7 days in a jars. The resulting mixture was filtered through a Whatman No 1 filter paper followed by the evaporation of filtrate through a rotary evaporator in order to remove the ethanol. The product of the rotary evaporation was concentrated herb from extract that were reconstituted in freshly prepared 2.5% dimethyl sulfoxide DMSO and kept in jar for evaluation of antihyperglycemic and antihyperlipidemic properties in diabetic rats. Standard treatment was given to the rats except positive controls through metallic feeding syringe orally for 28 days.

Total 42 rats were divided into 7 groups with 6 rats in each respectively. 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 b.w., Group-IV: diabetic rats were treated with Metformin at 10mg/kg bw, Group V: diabetic rats were treated with Rosuvastatin 10mg/kg/day b.w., Group VI: diabetic rats were treated with ethanolic extract of L. usitatissimum at a dose of 200mg/kg, Group VII: diabetic rats were treated with ethanolic extract of L. usitatissimum at a dose of 400mg/kg.

Blood Glucose level (FBS), serum insulin, glycalated hemoglobin (HbA1c) level, serum amylase levels, lipid profile including, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total cholesterol (TC) and very low density lipoprotein (VLDL), were estimated in serum samples by standard enzymatic methods using commercially available kits (Bartham, Trinder, Richmond and Schettler) according to manufacturer advice respectively.

The data was interpreted by using SPSS v. 20 and M.S Excel 2013. The lipid levels and fasting blood glucose was calculated as mean ± Standard deviation (SD). The mean and SD of different groups were expressed by using appropriate statistical test such as (ANOVA) test. The data were applied for interpretation of data by taking p-value $< 0.05$ as significance.

## RESULTS

Since no mortality occurred in this study, data obtained from all 42 Wistar albino rats with 6 rats in each group is given below:

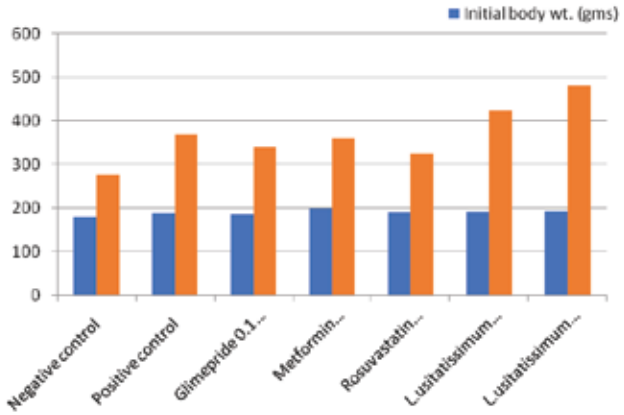


**Figure 1: Comparison of body weight in different groups at day 0 and 29.**

Figure 1 compares the mean weight of control groups, standard group i.e., metformin and glimepride and rosuvastatin group and treatment groups of 200mg and 400mg of L. usitatissimum (Lu) at 0 day and day 29.

The only significant weight gain was observed in positive control group (p value 0.010) in which no treatment was given as mentioned in Figure 1.

**Table 1: Comparison of glycemic indices (FBS, HbA1c and serum insulin) among different groups.**

| Groups | FBS DAY 3 | FBS DAY 29 | HbA1C | Serum Insulin |
|---|---|---|---|---|
| | (mg/dL) ISD | (mg/dL) ISD | (m.mol/L) ISD | (Ug/L) ISD |
| **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* |
| **Rosuvastatin** | 487.50± 26.07* | 196.29 ± 3.71* | 12.86 ± .45* | 5.71 ± .32* |
| ***L. usitatissimum* 200mg** | 453.66 ± 4.76 | 249.20 ±6.07 | 14.44 ± 0.28 | 7.44 ± 0.11 |
| ***L. usitatissimum* 400mg** | 454.00 ± 4.33 | 206.12 ± 3.80 | 13.64 ± 0.33 | 7.16 ± 0.50 |

• P- value >0.05
Lu: Linum usitatissimum, FBS: Fasting blood sugar.

Table 1 shows effects of L. usitatissium and standard drugs in improving fasting blood sugar, HbA1C and serum insulin level. A significant increase in level of FBS was observed in Positive control group at day 29. A rampant decrease was observed in the groups administered with Lu, a greater reduction was observed with a higher dose of Lu of 206.12mg/dl from 454 mg/dl. After applying ANOVA, this table shows that there is a significant difference among the groups (significant difference mentioned in*).

**Table 2: Comparison of different parameters of dyslipidemia among all groups.**

| Groups | TC | TG | HDL-C | VLDL |
|---|---|---|---|---|
| **Negative control** | 81.62 ± 1.78 | 39.43 ± 1.71 | 42.13 ± 1.59 | 7.79 ± 0.14 |
| **Positive control** | 264.88± 4.30* | 211.78 ± 13.41* | 32.79 ± 1.60* | 54.54 ± 3.49* |
| **Glimepride** | 82.28 ± 2.69 | 61.91 ± 2.46* | 53.49 ± 1.74* | 15.39 ± 0.43* |
| **Metformin** | 83.02 ±1.47* | 63.67 ± 1.27* | 50.93 ± 1.39* | 14.25 ± 0.43* |
| **Rosuvastatin** | 83.37 ± 1.80* | 60.88 ± 0.96* | 49.54 ± 0.50* | 11.90 ± 0.51* |

HDL: High-density lipoprotein cholesterol, LDL: Low density lipoprotein, TG: Triglycerides, Lu: L. usitatissimum, TC: Total cholesterol, VLDL: Very low-density lipoprotein cholesterol.

Table 2 shows the comparison of different parameters of dyslipidemia such TC, TG, LDL-C, HDL-C, VLDL and serum amylase in control and diabetic rats treated with standard drugs and herb L. usitatissimum. The PC group has reduced levels of HDL whereas, increased levels of TG LDL and VLDL. After applying ANOVA, the result proves that there was a significant difference exists among the groups (mentioned in*). It is to be noted that both the groups that were given Lu at doses of 200mg and 400mg did not show any significant differences in the levels of serum amylase though serum amylase levels were reduced.

## DISCUSSION

In this study a decrease in FBS level was observed in the group that was administered Lu 200 mg and a steep decline was observed with higher dose of Lu 400 mg. This hypoglycemic activity of flax seeds could be accredited to their high dietary fiber content. The typical viscous fibers in the flax seeds present in the form of the gum has an ability to decrease the glucose absorption from the gut[9] that in turn leads to a decline in the serum glycemic indices[12]. Another likely mechanism that clarify the effects of flaxseeds on blood glucose level may be the enhanced insulin secretion from the pancreas[13]. Furthermore, in a meta-analysis it was shown that the effects of flaxseed supplementation on blood glucose were more prominent in the studies whose participants had higher baseline glucose levels (>100 mg/dL)[14]. In our study, the baseline fasting blood sample of all the rats was recorded so that there is no difference in the blood glucose levels among all rats. After 3 days of inducing diabetes, the FBS values rose significantly as seen in Table 2, compared with other studies[15]. Therefore, greater than 100mg/dl FBS was present in almost all the groups and response of the Lu administration was positive[16].

L. usitatissimum (Lu) Flax seed is the richest source of omega-3 fatty acids (4:1 proportion of omega 3 to omega 6) which has many beneficial effects some of them are to help to reduce anxiety and depression and decrease cardiovascular problems as it reduces triglycerides and increases HDL[17]. In the following research diabetes was induced in the rats by intraperitoneal injection of Streptozotocin (STZ) which destroys β cells, present in the islets of Langerhans of pancreas, therefore, causing experimental diabetes[18].Though, many previously conducted studies were directed towards the positive effects of flax seeds such as hypolipidemic and hypoglycemic[19,20] including our prior study that was conducted to evaluate its combined effects with Glycyrrhiza glabra (GG)[21], but none of the previous studies evaluated the anti dyslipidemic effects of different doses of Lu on diabetic rats[22].

One of the major anthropometric measurements concerned with diabetes is weight. In our study, a considerable weight gain was seen in the diabetic control group which is not documented by other studies,[23] while in all other groups no significant weight gain has been reported using standard drugs and our research herb. Nevertheless a sharp increase in weight in the groups that were given Lu was observed which could be attributed to the fact that Lu also has omega 6 that leads to weight gain[24] and similar inconsequential weight increment in other groups may be age and diet related.

In our study, Lu did not have a major effect on the levels of glycated hemoglobin but it decreased other parameter associated with diabetes mellitus such as FBS, which is also consistent with the results of a Meta analysis conducted in 2008. This could be attributed to the short duration of this study, since significant results could be attained if the study had been done for more than 12 weeks [14].

Lipid disorder generally refers to the increased plasma TC, TG and LDL-C levels, and a decrease in HDL-C which is also known as "good cholesterol". In

the present study, the STZ-induced diabetic rats exhibited drastically higher serum triglycerides compared to the control rats. Controlled diabetes and isolated lipid disorders are often characterized with hypertriglyceridemia. Furthermore, increased levels of triglycerides can lead to pancreatitis, an inevitable complication hence instabilities in lipid metabolism in diabetes mellitus are often important determinants of the disease severity[16]. Several studies have shown that unusual blood glucose levels in T2DM is always accompanied with abnormal lipid levels[25]. An increase in serum levels of TC and TG can inadvertently lead to cardiovascular disease due to the ectopic lipid deposition in the heart and blood vessels[16]. However, in our study the serum values dropped to within normal levels as a consequence of Lu extract intake of both doses supplementation. L. usitatissimum is rich in omega 3 fatty acids i.e., alpha linolenic acid[26] and lignans (phytoestrogens)[27] which may help in improving dyslipedemic profile including improved HDL levels as shown in Table 2.

Pancreatitis sometimes is encountered as adverse effect of certain anti hyperglycemic agents therefore, serum amylase levels were evaluated after 28 days of treatment with Lu and we found no derangement in these enzymes[28]. On the other hand our study showed increase level of serum amylase in the control group of diabetic rats which could be due to diabetic ketoacidosis or renal insufficiency[29,30]. Data is scarce regarding protective effects of Lu on pancreas but it might be due to its rich flavonoids and anti oxidant compounds[31].

## CONCLUSION

Lu possesses significant potential to correct altered blood glucose and dyslipidemia associated with diabetes without any deleterious effects on serum amylase levels and may be a suitable candidate as alternative or adjuvant treatment option for the same disease.

## FUTURE RECOMMENDATIONS

Further studies must be conducted for longer duration to obtain appropriate results for all glycemic indices and clinical trials may be conducted for analysis of Lu as adjuvant therapy with standard anti diabetic regimen initially and if successful as alternate therapy for significant translational value.

## LIMITATIONS

Besides various strengths of this study, there are some limitations that should be considered. Firstly, the recorded values of TG, cholesterol, HDL, LDL are the final values that were observed at the end of the study, for better comparison initial values of the parameters mentioned above should have been recorded. Secondly, our study was conducted for a short period of time which could have led to an insignificant impact on the glycemic parameters.

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## CONFLICT OF INTEREST

The authors have no known conflict of interest associated with the study.

## ETHICS APPROVAL

This study was approved by Ethics Review Committee of Ziauddin University.

## AUTHORS CONTRIBUTION

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

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