Full Length Research Paper

***In vivo* assessment of antihyperglycemic and antioxidant activity from oil of linseed in streptozotocin induced diabetic rats**

Manoj Kumar¹, Sunil Sharma^{2*} and Neeru Vasudeva²

¹Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana-125001, India.

²Pharmacology Divisions, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Post Box: 38, Hisar-125001, India.

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In the present study, the antihyperglycemic and antioxidant effects of oil from seeds of *Linum usitatissimum* Linn. (LO) were investigated. A streptozotocin-nicotinamide (STZ) rat model of hyperglycaemia was used to evaluate the antihyperglycemic and antioxidant properties of oil of linseed. The body weight, oral glucose tolerance test and biochemical parameters namely; glucose level, insulin level, liver glycogen content, glycosylated hemoglobin and antioxidant parameters were estimated for all treated groups and compared against diabetic control group. LO (500 and 1000 mg/kg/day) in respective groups of diabetic animals administered for 28 days reduced the blood glucose level in streptozotocin-nicotinamide induced diabetic rats. There was significant increase in body weight, liver glycogen content, plasma insulin level and decrease in the blood glucose and glycosylated hemoglobin in test groups as compared to control group. *In vivo* antioxidant studies on STZ-nicotinamide induced diabetic rats revealed decreased malondialdehyde (MDA) and increased reduced glutathione (GSH). The findings demonstrate that LO have excellent antihyperglycemic and antioxidant activities and thus have great potential as a source for natural health products.

Key words: Streptozotocin, *Linum usitatissimum*, fixed oil, malondialdehyde (MDA), diabetes.

INTRODUCTION

A number of plants are being used from ancient times for the management of various disease like diabetes, hyperlipidemia, cancer, inflammation, pain etc. Many herbal products including several metals and minerals have been described for the care of diabetes mellitus in ancient literature (Nadkarni et al., 1992). Ayurveda, an ancient Indian form of medicine, deals with plants and plant extracts. This indigenous form of medicine uses the active ingredients present in plants for treating diseases

(Lewis and Elvin-Lewis, 1997). Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones (Momin, 1987). Many herbs have shown to possess hypoglycaemic action in animals and humans (Twaij and Al-Badr, 1988; Gupta, 1994).

Linum usitatissimum Linn. (Linaceae) is one of the important medicinal plants being used in various systems of medicine and food (Velisek et al., 1995). Human being has consumed flaxseed since the beginning of the

*Corresponding author. E-mail: sharmask71@rediffmail.com. Tel: 01662-263561. Fax: 01662-278181.

earliest civilizations. It was used traditionally to relieve abdominal pains and also as energy source from ancient time. Flax is considered a source of food as it contains alpha-linolenic acid lignans and polysaccharides (other than starch), all of which have positive effects in disease prevention (Rubilar et al., 2010).

Diabetes mellitus is a metabolic disorder which causes great morbidity in developed and developing countries. Diabetes was known to ancient Indian physicians as 'madumeha'. The metabolism of carbohydrates, fats and proteins are malformed in diabetes and patients with this disorder are at an increased risk of various complications like coronary heart disease, peripheral vascular disease and cerebrovascular disease (Brown, 1994; Stamler et al., 1993). It is associated with profound alterations in the plasma lipid and lipoprotein profile thereby an increased risk of premature atherosclerosis, coronary insufficiency and myocardial infarction exists (Betteridge, 1997). Accumulation of lipids in diabetes is mediated through a variety of regulatory processes, especially insulin deficiency, thereby rendering the diabetic patient more prone to hypercholesterolemia and hypertriglyceridemia [Jaiprakash et al., 1993].

The LO has been widely used in the food industries from centuries. As far as we know, the effect of LO on the blood profiles in diabetic models has not been studied. In light of these findings, we carried out this study for the evaluation of antihyperglycemic, and antioxidant potential of LO.

MATERIALS AND METHODS

Drugs and chemicals

The drugs and chemicals used in the study were glibenclamide (Torrent Pharmaceutical, Ahmadabad), streptozotocin, heparin (SRL, India), EDTA (Hi-media Lab. Pvt Ltd., Mumbai, India), Ellman's reagent (5,5'-dithiobis-(2-nitro-benzoic acid); DTNB), sodium sulphate, methanol, pyridine, anthrone, thiourea, benzoic acid, sodium chloride (SD Fine Chem Ltd., Mumbai, India). All the chemicals used in the study were of analytical grade.

Isolation of oil

The dried seeds of linseed were purchased from Chaudhary Charan Singh Haryana Agriculture University, Hisar, India. The seeds were crushed and oil was extracted by hot compression method. The percentage yield of oil was found to be 35% w/w.

Experimental animals

Healthy albino wistar rats (150 to 250 g) were procured from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana). The rats were housed in (Polycarbonate cage size: 29 × 22 × 14 cm) under laboratory standard conditions (25 ± 3°C: 35 to 60% humidity) with alternating light and dark cycle of 12 h each and were fed with a standard rat pellet diet (Hindustan Lever Ltd, Mumbai, India) and

water *ad libitum*. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) and Animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India (Registration No. 0436).

Induction of diabetes

Type II diabetes mellitus (NIDDM) was induced in overnight fasted animals by a single intraperitoneal injection of 50 mg/kg STZ in 0.1 M citrate buffer (pH-4.5) in a volume of 1 ml/kg body weight 15 min after the i.p. administration of 110 mg/kg nicotinamide. Diabetes was developed and stabilized over a period of 7 days. Diabetes was confirmed by the elevated blood glucose levels determined at 72 h and on 7th day after injection. Only rats confirmed with permanent NIDDM (glucose level above 250 mg/dl) were used in the study. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in plastic cages as per the guidelines of Institute of Animal Ethics committee. Blood was collected by intraocular route (Kumar et al., 2012).

Experimental design

Rats were divided into the following groups comprising six rats in each group after the induction and confirmation of diabetes.

For acute antihyperglycemic model

In the acute antihyperglycemic models, the study was carried out for 4 h to check if the plant possess some antidiabetic effect.

Group 1: Normal rats.

Group 2: Diabetic control.

Group 3: Diabetic animals were administered glibenclamide (0.6 mg/kg p.o).

Group 4: Diabetic animal were administered orally 500 mg/kg/day of LO.

Group 5: Diabetic animal were administered orally 1000 mg/kg/day of LO.

For chronic antihyperglycemic model

In the chronic antihyperglycemic models, the animal were treated with different doses that is, 500 and 1000 mg/kg for 28 days to study the various parameters of the diabetes to confirm the antihyperglycemic activity of LO in streptozotocin induced diabetes in rats.

Group 1: Normal rats.

Group 2: Diabetic control.

Group 3: Diabetic animals were administered glibenclamide (0.6 mg/kg p.o).

Group 4: Diabetic animal were administered orally 500 mg/kg/day of LO.

Group 5: Diabetic animal were administered orally 1000 mg/kg/day of LO.

Sample collection

Blood sample

In acute model, the blood was collected from retroorbital vein of the

rats previously fasted for 24 h. In chronic model, the 24 h fasted animals were sacrificed by cervical decapitation on 28th day of treatment. Trunk blood was collected in heparinized tubes. Plasma was obtained by centrifugation at 5000 rpm for 5 min to determine the biochemical parameters; glucose, insulin, cholesterol etc.

Estimation of plasma glucose and cholesterol

Plasma cholesterol and glucose level were measured by commercial supplied biological kit Erba Glucose Kit (GOD-POD Method) and Erba Cholesterol Kit (CHOD-PAP Method), respectively using Chem 5 Plus-V₂ Auto-analyser (Erba Mannheim Germany) in plasma sample. Glucose and cholesterol values were calculated as mg/dl blood sample.

Estimation of glycosylated hemoglobin (Hb1Ac)

Glycosylated hemoglobin was measured using commercial supplied biological kit (Erba Diagnostic) in plasma sample using Chem 5 Plus-V₂ Auto-analyser (Erba Mannheim Germany). Values are expressed as the percent of total hemoglobin.

Estimation of insulin

Serum insulin level was measured by an enzyme-linked immunosorbent assay (ELISA) procedure using Mercodia rat insulin ELISA kit. Briefly, the solid phase two-site enzyme immunoassay is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants 35 (epitopes) on the insulin molecule. During incubation, insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to the micro titration well. After washing three times, unbound enzyme labeled antibody was removed. The bound conjugated insulin was detected by reacting with 3, 3', 5, 5'-tetramethylbenzidine. The reaction was stopped by adding acid to give a colorimetric end-point, and optical density was measured with a micro plate auto reader (Bio-tek Instrument Inc., USA) at a wavelength of 450 nm. The serum insulin is expressed as µg/l.

Estimation of liver glycogen content

Liver glycogen estimation was done by the method as described by Seifter et al. (1950) with some modifications (Seifter et al., 1950). Immediately after excision from the animal, 1 g of the liver was dropped into a previously weighed test tube containing 3 ml of 30% potassium hydroxide solution. The weight of the liver sample was determined. The tissue was then digested by heating the tube for 20 minutes in boiling water bath. The digest was then cooled, transferred quantitatively to a 50 ml volumetric flask, and diluted to the mark with water. The contents of the flask were then thoroughly mixed and a measured portion was then further diluted with water in a second volumetric flask so as to yield a solution of glycogen (3 to 30 µg/ml). Five ml aliquots of the final dilution were then pipetted into Evelyn tube and the determination with anthrone was carried out. The amount of glycogen in the aliquot used was then calculated using the following equation:

$$\mu\text{g of glycogen in aliquot} = 100 \text{ U} / 1.11 \text{ S}$$

U is the optical density of unknown solution. S is the optical density of the 100 µg glucose and 1.11 is the factor determined by Morris in

1948 for the conversion of the glucose to the glycogen (Seifter et al., 1950).

In vivo antioxidant activity

Estimation of MDA level

Malondialdehyde (MDA), an index of free radical generation/lipid peroxidation, was determined as described by Okhawa et al. (1979) with some modification (Okhawa et al., 1979). Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium lauryl sulphate, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid added to 0.2 ml of blood plasma. The mixture was made up to 4.0 ml with distilled water and heated at 95°C for 60 min. After cooling the contents under running tap water, 5.0 ml of n-butanol and pyridine (15:1 v/v) and 1.0 ml of distilled water was added. The contents were centrifuged at about 3,000 rpm for 10 min. The organic layer was separated out and its absorbance was measured at 532 nm using double beam UV-Visible spectrophotometer (Systronics 2203, Bangalore, India) against a blank. MDA values were calculated using the extinction coefficient of MDA-thiobarbituric acid complex 1.56×10^5 l/mol cm and expressed as nmol/ml.

Estimation of reduced glutathione level

The tissue sample (liver 200 mg) was homogenized in 8.0 ml of 0.02 M EDTA in an ice bath. The homogenates were kept in the ice bath until used. Aliquots of 5.0 ml of the homogenates were mixed in 15.0 ml test tubes with 4.0 ml distilled water and 1.0 ml of 50% trichloroacetic acid (TCA). The tubes were centrifuged for 15 min at approximately 3,000 rpm, 2.0 ml of supernatant was mixed with 4.0 ml of 0.4 M Tris buffer pH 8.9, 0.1 ml Ellman's reagent [5,5-dithiobis-(2-nitro-benzoic acid)] (DTNB) added and the sample shaken. The absorbance was read within 5 min of the addition of DTNB at 412 nm against a reagent blank with no homogenate. Results are expressed as µmol GSH/g tissue.

Statistical analysis

The data for various biochemical parameters were evaluated by use of one-way analysis of variance (ANOVA), followed by Dunnett's t-test using the software Sigma-Stat 3. In all the tests, the criterion for statistical significance was $p < 0.05$.

RESULTS

Oral glucose tolerance test

The effect of LO on plasma glucose level after glucose loading of 2 g/kg body weight orally to the STZ diabetic rats is expressed in the Table 1. The blood glucose level rises to a maximum in 60 min after glucose loading. The oil (500 mg/kg/day and 1000 mg/kg/day body weight) treated groups showed a significant decrease ($p < 0.01$) in level of glucose as compared to control group. The oil treated group showed a marked fall in glucose level in 90 to 120 min interval.

Table 1. Effect of *Linum usitatissimum* oil in oral glucose tolerance test (OGTT).

Treatment	Dose (mg/kg)	Mean blood glucose concentration (mg/dl) $\pm$ SEM				
		0 min	30 min	60 min	90 min	120 min
Normal	-	80 $\pm$ 2.6	87 $\pm$ 2.8	90 $\pm$ 3.8	86 $\pm$ 2.5	83 $\pm$ 2.7
Diabetic control	-	290 $\pm$ 4.6	390 $\pm$ 5.3	413.2 $\pm$ 4.3	360 $\pm$ 2.7	331 $\pm$ 2.8
LUO	500	320 $\pm$ 2.9	332 $\pm$ 2.7	350 $\pm$ 3.0	317 $\pm$ 3.6*	288 $\pm$ 4.1**
LUO	1000	372 $\pm$ 3.6	383 $\pm$ 4.5	401 $\pm$ 4.1	360 $\pm$ 3.4**	301 $\pm$ 3.3**

Values are presented as mean $\pm$ SEM.; n = 6 in each group. One way ANOVA followed by Dunnett's test
 **p < 0.01 versus diabetic control; LUO: *Linum usitatissimum* oil

Table 2. Effect of *Linum usitatissimum* oil in STZ induced diabetic rats in acute antihyperglycemic study.

Treatment	Dose (mg/kg p.o)	Mean blood glucose concentration (mg/dl) $\pm$ SEM				
		0 h	1/2 h	1 h	2 h	4 h
Normal	-	76 $\pm$ 4.2	80 $\pm$ 3.2	77 $\pm$ 2.5	82 $\pm$ 4.1	79 $\pm$ 5.3
Control	-	340.5 $\pm$ 10.2	342 $\pm$ 11.3	346 $\pm$ 7.6	341.0 $\pm$ 6.7	332.0 $\pm$ 7.2
LUO	500	350 $\pm$ 5.7	340.1 $\pm$ 5.4	310 $\pm$ 4.5**	305 $\pm$ 3.4**	288 $\pm$ 3.6**
LUO	1000	338 $\pm$ 1.8	314 $\pm$ 1.9*	296 $\pm$ 2.3**	277 $\pm$ 2.4**	262 $\pm$ 4.9**
Glibenclamide	0.6	348 $\pm$ 7.1	328 $\pm$ 3.2**	288 $\pm$ 6.4**	260 $\pm$ 5.2**	249.0 $\pm$ 6.8**

Values are presented as mean $\pm$ SEM; n = 6 in each group. One way ANOVA followed by Dunnett's test *p < 0.05; **p < 0.01 versus diabetic control; LUO: *Linum usitatissimum* oil.

Table 3. Effect of *Linum usitatissimum* oil in STZ induced diabetic rats in chronic antihyperglycemic study.

Treatment	Dose (mg/kg p.o)	Mean blood glucose concentration (mg/dl) $\pm$ SEM				
		0 th Day	7 th Day	14 th Day	21 st Day	28 th
Normal	-	80 $\pm$ 4.2	79 $\pm$ 3.2	82 $\pm$ 2.5	85.5 $\pm$ 4.1	78 $\pm$ 2.1
Control	-	380 $\pm$ 7.3	379 $\pm$ 7.6	384 $\pm$ 6.7	416 $\pm$ 7.2	410 $\pm$ 5.4
LUO	500	345 $\pm$ 7.4	299 $\pm$ 4.5**	278 $\pm$ 5.2**	245 $\pm$ 6.1**	170 $\pm$ 6.2**
LUO	1000	370 $\pm$ 5.2	286 $\pm$ 5.9**	210 $\pm$ 5.8**	190 $\pm$ 4.4**	155 $\pm$ 5.8**
Glibenclamide	0.6	344 $\pm$ 8.4	259.2 $\pm$ 7.7**	166.3 $\pm$ 4.9**	140 $\pm$ 2.6**	124 $\pm$ 1.8**

Values are presented as mean $\pm$ SEM; n = 6 in each group. One way ANOVA followed by Dunnett's test **p < 0.01 versus diabetic control; LUO: *Linum usitatissimum* oil

Effect of LO on STZ diabetic rats in acute study

Administration of LO at a dose 500 mg/kg/day body weight p.o. to STZ diabetic rats showed reduction in blood glucose level from 350 to 288 mg/dl at 4th h. When the dose was increased as 1000 mg/kg/day then the blood glucose level decreased from 338 to 262 mg/dl which was found significant (p < 0.01) when compared with diabetic control (Table 2).

Effect of LO on STZ diabetic rats in chronic study

In chronic study, administration of LO at the dose of 500 mg/kg body weight to STZ diabetic rats for 28 days

showed a fall in plasma glucose level from 345 to 170 mg/dl on 28th day when compared to 0 day value. LO at the dose of 1000 mg/kg/day body weight showed a significant (p < 0.01) fall in plasma glucose level from 370 to 155 mg/dl on 28th day (Table 3).

Effect of LO on body weight

An increase in the body weight of normal rats was observed whereas the weight of diabetic control rats decreased from day 1 to day 28. LO at the dose of 500 and 1000 mg/kg/day body weight when administered to respective groups of diabetic rats showed a significant increase in body weight as compared to the diabetic

control group ($p < 0.01$) (Table 4).

Effect of LO on insulin level

Table 5 shows the level of plasma insulin in the control and experimental groups of rats. Diabetic rats showed a significant decrease ($p < 0.01$) in plasma insulin compared with normal rats. Oral administration of LO at the dose of 500 mg/kg/day and 1000 mg/kg/day body weight respectively showed a significant increase in plasma insulin level in respective groups as compared to control rats.

Effect of LO on glycosylated hemoglobin (HbA1c)

The effect of LO on HbA1c in STZ diabetic rats is shown in the Table 5. The level of glycosylated hemoglobin significantly increased ($p < 0.01$) in diabetic rats as compared to normal control group. The diabetic rats when treated with LO for 28 days showed a significant ($p < 0.01$) decrease in level of glycosylated Hb as compared to untreated diabetic group. The fall in glycosylated hemoglobin level was found to be dose dependent (Table 5).

Effect of LO on hepatic glycogen

The hepatic glycogen content in diabetic rats decreased sharply as compared to control animal (Table 5). Chronic administration of LO at both the doses to diabetic rats, a significant increase ($p < 0.01$) in liver glycogen content, was observed as compared to diabetic control group.

Effect of LO on lipid profile

Table 6 shows the level of lipids in normal and tested animals. There was a significant decrease ($p < 0.01$) in the level of HDL-cholesterol and a significant increase in the levels of total cholesterol and triglycerides in diabetic rats when compared to normal rats. The administration of LO reversed the level of lipids significantly ($p < 0.05$ and $p < 0.01$).

Effect of LO on *in vivo* antioxidant parameters

The data depicted in Table 7 shows the effect of LO on plasma malonaldehyde and reduced glutathione level. Plasma MDA level was found to be significantly higher in STZ diabetic rats compared to normal rats. The LO at dose 1000 mg/kg body weight p.o significantly reduced ($p < 0.01$) the level of MDA in diabetic rats. Plasma GSH level was found to be significantly lowered at both the

doses of LO in STZ diabetic rats as compared to normal rats. The chronic administration of LO at 1000 mg/kg body weight significantly increased the level of glutathione in diabetic rats.

DISCUSSION

The aim of the study was to evaluate the antidiabetic and antioxidant potential of the LO in STZ induced diabetic rats. Diabetes mellitus causes a disturbance in the uptake of glucose as well as glucose metabolism. A dose of STZ as low as 50 mg/kg produces an incomplete destruction of pancreatic beta cells even though the rats become permanently diabetic (Kumar et al., 2012). After treatment with a low dose of STZ, many beta cells survive and regeneration is also possible (Kumar et al., 2012). Hyperglycemia generates a high level of free radicals by autooxidation of glucose and protein glycation, and oxidative stress has been reported to be a positive factor of cardiovascular complications in STZ-induced diabetes mellitus (Okutan et al., 2005). Hyperglycemia is associated with the generation of reactive oxygen species (ROS), causing oxidative damage particularly to heart, kidney, eyes, nerves, liver, small and large vessels and gastrointestinal system (Tunali and Yanardag, 2006). The increased levels of plasma glucose in STZ-induced diabetic rats were lowered by LO administration. The plasma glucose lowering activity of LO was comparable to glibenclamide, a standard hypoglycemic drug that stimulates insulin secretion from pancreatic beta cells (Tian et al., 1998). From the results of the present study, it appears that still insulin producing cells are functioning and the stimulation of insulin release could be responsible for most of the metabolic effects. It may be suggested that the mechanism of action of LO is similar to glibenclamide. The glucose lowering activity of LO may be related to both pancreatic (enhancement of insulin secretion) and extra pancreatic (peripheral utilization of glucose) mechanism.

An increase in the level of glycosylated hemoglobin (HbA1c) in the diabetic control group of rats is due to the presence of large amount of blood glucose which reacts with hemoglobin to form glycosylated hemoglobin (Chattopadhyay, 1999). Oxidative stress increases due to the activation of transcription factors, advanced glycated end products (AGEs), and protein kinase C. If diabetes is persistent for a long time, the glycosylated hemoglobin is found to increase (Sheela and Augusti, 1992). The level of HbA_{1c} was decreased after the administration of LO 1000 mg/kg as compared to diabetic control group (** $p < 0.01$).

In STZ induced diabetes mellitus, the loss of body weight is caused by increase in muscle wasting and catabolism of fat and proteins (Chakravarti et al., 1981). Due to insulin deficiency, protein content is decreased in

Table 4. Effect of *Linum usitatissimum* oil on body weight in diabetic rats.

S/No.	Treatment	Dose (mg/kg p.o)	Initial body weight (g)	Final body weight (g)	Change in weight
1	Normal	-	220±1.1	240±1.5	+20
2	Diabetic control	-	215±1.8	194±2.0	-21 ^a
3	LUO	500	250±2.2	255±1.0	+5
4	LUO	1000	245±1.3	255±1.4	+10 ^{**}
5	Glibenclamide	0.6	220±4.7	250±1.8	+30 ^{**}

Values are presented as mean ± SEM; n = 6 in each group. One way ANOVA followed by Dunnett's test ^ap < 0.01 versus normal; ^{**}p < 0.01 versus diabetic control; LUO: *Linum usitatissimum* oil.

Table 5. Effect of *Linum usitatissimum* oil on glycosylated hemoglobin (HbA1c), hepatic glycogen and insulin in the study.

Treatment	Dose (mg/kg)	HbA1c (% of Hb)	Hepatic glycogen (mg/g wt of tissue)	Insulin (micro U/ml)
Normal	-	6±1.4	74±6.6	14±2.1
Diabetic control	-	11.3±2.4 ^a	27±4.5 ^a	7.9±1.1 ^a
LUO	500	9.0±2.2	47±1.8*	10±2.0
LUO	1000	7.2±2.6 ^{**}	64±3.6 ^{**}	12±2.5*
Glibenclamide	0.6	7.0±0.7 ^{**}	66±2.8 ^{**}	11.9±1.0*

Values are presented as mean ± SEM; n = 6 in each group. One way ANOVA followed by Dunnett's test ^ap < 0.01 versus normal; *p < 0.05; ^{**}p < 0.01 versus diabetic control; LUO: *Linum usitatissimum* oil.

Table 6. Effect of *Linum usitatissimum* oil on Lipid profile.

Treatment	Dose (mg/kg)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)
Normal	-	85±1.5	16±2.5	66±1.9
Diabetic control	-	232±2.4 ^a	43±3.1 ^a	37.4±1.2 ^a
LUO	500	177±3.6 ^{**}	29±1.6 ^{**}	44±2.1
LUO	1000	111±2.5 ^{**}	21±1.3 ^{**}	48±1.3*
Glibenclamide	0.6	138±1.8 ^{**}	14.4±1.2 ^{**}	53±1.8*

Values are presented as mean ± SEM; n = 6 in each group. One way ANOVA followed by Dunnett's test ^ap < 0.01 versus normal; *p < 0.05; ^{**}p < 0.01 versus diabetic control; LUO: *Linum usitatissimum* oil.

muscular tissue by proteolysis (Flat et al., 1990). A decrease in body weight was registered in case of STZ diabetic control group rats while in tested groups the weight loss was reversed. Fatty acid mobilisation from adipose tissue is sensitive to insulin. Insulin's most potent action is the suppression of adipose tissue lipolysis (Campbell et al., 1992). A rise in plasma insulin concentration of only 5 IU/ml inhibits lipolysis by 50%, whereas a reduction in basal insulin levels result in a marked acceleration of lipolysis (Bonadonna et al., 1990). We demonstrated that LO increased plasma insulin concentrations in diabetic rats. Insulin levels higher than those of the control group may result in inhibition of lipolysis and decreased plasma triglyceride and cholesterol levels. Some studies suggest that the antihyperglycemic action of traditional antidiabetic plant

extracts may be due in part to decreased glucose absorption *in vivo* (Gallagher et al., 2003). This mechanistic explanation may also apply to the actions of LO in lowering the triglyceride and cholesterol level.

The conversion of glucose to glycogen in the liver cells is dependent on the extracellular glucose concentration and on the availability of insulin which stimulates glycogen synthesis over a wide range of glucose concentration (Kumar et al., 2011). Diabetes reduces activity of glycogen synthase thereby affecting the glycogen storage and synthesis in rat liver and skeletal muscle (Kumar et al., 2011). Oral administration of LO 1000 mg/kg body weight significantly increased hepatic glycogen levels in STZ diabetic rats possibly because of the reactivation of the glycogen synthase system as a result of increased insulin secretion.

Table 7. Effect of *Linum usitatissimum* oil on antioxidant parameters (MDA and GSH).

Treatment	Dose (mg/kg)	MDA (nmol/dl)	GSH (μ mol/g)
Normal	-	2.8 $\pm$ 0.2	41.2 $\pm$ 2.8
Diabetic control	-	5.4 $\pm$ 0.4 ^a	14 $\pm$ 1.15 ^a
LUO	500	3.8 $\pm$ 0.7	22 $\pm$ 2.4
LUO	1000 mg/kg	3.1 $\pm$ 0.3**	34 $\pm$ 2.1**
Glibenclamide	0.6 mg/kg	3.0 $\pm$ 0.2**	38 $\pm$ 1.8**

Values are presented as mean $\pm$ SEM; n = 6 in each group. One way ANOVA followed by Dunnett's test ^ap < 0.01 versus normal; **p < 0.01 versus diabetic control; LUO: *Linum usitatissimum* oil.

Conclusion

The present study showed that oral administration of *Linum usitatissimum* has potential antidiabetic, anti-hyperlipidemic and antioxidant effect in STZ induced diabetic rats. The potent antioxidant activity may be responsible for the antihyperglycemic and antihyperlipidemic effects. Thus the investigation reveals that *L. usitatissimum* can be used as a natural oral agent with antihyperglycemic, antihyperlipidemic and antioxidant effects.

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