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Antidiabetic and Anti-Hyperlipidemic Potential of *Linum usitatissimum* (Linn.) Seeds in Streptozotacin Induced Diabetic Rat

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Abstract: ***The present study was designed to evaluate the anti-diabetic and Anti-hyperlipidemic potential of Linum usitatissimum (Linn.) seeds. The dried, crushed seeds were extracted successively by methanol and water. These extract then subjected for phytochemical screening. Animals were divided in seven groups and treated high dose (400 mg/kg) and low dose (200 mg/kg) of methanolic and aqueous extract and compared with diabetic control group. Results indicates that administration of methanolic and aqueous extract of Linum usitatissimum (Linn.) seeds daily for 28 days showed significant decrease in the blood glucose, cholesterol, triglyceride, LDL and significant increase in HDL of the STZ induced diabetic rats compared to the diabetic control rats. The findings of this research suggest that the methanolic and aqueous extract of Linum usitatissimum (Linn.) seeds may contains bioactive constituents with anti-diabetic potential which can be used for the treatment of diabetes mellitus and hyperlipidemia.***

Keyword: *Linum usitatissimum* (Linn.) , Diabetes, antidiabetic agents, Hyperlipidemia, Anti-hyperlipidemic agents, cholesterol, LDL

1. Introduction

Diabetes mellitus is the commonest endocrine disorder that affects more than 100 million people worldwide. The estimated prevalence rate of diabetes in India is about 1- 5%. In India, indigenous remedies have been used in the treatment of DM since the time of Charaka and Sushruta. (Grover J. K. et. al., 2002).

Hyperlipidemia is the greatest risk factor contributing to atheroselerosis and other cardiovascular disorders. Since synthetic agents have shown to have side effects clinical mimportance of herbal drugs in treatment of hyperlipidemia has received considerable attention in recent years (Konda V.R. et.al., 2013).

Herbal medicines are used for primary healthcare, by about 801% world population particularly in the developing countries, because of better cultural acceptability, safety, efficacy, potent, inexpensive and lesser side effects. The plant drugs are considered less toxic when compared to synthetic drugs. More than 1123 plant species have been found to treat the diabetes and more than 200 pure compounds have showed lowering blood glucose activity. The WHO expert committee recommended the importance to investigate hypoglycaemic antihyperlipidemic agents from plant origin, used in traditional medicine for treatment of DM and hyperlipidemia. The antihyperglycemic agents have been focused on plants used in traditional medicine because that may be better treatment than currently used synthetic drugs.(Sellamuthu Periyar et.al., 2009)

Inspite of presence of known antidiabetic medicines in the market, remedies from the medicinal plants are used with success to treat this disease possibly because they are considered to be less toxic and free from side effects compared to synthetic one(Patel S.S. et.al., 2009)

Ethanobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes.

Flaxseed or linseed (*Linum usitatissimum* L.) is an annual herb belongs to the *Linaceae* family. It is cultivated worldwide and has been used for its oil seed and fiber since ancient times in Egypt, Rome and Greece. Nowadays it is considered as a medicinal plant in Asia, Europe and North America. (Mohammad Hassan Houshdar Tehrani et.al, 2014)

2. Material and Method

Eight weeks old healthy Sprague-dawley rats (weighing 180-250g) was used for this study. Animals were housed in polypropylene cages with wire mesh top and husk bedding and maintained under controlled conditions of light (10h-light: 14h- dark), temperature (22±3°C), and humidity and fed with standard pellet diet and water, were used for the entire animal study.

3. Extraction Procedure

a) Collection and authentication of plant materials

The *Linum usitatissimum* (Linn.) seeds were collected from different parts of India. The plant material were identified and authenticated by Mrs. A. M. Gaharwar, Programme Coordinator, Krishi Vigyan Kendra, Yavatmal (Ref No. KVK/Ytl/Hort/22/2017).

b) Processing of sample

The *Linum usitatissimum* (Linn.) seeds were dried in shade and then crushed to get a coarse powder. This powder was stored in an air tight container and used for extraction.

c) Extraction

The coarse powder of *Linum usitatissimum* (Linn.) seeds were subjected to successive extraction with methanol and thereafter water by maceration. Then filtered and dried on water bath.

Preliminary Phytochemical Screening

A combined knowledge of chemical constituents is desirable for supportive evaluation of natural drug or drug component as well as phytopharmaceuticals, for knowledge of phytopharmacology screening. Hence, all the extracts were subjected for preliminary phytochemical screening for identification of different constituents and plant metabolites present /extracted in different extracts.

4. Experimental Design

The animals were grouped into five following groups. (n=6)

1) **Control Group:-** Animals were treated with vehicle alone
2) **Negative control Group :** Animals were treated with STZ (60 mg/kg)
3) **STD Group:** Diabetic rats were treated with standard drug Glibenclamide 0.5 mg/kg once daily *p.o.*
4) **Low dose MLU Group (MLU 200 mg/kg *p.o.*):** Rats were treated with STZ (60 mg/kg) and MLU 200 mg/kg once daily *p.o.*
5) **High dose MLU Group (MLU 400 mg/kg *p.o.*):** Rats were treated with STZ (60 mg/kg) and MLU 400 mg/kg once daily *p.o.*
6) **Low dose WLU Group (WLU 200 mg/kg *p.o.*):** Rats were treated with STZ (60 mg/kg) and MLU 200 mg/kg once daily *p.o.*
7) **High dose WLU Group (WLU 400 mg/kg *p.o.*):** Rats were treated with STZ (60 mg/kg) and MLU 400 mg/kg once daily *p.o.*

Induction of diabetes in rats by Streptozotocin (Ojha S, 2014**)**

Rats were made diabetic by intraperitoneal injection of STZ at dose of 60 mg/kg. STZ was first weighed individually for each animal according to the body weight and solubilized with 0.05 M citric buffer pH 4.5. It was then injected. 5% glucose was provided for 24 hrs to inhibit hypoglycemia. Administration of single dose of STZ in rats results in hyperglycemia within 72 hrs.

Sample Collection

Blood samples were collected by retro-orbital plexus puncture method and blood glucose levels, blood lipid parameters were estimated by semi- automated electronic bioanalyser (Ambika Diagnostic, Parbhani, India)

5. Result

Table: Phytochemical screening of *Withania coagulans* dunal flower

Sr. No.	Phytoconstituents	Test	MLU	WLU
1	Alkaloid	Mayer's Test	+	+
		Dragendroff's Test	+	+
2	**Flavonoids**	Ferric Chloride Test	+	+
		Lead Acetate Test	+	+
3	**Carbohydrates**	Molisch Test	-	+
		Fehling Test	-	-
		Benedict's Test	+	+
4	**Steroid**	Salkowski's Test	+	+
		Libermann Barchared test	+	+
5	**Tannins**	Lead Acetate Test	+	+
		Gelatin Test	+	+
6	**Proteins**	Xanthoprotein test	+	-
		Biuret Test	-	+
		Lead Acetate Test	+	+
7	**Glycoside**	Keller Kiliani Test	-	-

+ Present, - Absent.

MLU- Methanolic extract of *Linum usitatissimum* (Linn.) seeds, **WLU-** Aqueous extract of *Linum usitatissimum* (Linn.) seeds,

Table 2: Effect of *Linum usitatissimum* (Linn.) seeds on blood serum glucose level in STZ induced diabetic rats at 0, 3, 14 and 28 days

Sr. No.	Groups	Glucose (mg/dl)			
		0 days	3 days	14 days	28 days
1	Control	88.7± 4.28	85.2±3.96	90.5±5.25	88.9±4.95
2	Negative control	91.6±5.10	273.9±15.7@	285.6±16.6@	270.6±15.2@
3	Standard	90.4±4.85	270.8±14.2	164.83±10.5**	94.5±5.46**
4	MLU (200mg/kg)	88.96±4.10	269.5±13.9	232.24±12.9*	214.6±12.0**
5	MLU (400mg/kg)	90.37±4.84	271.4±14.15	190.78±11.7**	168.65±10.6**
8	WLU (200mg/kg)	90.44±4.85	271.9±14.25	190.69±11.6**	171.5±10.3**
9	WLU (400mg/kg)	89.65±4.65	272.23±14.30	165.80±10.18**	128.95±7.86**

Results are expressed as mean ± SD in mg/dl. (n=6)

@p<0.01 Compared with corresponding normal control group, **p<0.01 Compared with diabetic control group, *p<0.05 compared with diabetic control group

Table 3: Effect of *Linum usitatissimum* (Linn.) on serum Cholesterol level in STZ induced diabetic rats at 0, 3, 14 and 28 days

Sr. No.	Groups	Cholesterol (mg/dl)			
		0 days	3 days	14 days	28 days
1	Control	48.10 ± 1.91	48.20 ± 1.97	47.33 ± 1.75	48.16 ± 1.75
2	Negative control	48.15 ± 1.98	48.35 ± 1.99	52.66 ± 2.36@	87.16 ± 3.98@#
3	Standard	48.45 ± 2.15	47.95 ± 1.39	47.50 ± 1.94 **	48.59 ± 1.90**
4	MLU(200mg/kg)	47.81 ± 1.41	48.15 ± 1.97	48.79 ± 1.87 **	57.65 ± 2.95**
5	MLU(400mg/kg)	48.21 ± 1.40	47.97 ± 1.79	47.87 ± 1.95 **	53.17 ± 2.10**
6	WLU(200mg/kg)	48.19 ± 1.35	48.21 ± 1.98	48.84 ± 1.98 **	56.77 ± 2.75**
7	WLU(400mg/kg)	47.65 ± 1.57	48.17 ± 1.97	47.77 ± 1.81 **	53.25 ± 2.57**

Table 4: Effect of *Linum usitatissimum* (Linn.) on serum Triglyceride level in STZ induced diabetic rats at 0, 3, 14 and 28 days

Sr. No.	Groups	Triglyceride (mg/dl)			
		0 days	3 days	14 days	28 days
1	Control	67.27 ± 2.65	67.83 ± 2.79	67.16 ± 2.51	68.00 ± 1.32
2	Negative control	67.17 ± 2.45	66.95 ± 2.47	83.66 ± 3.96@	104.16 ± 4.89@
3	Standard	67.39 ± 2.69	67.17 ± 2.65	67.50 ± 2.64 **	74.33 ± 3.01**
4	MLU(200mg/kg)	67.17 ± 2.12	66.97± 2.31	73.15 ± 2.97**	85.49 ± 3.79**
5	MLU(400mg/kg)	67.29 ± 2.67	67.22 ± 2.61	70.87 ± 2.88 **	82.10 ± 3.11**
6	WLU(200mg/kg)	67.14 ± 2.37	67.10± 2.25	72.99 ± 2.96 **	85.15 ± 3.28**
7	WLU(400mg/kg)	67.31 ± 2.68	66.31± 2.28	70.91± 2.89**	81.48 ± 3.92**

Results are expressed as mean ± SD in mg/dl. (n=6)
@p<0.01 Compared with corresponding normal control group, **p<0.01 Compared with diabetic control group, ns p>0.05 compared with diabetic control group.

Table 5: Effect of *Linum usitatissimum* (Linn.) on serum LDL level in STZ induced diabetic rats at 0, 3, 14 and 28 days

Sr. No.	Groups	LDL			
		0 days	3 days	14 days	28 days
1	Control	25.23 ± 0.63	24.43 ± 0.79	23.43 ± 0.77	23.60 ± 0.67
2	Negative control	24.94 ± 0.72	24.14 ± 0.59	34.06 ± 0.97@	74.83 ± 1.28@
3	Standard	25.04 ± 0.60	24.44 ± 0.81	23.83 ± 0.64 **	26.83 ± 0.46**
4	MLU(200mg/kg)	25.25 ± 0.69	25.21 ± 0.91	27.75 ± 0.94 **	37.43 ± 0.94**
5	MLU(400mg/kg)	25.59 ± 0.71	24.93 ± 0.85	26.65 ± 0.87**	35.88 ± 0.88**
6	WLU(200mg/kg)	25.70 ± 0.85	24.64 ± 0.84	26.94 ± 0.71 **	38.27 ± 1.05**
7	WLU(400mg/kg)	24.97 ± 0.77	25.25 ± 0.75	25.49 ± 0.65**	36.47 ± 0.94**

Results are expressed as mean ± SD in mg/dl. (n=6)
@p<0.01 Compared with corresponding normal control group, **p<0.01 Compared with diabetic control group, ns p>0.05 compared with diabetic control group.

Table 6: Effect of *Linum usitatissimum* (Linn.) on serum HDL level in STZ induced diabetic rats at 0, 3, 14 and 28 days

Sr. No.	Groups	HDL			
		0 days	3 days	14 days	28 days
1	Control	36.83 ± 0.75	37.33 ± 0.81	38.16 ± 0.75	37.36 ± 0.75
2	Negative control	36.92± 0.78	37.37 ± 0.82	34.33 ± 0.51@	33.16 ± 0.75@
3	Standard	36.72± 0.69	36.99 ± 0.78	37.96 ± 0.75**	37.34 ± 0.51 **
4	MLU(200mg/kg)	37.10± 0.79	37.35 ± 0.81	35.61 ± 0.78 **	36.66 ± 0.81**
5	MLU(400mg/kg)	36.98± 0.78	37.25 ± 0.77	36.24 ± 0.77 **	37.16 ± 0.75**
6	WLU(200mg/kg)	36.89± 0.71	37.36 ± 0.82	35.84 ± 0.75 **	36.83 ± 0.75**
7	WLU(400mg/kg)	36.69± 0.68	37.29 ± 0.80	37.21 ± 0.71 **	37.07 ± 0.72**

Results are expressed as mean ± SD in mg/dl. (n=6)
@p<0.01 Compared with corresponding normal control group, **p<0.01 Compared with diabetic control group, ns p>0.05 compared with diabetic control group.

Qualitative phytochemical analysis of MWC and WWC are indicated in table 1. Result shows presence of Alkoaloids, Flavonoids, Carbohydrates, steroids, tannins, proteins and absence of glycosides in all plant extracts.

Table 2 shows the effect of MLU and WLU on blood glucose level in STZ induced diabetes rats. There were significant increase (p<0.01) in the blood glucose level in all the groups of rats compared to normal control group of rats on 3rd to 28th day. After the confirmation of diabetes, rats were treated with MLU and WLU. There were significant (p<0.05) reduction in the blood glucose level in MLU (200 mg/kg) and WLU (200 mg/kg) and a significant (p<0.01) in blood glucose level in MLU (400 mg/kg) and WLU (400 mg/kg) treated group compared to diabetic control group on 14th and 28th day.

Table 3, 4, 5 and 6 shows the effect of MLU and WLU on blood lipid profile in STZ induced diabetic rats. There were significant increase (p<0.01) in the cholesterol, triglyceride

and LDL level whereas significant decrease in the HDL level in negative control group compared to normal control group on 14 th and 28th day. After treatment with plant extracts, significant ($p<0.01$) reduction was observed in the cholesterol, triglyceride and LDL level and significant increase in the level HDL in MLU and WLU (200 mg/kg and 400 mg/kg) treated group compared to diabetic control group on 14 th and 28th day.

6. Discussion

Diabetes is a complex and heterogeneous disorder presently affecting more than 100 million people worldwide and causing serious socio-economic problems. Appropriate experimental models are essential tools for understanding the pathogenesis, complications, and genetic or environmental influences that increase the risks of diabetes and testing of various therapeutic agents. The animal models of diabetes can be obtained either spontaneously or induced by chemicals or dietary or surgical manipulations and/or by combination thereof. In recent years, large number of new genetically modified animal models including transgenic, generalized knock-out and tissue specific knockout mice have been engineered for the study of diabetes (Srinivasan K, 2007).

Streptozotocin (STZ) and Alloxan (ALX) are the most frequently used drugs and this model has been useful for the study of multiple aspects of the disease. Both drugs exert their diabetogenic action when they are administered parenterally (intravenously, intraperitoneally or subcutaneously). (Sharma R, 2013). Because of high mortality, ALX is now almost replaced by STZ for induction of diabetes in laboratory animals (Rees D. A, 2005) and hence for this study STZ was used to induce diabetes.

Current therapeutic agents are generally had inadequate efficacy and number of serious adverse effects. Thus, there is wide variety of newer therapeutic agents/strategies being examined for the treatment of diabetes (Cheng D, 2005; Bailey C. J, 2011).

About 75 - 80% of world population seeking herbal therapy for primary health care. The synthesis of Herbal medicine is the backbone for the generation of therapeutic experiences of practicing physicians for over hundreds of years. Herbal medicines are now in great demand in the developing world not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. (Pal S. K, 2013)

Diabetes mellitus is the most common endocrine disorder, affecting more than 300 million people worldwide. For this, therapies developed along the principles of western medicine (allopathic) are often limited in efficacy, carry the risk of adverse effects & are often too costly, especially for the developing world. Therefore, treating diabetes mellitus with plant derived compounds which are accessible & do not require laborious pharmaceutical synthesis, seems highly attractive. There are various herbal drugs such as a *Allium cepal* Linn(Gazuwa S. Y,2013; Jevas C,2011), *Allium sativum* Linn(Gazuwa S. Y,2013; Thomson M, 2007) *Aloe vera*(Hussein A. M, 2013; Sahu. P. K, 2013), *Bidens pilosa*(Diego F, 2013), *Elephantopus scaber* (Prathapan R, 2014), *Magnifera indica* (Irondi A. E, 2016) etc. have significant antidiabetic activity due to the presence of chemical constituensts like alkaloids, glycosides, steroids, flavonoid, carbohydrates, proteins, amino acids etc. shows antidiabetic activity may be due to the insulin like effect on peripheral tissues either by promoting glucose uptake and metabolism and/or inhibiting hepatic gluconeogenesis (Gaikwad S. B,2014).

As *Linum usitatissimum* (Linn.) seeds also contain alkaloids, Steroids, amino acids, flavonoids, proteins, carbohydrates and tannins.(Gupta V., 2013;Prasad S. K, 2010) Hence this plant was selected for screening antidiabetic activity and extracted by using Methanol and water solvent system (Salwaan C, 2012; Srivastava S. K, 2015).

The phytochemical study was carried out for confirmation of various phytochemicals and observed the presence of alkaloids. Steroids, amino acids, flavonoids, proteins, carbohydrates and tannins in Methanolic and aqueous extract of seeds of *Linum usitatissimum* (Linn.).

The treatment with MLU and WLU showed the significant ($p<0.05$) decrease in blood glucose level at 200 mg/kg and ($p<0.01$) significant at 400 mg/kg dose of extract in blood glucose level after 14 days of treatment as compared to the diabetic control group of rat. After the 28 days of treatment there were significant ($p<0.01$) decrease in blood glucose level in all treated groups with extracts as compared to the diabetic control groups of rats.

It might be due to the insulin production, stimulation, release from β cells and/or inhibiting hepatic gluconeogenesis, by decreasing the intestinal absorption of glucose by retarding the release of glucose during digestion, inhibit the α-glucosidase and pancreatic α-amylase possibly by the chemical constituents presents in the extracts of *Linum usitatissimum* (Linn.) seeds.

Our data showed significant ($p<0.01$) increase in the level of cholesterol, triglyceride, LDL and decreased level of HDL in the diabetic control group as compared to the normal control group of rats on 14th and 28th day. After treatment with MLU and WLU 200 mg/kg and 400 mg/kg of dose, the level of cholesterol, triglyceride, LDL were significantly ($p<0.01$) decreased and level of HDL was increased on 14th and 28th day.(Stalin S, 2014)

Hence, the results obtained in the present study indicated that seeds of *Linum usitatissimum* (Linn.) have potential to treat diabetes and prevent diabetes mellitus associated altered lipid profile.

7. Conclusion

The present study was designed to evaluate the anti-diabetic and antihyperlipidemic potential of flower of *Linum usitatissimum* (Linn.) seeds based on its chemical constituents and its use as folk medicine for the control of diabetes and associated problems. The antidiabetic study indicates that administration of methanolic and aqueous extract of *Linum usitatissimum* (Linn.) seeds daily for 28

days showed significant decrease in the blood glucose, cholesterol, triglycerides, and LDL level and significant increase in HDL level of the STZ induced diabetic rats compared to the diabetic control rats. The findings of this research suggest that the methanolic and aqueous extract of *Linum usitatissimum* (Linn.) seeds flower may contains bioactive constituents with anti-diabetic potential which can be used for the treatment of diabetes mellitus and related complications.

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