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Antidiabetic activity of aqueous extracts of *Linum usitatissimum* in streptozotocin induced diabetic rats

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Abstract

Background: The aim of the present study was to evaluate antidiabetic activity of aqueous extract of *Linum usitatissimum* seeds in streptozotocin (STZ) induced diabetic rats.

Methods: *Linum usitatissimum* seeds extract or glibenclamide was administered to the rats orally daily for 28 days. Levels of blood glucose at weekly interval and on 29th day various hematological parameters, serum biochemical parameters as well as histopathology were carried out to evaluate its antidiabetic effects in diabetic rats.

Results: Blood glucose, creatinine, BUN, AST, ALT and total cholesterol levels were significantly increased along with alterations in pancreas tissues in STZ induced diabetic rats as compared to vehicle control rats. After 28 days administration of aqueous extract *Linum usitatissimum* showed the significant decrease in above levels along with no defined pathological lesions in histoarchitecture of pancreas as compared to diabetic control rats.

Conclusion: Our results suggest that the aqueous extract of *Linum usitatissimum* seeds clearly demonstrated the antidiabetic activity in an experimental model of rats.

Keywords: *Linum usitatissimum*, streptozotocin, glibenclamide, diabetic rats

1. Introduction

Diabetes is a complex multisystemic disorder characterized by a relative or absolute insufficiency of insulin secretion and disturbances in carbohydrate, protein and lipid metabolism^[1]. The worldwide prevalence of diabetes has continued to increase dramatically. According to International Diabetes Federation 7th edition, the number of individuals with diabetes in 2015 crossed 415 million and by 2040, this will rise up to 642 million^[2]. Therapies currently available for diabetes include oral hypoglycemic agents like glibenclamide, glipizide, gliclazide, nateglinide, repaglinide, acarbose, miglitol, dipeptidylpeptidase, sitagliptin, linagliptin, alogliptin, dutogliptin, gemigliptin and injectable insulin but they have their own limitations due to selective mechanism of action^[3]. Drawbacks of insulin therapy are like local pain, inconvenience of multiple injections, insulin edema, lipohypertrophy, allergy and resistance. Therefore, searching for effective, low cost and less side effect containing herbal hypoglycemic agents is important. In recent findings, extracts of various plant materials are capable of decreasing blood sugar level in experimental animal models and are considered to be less toxic than synthetic ones.

Linum usitatissimum plant is a blue or purple flowering crop and a member of family Linaceae commonly called Flax seed or linseed in english, Alsi or Tisi in hindi, Alsi in gujarati and Atasi in sanskrit and it is distributed in tropical India^[4]. Flax seed playing a major role in the field of diet and disease research due to its health benefits associated with high content of α -linolenic acid and major lignan namely secoisolariciresinol diglucoside (SDG). Based on previous study reports, medicinal activities of flaxseed include antioxidant, anticancer, antiviral, bactericidal, anti-inflammatory and antiatherosclerotic^[5], decreases the blood glucose and cholesterol levels^[6] and decrease the risk of obesity, dyslipidemia and resistance to insulin^[7]. Hence in the present study research was done to evaluate the antidiabetic activity of aqueous extract of *Linum usitatissimum* seeds in streptozotocin (STZ) induced diabetic rats.

2. Materials and Methods

2.1 Experimental Animals

The study was conducted on 36 healthy male Sprague-Dawley rats of 8-12 weeks of age. Rats were procured from Zydus Research Centre (ZRC), Ahmedabad, India.

The experimental protocol was approved by Institutional Animal Ethics Committee (Project No. 258/VPT/2017) at College of Veterinary Science and Animal Husbandry, Anand, Gujarat and protocols were followed according to the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA). The animals were housed in standard polypropylene cages and maintained under controlled room temperature (22 ± 2 °C) and humidity ($55 \pm 5\%$) with 12 h light and 12 h dark cycle. All the rats were fed normal pellet diet and deionized water was provided *ad libitum* throughout the course of the experiment. All the rats were kept under acclimatization for 5 days prior to grouping and initiation of experiment. Rats were kept under constant observation during entire period of study. All necessary management procedures were adopted to keep the rats free from stress.

2.2 Drugs and Chemicals

Streptozotocin was purchased from Hi-Media Laboratory Pvt. Ltd., India and glibenclamide was purchased from Sigma-Aldrich (Spruce Street, St. Louis, MO, U.S.A.). Serum biochemical kits for estimation of serum biochemical parameters were purchased from Coral Clinical System (Goa, India).

2.3 Collection and authentication of plant materials

Seeds of *L. usitatissimum* were purchased from local market of Anand district (Gujarat) and identified and authenticated by Botanist of Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand.

2.4 Preparation of the plant extracts

Seeds of *L. usitatissimum* were taken and dried under shade, then powdered by mechanical grinder and stored in air tight containers. The dried powder of *L. usitatissimum* was used for the preparation of aqueous extract. For this purpose, about 100 g of the powdered material was soaked in 2 liters of distilled water with shaking thrice daily. The extracts obtained were concentrated in rotary evaporator at 50-60 °C under reduced pressure leaving a brown residue. Aqueous extracts of *L. usitatissimum* obtained was transferred to a petri dish and kept over water bath (50 °C) until the solvent gets completely evaporated. It was stored in air tight glass containers in refrigerator at 2-8 °C for further use in experiments. Respective doses for the study were prepared by reconstituting the extract in distilled water.

2.5 Acute toxicity testing of plant extracts

The acute oral toxicity study was carried out as per Organization for Economic Cooperation and Development (OECD) guideline No. 423. Sprague Dawley rats were taken for the study and dosed once with 2000 mg/kg, orally. The treated rats were monitored for 24 hours and up to 14 days for general clinical signs and symptoms as well as mortality. It was observed that the aqueous extracts of *L. usitatissimum* showed no mortality in rats even at 2000 mg/kg dose. Hence, 100, 200 and 400 mg/kg dose of this plant extracts were selected and considered safe for further study.

2.6 Induction of diabetes in rats

Experimentally induction of diabetes in rats by administered single intraperitoneal (i.p.) injection of STZ @ 60 mg/kg in overnight fasted rats. STZ formulation was prepared by using

0.1 M citrate buffer (pH 4.5) solution. Citrate buffer solution was prepared by dissolving 1.47 g of sodium citrate in 50 ml of distilled water and adjust the pH to 4.5 by citric acid solution and kept it in ice cold. STZ was dissolved in freshly prepared citrate buffer solution and used immediately because STZ is pH sensitive compound.

2.7 Experimental design

The present study was conducted on thirty six (36) rats dividing them in various groups, having six rats in each group (n=6).

Group I- Vehicle control (received normal saline)

Group II- Diabetic control (given STZ 60 mg/kg)

Group III- Treatment control treated with glibenclamide (5 mg/kg)

Group IV- Diabetic rats treated with LU extract (100 mg/kg)

Group V- Diabetic rats treated with LU extract (200 mg/kg)

Group VI- Diabetic rats treated with LU extract (400 mg/kg)

2.8 Body weight and feed consumption

Body weight and feed consumption of all rats were measured weekly interval for 28 days.

2.9 Estimation of blood glucose

Blood was collected from the dorsal vein of the tail and blood glucose was estimated by Dr. Morepen Gluco One (Model: BG-03, Morepen Laboratories Ltd., Delhi, India) on day 0 of treatment and weekly for 28 day.

2.10 Hematological and biochemical estimation

On termination of experiment, blood samples were collected from retro-orbital plexuses under light anesthesia with the help of capillary tube. Blood samples collected in test tubes with K_3EDTA were utilized for estimation of various hematological parameters (Hb, RBC, PCV, TLC, MCV, MCH and MCHC) by hematology auto analyzer (Mindray, BC-2800 Vet, Garnerville, New York). Serum biochemical parameters like serum creatinine, blood urea nitrogen (BUN), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and total cholesterol (TC) were estimated by using auto serum chemistry analyser (Mindray BS-120, Mumbai, India).

2.11 Histopathology

After opening the carcass, gross lesions were recorded and collected tissues like pancreas, kidney, liver, spleen and heart were fixed in 10% formalin. The formalin fixed tissues were processed by paraffin wax embedding method of tissue sectioning. Sections from the tissues were cut at 5-6 microns thickness with automatic section cutting machine (Leica, Germany) and were stained with Haematoxylin and Eosin (H & E) stains.

2.12 Statistical analysis

All the data have been presented as mean \pm SE. Statistical comparisons of the results were made using one way analysis of variance (ANOVA) by using software SPSS (Version 20). Significant differences ($p < 0.05$) between different experimental groups were analyzed by Duncan's test.

3. Results

3.1 Effect on body weight and feed consumption

There was significant reduction in body weight gain from 7th to 28th day of study and increase feed consumption from 1st,

3rd and 4th week of study in diabetic rats compared to vehicle control rats. Following 28 days oral administration of aqueous extracts of *L. usitatissimum* at the dose 200 and 400 mg/kg showed significant increase body weight gain at 21 and 28 days and decrease feed consumption in diabetic treated rats.

3.2 Effect on blood glucose

There was significant increase in blood glucose level in diabetic control rats as compared to vehicle control rats from 0 to 28th day of study. Whereas administration of aqueous extract of *L. usitatissimum* at the dose 100, 200 and 400 mg/kg and glibenclamide in diabetic rats for 28 days showed significant reduction in the elevated level of blood glucose at 21st and 28th days of study as depicted in Table 1. Aqueous extracts of *L. usitatissimum* showed their effectiveness in dose dependent manner.

3.3 Effect on hematological parameters

Diabetic control rats showed significant decrease in Hb, RBC and PCV (14.85 ± 0.39 g/dl, 6.67 ± 0.20 $10^6/\mu\text{l}$ and $31.85 \pm 2.21\%$ respectively) as compared to vehicle control rats (16.52 ± 0.66 g/dl, 8.14 ± 0.35 $10^6/\mu\text{l}$ and $42.25 \pm 1.57\%$, respectively), whereas significant increase in TLC (12.03 ± 0.39 $10^3/\mu\text{l}$) as compared to vehicle control rats (8.95 ± 0.29 $10^3/\mu\text{l}$). While daily oral administration of aqueous extracts of *L. usitatissimum* at the dose 100, 200 and 400 mg/kg body weight for 28 days showed significant increase in the Hb, PCV and also significant increase in RBC count at the dose 200 mg/kg and 400 mg/kg body weight extract treated rats, whereas significant decrease in TLC within normal physiological range in at higher dose (400 mg/kg) extract treated rats as compared to diabetic control rats as depicted in Table 2.

3.4 Effect on biochemical parameters

There was significant increased serum concentration of creatinine, BUN, AST, ALT and total cholesterol level (1.03 ± 0.02 mg/dl, 32.19 ± 0.44 mg/dl, 161.51 ± 9.87 U/l, 83.98 ± 2.69 U/l and 149.48 ± 5.07 mg/dl, respectively) in diabetic control rats as compared to vehicle control rats (0.36 ± 0.02 mg/dl, 18.44 ± 0.30 mg/dl, 103.98 ± 1.54 U/l, 41.58 ± 3.77 U/l, 82.95 ± 4.84 mg/dl, respectively). While daily oral administration of aqueous extracts of *L. usitatissimum* at the dose 100, 200 and 400 mg/kg in diabetic rat for 28 days produced significant reduction in the serum creatinine, BUN, AST, ALT and TC level as compared to diabetic control rats as depicted in Table 3.

3.5 Histopathology

In the present study, histopathological examination of section of pancreas from vehicle control rats showed normal architecture of β cells of islets of Langerhans and acinar cells as depicted in Figure 1. Pancreas of diabetic control rats revealed destruction and decrease in number, derangement, diminished size and shape of β cells and damaged acinar cells with abnormal architecture of islets of Langerhans (Figure 2), while pancreas of glibenclamide and aqueous extracts treated rats at dose of 400 mg/kg daily for 28 days showed no defined pathological lesions in histoarchitecture of islets of Langerhans and acinar cells (Figure 3). Whereas other organs like kidney, liver, spleen and heart from diabetic control, treatment control and aqueous extracts treated rats did not showed any marked gross and microscopic alterations.

4. Discussion

At present, therapeutic options for diabetes are diet, exercise, oral hypoglycemic drugs and insulin therapy. The management of diabetes with the agents devoid of any side effects is still a challenge to the medical system. This concern has led to an increased demand for safe and natural herbal derived treatment of diabetes. Hence the present study aimed to evaluate the antidiabetic effect of aqueous extract of *L. usitatissimum* seeds in comparison with Glibenclamide in STZ induced diabetic rat model.

In our study, the significant increase in blood glucose was observed in STZ induced diabetic rats from 0 to 28th day of study and when diabetic rats administered orally with aqueous extract of *L. usitatissimum* (100, 200 and 400mg/kg) and standard drug glibenclamide, a reduction in glucose levels at 21st and 28th days in dose dependent manner were observed. Similar observations were reported for the ethanolic extracts of *L. usitatissimum* administered at dose of 200 and 400 mg/kg [8]. Likewise, linseed oil given at dose of 500 and 1000 mg/kg/day for 28 days showed significant decrease in blood glucose level in diabetic rats [9]. Similar results were also reported for 10% and 20% flax seed treated group showed significant reduction in fasting blood sugar at 14th day compare to standard drug metformin [10]. Flaxseed was given at 0.714 g/kg/day orally in 0.1% carboxymethyl cellulose for 12 weeks showed significant decrease in blood glucose level compare to diabetic control rats [11]. Oral administration of herbal formulation of flaxseed, fenugreek and jamun seeds (1:1:1) given at dose of 200 mg/kg and their individual compounds for 28 days showed significant reduction in blood glucose level [12]. Additionally in agreement to our study, decreased glucose level in rats after administration of flaxseed extract orally at dose of 400 mg/kg for 4 weeks [13].

In the present study, finding showed significant increased Hb, PCV and RBC count in *L. usitatissimum* aqueous extract treated diabetic rats. Similar result was also reported as feeding of 10% flaxseed for 56 days showed significantly increase in total RBC and PCV count in diabetic rats [14]. Likewise, oral administration of herbal formulation of flaxseed, fenugreek and jamun seeds (1:1:1) at dose of 200 mg/kg and their individual drug for 28 days showed significant increased Hb level in diabetic rats [12].

The results of present study showed significant reduction in the serum creatinine, BUN, AST, ALT and TC levels in aqueous extracts of *L. usitatissimum* @ 100, 200 and 400 mg/kg treated diabetic rats. Similarly, feeding of flaxseed oil for eight weeks showed significant reduction in serum creatinine and urea levels in diabetic rats [15]. Likewise another report on flaxseed supplemented (10% w/w) for 8 weeks showed significant decrease in serum creatinine, BUN, AST and ALT level in rats [16]. Additionally, administration of flaxseed oil @ 500 mg/kg daily for 8 weeks showed significant decreases in the elevated creatinine, BUN, AST and ALT levels in rats [17]. In another report showed significant decreased serum creatinine and BUN level at different levels of flaxseed oil (20, 30 and 40 g/kg) given to rats [18]. Similarly, administration of flax and pumpkin seed mixture (33%) for 30 days leads to significant decrease in plasma and liver AST, ALT and TC level in alloxan induced diabetic rats [19]. In another report, administration of linseed oil at dose of 500 and 1000 mg/kg/day for 28 days showed significant decrease in total cholesterol level in diabetic rats [9], also administration of 4% flaxseed oil in diabetic rats showed significantly decrease in TC level [20]. Flaxseed given

(0.714 g/kg/day, orally) for 12 weeks showed significant decrease in AST and ALT level in diabetic rats [11]. The flaxseed extract given orally at dose of 20 mg/kg body weight for 14 days showed significant decrease in total cholesterol in rats [21]. Similarly, flaxseed oil administered at the dose 1.8 mg/kg for 8 week showed significant decrease in total cholesterol level in rats [22].

The findings of the present study showed no defined pathological lesions in histoarchitecture of pancreas of aqueous extract treated diabetic rats. In agreement to our report, administration of (33%) flax and pumpkin seed

mixture supplemented for 30 days showed normal β cell architecture in pancreas [19]. Similarly, 8 weeks feeding of flaxseed oil showed protection of the pancreatic β -cells and preserving pancreatic normal structure with no histopathological changes in diabetic rats [15]. In another report, treatment of mice orally at dose of 100 μ g/200 μ L of *Linum usitatissimum* fraction suspended in distilled water with 0.5% DMSO for 21 days showed potential for the formation of new islets *in vitro* as well as *in vivo* in the pancreas [23].

Table 1: The effect of 28 days oral administration of aqueous extracts of *L. usitatissimum* on blood glucose of rats.

Group	Treatment	Blood glucose (mg/dl) (Mean \pm S.E.) (n=6)				
		0 day	7 day	14 day	21 day	28 day
I	VC	107.83 \pm 5.54 ^a	118.17 \pm 3.94 ^a	103.17 \pm 3.77 ^a	107.00 \pm 6.10 ^a	118.17 \pm 3.32 ^a
II	DC	368.00 \pm 26.66 ^b	370.33 \pm 27.48 ^b	374.50 \pm 27.70 ^c	375.33 \pm 27.74 ^c	376.83 \pm 26.98 ^c
III	TC	390.17 \pm 27.08 ^b	346.17 \pm 20.92 ^b	298.17 \pm 20.90 ^{bc}	227.50 \pm 17.10 ^b	143.00 \pm 7.97 ^{ab}
IV	LU Aq-100	304.17 \pm 29.49 ^b	303.00 \pm 29.69 ^b	291.17 \pm 30.06 ^{bc}	264.17 \pm 22.86 ^b	244.50 \pm 21.99 ^d
V	LU Aq-200	365.33 \pm 51.88 ^b	360.67 \pm 53.88 ^b	318.50 \pm 44.78 ^{bc}	251.67 \pm 38.03 ^b	206.83 \pm 27.95 ^{cd}
VI	LU Aq-400	330.00 \pm 32.42 ^b	324.00 \pm 31.98 ^b	281.33 \pm 29.14 ^b	209.00 \pm 16.41 ^b	159.50 \pm 12.09 ^{abc}

Mean value with dissimilar superscript in a column vary significantly at $p < 0.05$. VC: Vehicle Control, DC= Diabetic Control, TC: Treatment Control

Table 2: The effect of 28 days oral administration of aqueous extracts of *L. usitatissimum* on Hb, RBC, PCV, TLC MCV, MCH and MCHC of rats.

Group	Treatment	Mean \pm SE values of Hb (g/dl), RBCs ($10^6/\mu$ L), PCV (%) and TLC ($10^3/\mu$ L) in rats (n=6)						
		Hb (g/dl)	RBC ($10^6/\mu$ L)	PCV (%)	TLC ($10^3/\mu$ L)	MCV (fl)	MCH (pg)	MCHC (g/dl)
I	VC	16.52 \pm 0.66 ^b	8.14 \pm 0.35 ^{bcd}	42.25 \pm 1.57 ^c	8.95 \pm 0.29 ^{ab}	52.46 \pm 3.37	20.64 \pm 1.68 ^{ab}	39.41 \pm 2.29 ^a
II	DC	14.85 \pm 0.39 ^a	6.67 \pm 0.20 ^a	31.85 \pm 2.21 ^a	12.03 \pm 0.39 ^c	48.27 \pm 4.41	22.40 \pm 0.98 ^{bc}	47.60 \pm 2.99 ^{bc}
III	TC	17.13 \pm 0.21 ^b	8.09 \pm 0.35 ^{bcd}	41.05 \pm 1.64 ^{bc}	9.89 \pm 0.28 ^{ab}	50.98 \pm 2.05	21.39 \pm 1.02 ^{ab}	42.14 \pm 2.04 ^{ab}
IV	LU Aq-100	17.83 \pm 0.16 ^b	7.18 \pm 0.24 ^{ab}	37.05 \pm 0.92 ^b	10.28 \pm 0.27 ^{abc}	51.72 \pm 1.32	24.99 \pm 0.99 ^c	48.29 \pm 1.32 ^c
V	LU Aq-200	16.57 \pm 0.29 ^b	7.72 \pm 0.36 ^{bc}	41.89 \pm 0.97 ^c	10.80 \pm 0.43 ^{bc}	55.02 \pm 3.41	21.75 \pm 1.30 ^{abc}	39.68 \pm 1.37 ^a
VI	LU Aq-400	16.92 \pm 0.33 ^b	8.58 \pm 0.27 ^{cd}	42.10 \pm 0.82 ^c	9.84 \pm 0.39 ^{ab}	49.37 \pm 1.99	19.80 \pm 0.59 ^{ab}	40.29 \pm 1.30 ^a

Mean value with dissimilar superscript in a column vary significantly at $p < 0.05$

Table 3: The effect of 28 days oral administration of aqueous extracts of *L. usitatissimum* on creatinine and BUN, AST, ALT and TC of rats.

Group	Treatment	Mean \pm SE values of creatinine (mg/dl) and BUN (mg/dl) in rats of all groups (n=6)				
		Creatinine (mg/dl)	BUN (mg/dl)	AST (U/l)	ALT (U/l)	TC (mg/dl)
I	VC	0.36 \pm 0.02 ^a	18.44 \pm 0.30 ^a	103.98 \pm 1.54 ^a	41.58 \pm 3.77 ^a	82.95 \pm 4.84 ^a
II	DC	1.03 \pm 0.02 ^b	32.19 \pm 0.44 ^f	161.51 \pm 9.87 ^d	83.98 \pm 2.69 ^d	149.48 \pm 5.07 ^c
III	TC	0.36 \pm 0.02 ^a	19.52 \pm 0.39 ^b	117.07 \pm 5.70 ^{ab}	46.88 \pm 3.41 ^{ab}	97.95 \pm 1.63 ^{bc}
IV	LU Aq-100	0.42 \pm 0.03 ^a	25.44 \pm 0.28 ^e	139.61 \pm 7.89 ^c	64.64 \pm 4.20 ^c	119.62 \pm 3.43 ^d
V	LU Aq-200	0.39 \pm 0.01 ^a	23.49 \pm 0.26 ^d	125.62 \pm 4.04 ^{bc}	55.64 \pm 4.64 ^{abc}	103.22 \pm 3.43 ^{bc}
VI	LU Aq-400	0.35 \pm 0.03 ^a	20.73 \pm 0.37 ^c	116.80 \pm 3.61 ^{ab}	50.80 \pm 9.49 ^{abc}	90.87 \pm 5.83 ^{ab}

Mean value with dissimilar superscript in a column vary significantly at $p < 0.05$

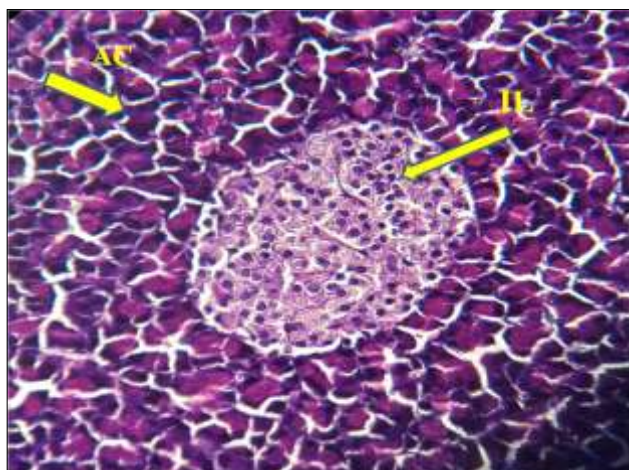


Fig 1: Section of pancreas from vehicle control rats (group I) showing normal architecture of β cells of islets of Langerhans (II) and acinar cells (AC) (H & E stain X 120).

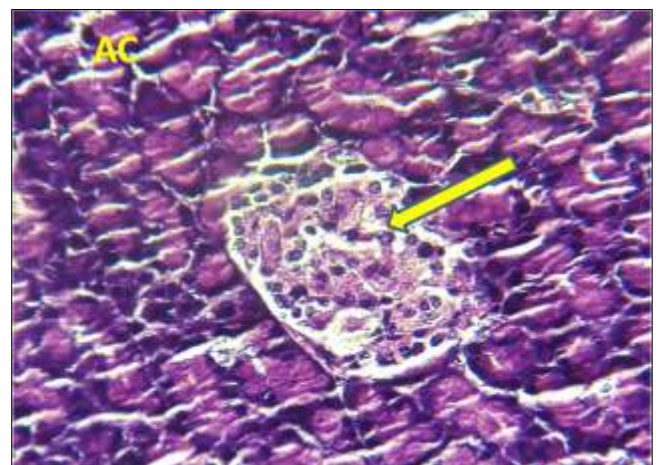


Fig 2: Section of pancreas from diabetic control rats (group II) showing destruction and decrease number of β cells (H & E stain X 240).

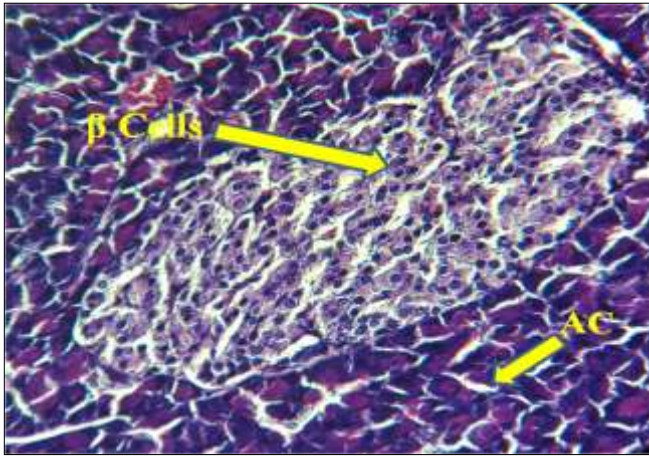


Fig 3: Section of pancreas from diabetic rats treated with aqueous extracts of *L. usitatissimum* @ 400 mg/kg daily for 28 days (group VI) showing no pathological alteration of the β cells of islets of Langerhans and normal appearance of acinar cells (AC) (H & E stain X 120).

5. Conclusions

The present study revealed that oral administration of aqueous extracts of *L. usitatissimum* has dose dependent antidiabetic effect in an experimental model of rats. The extract was found safe following repeated oral administration at dose of 400 mg/kg for 28 days in rats.

6. Acknowledgements

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7. References

- Barik R, Jain S, Qwatra D, Joshi A, Tripathi GS, Goyal R. Antidiabetic activity of aqueous root extract of *Ichnocarpus frutescens* in streptozotocin-nicotinamide induced type-II diabetes in rats. *Indian Journal of Pharmacology*. 2008; 40(1):19.
- International Diabetes Federation IDF Diabetes Atlas, Edn 7. 2015
- Mangesh K, Ashwini K, Srinivas B, Shivram G, Aaditi K. Phytochemical and Pharmacological Accounts of Some Reviewed Plants with Antidiabetic Potential. *Bridges*. 2015; 11:14.
- Anjaria J, Parabia M, Dwivedi S. *Indian Ethnoveterinary Medicine, an overview*, pathik enterprise. Ahmedabad, India. 2002, 420.
- Halligudi N. Pharmacological properties of flax seeds: a Review. *Hygeia. JD Med*. 2012; 4(2):70-7.
- Kristensen M, Jensen MG, Aarestrup J, Petersen KE, Søndergaard L, Mikkelsen MS *et al*. Flaxseed dietary fibers lower cholesterol and increase fecal fat excretion, but magnitude of effect depend on food type. *Nutrition & metabolism*. 2012; 9(1):8.
- Saxena S, Katare C. Evaluation of flaxseed formulation as a potential therapeutic agent in mitigation of dyslipidemia. *Biomed J*. 2014; 37(6):386-90.
- Ghule AE, Jadhav SS, Bodhankar SL. Effect of ethanolic extract of seeds of *Linum usitatissimum* (Linn.) in

- hyperglycaemia associated ROS production in PBMNCs and pancreatic tissue of alloxan induced diabetic rats. *Asian Pacific Journal of Tropical Disease*. 2012; 2(5):405-10.
- Kumar M, Sharma S, Vasudeva N. *In vivo* assessment of antihyperglycemic and antioxidant activity from oil of linseed in streptozotocin induced diabetic rats. *African Journal of Pharmacy and Pharmacology*. 2013; 7(23):1568-74.
- Venkatachalam S, Ponnuswamy TK, Bindu G, Yamunadevi MS, Amudhan Arvind E. Comparison of hypoglycemic effect of flax seeds and metformin in streptozotocin induced diabetic rats. *European Journal of Pharmaceutical and Medical Research*. 2015; 2(5):594-60.
- Gök M, Ulusu NN, Tarhan N, Tufan C, Ozansoy G, Arı N *et al*. Flaxseed protects against diabetes-induced glucotoxicity by modulating pentose phosphate pathway and glutathione-dependent enzyme activities in rats. *Journal of dietary supplements*. 2015; 13(3):339-51.
- Latha S, Vijaya Kumar R, Senthil Kumar BR, Bupesh G, Kumar TS. Acute and Repeated Oral Toxicity of Antidiabetic Polyherbal Formulation Flax Seed, Fenugreek and Jamun Seeds in Wistar Albino Rat. *J Diabetes Metab*. 2016; 7(656):2.
- Al-Ania IM, Abireda AN, Mustafab BE, Wahaba EN, Azzubaidi MS. Effect of Flaxseed Extract on the Liver Histological Structure in Streptozotocin Induced Diabetic Rats. *International Medical Journal Malaysia*. 2017; 16(1).
- Babu S, Geraldine V. Mitchell, Paddy Wiesenfeld, Mamie Y. Jenkins, Hemavathi Gowda U. Nutritional and hematological impact of dietary flaxseed and defatted flaxseed meal in rats. *International journal of food sciences and nutrition*. 2000; 51(2):109-17.
- Soltan SS. The effects of varieties sources of omega-3 fatty acids on diabetes in rats. *Food and Nutrition Sciences*. 2012; 3(10):1404.
- Al-Bishri WM. Favorable effects of flaxseed supplemented diet on liver and kidney functions in hypertensive Wistar rats. *Journal of oleo science*. 2013; 62(9):709-15.
- Wahba HM, Ibrahim TA. Protective effect of flaxseed oil and vitamin E on potassium bromate-induced oxidative stress in male rats. *Int. J Curr. Microbiol. App. Sci*. 2013; 2(9):299-309.
- Sayeda EL, El-Sahar GE, Abor MM, Abed ER. Study on the biological effect of use flaxseed oil as a source of fat on the biomarkers of experimental rats. *Journal of American Science*. 2014; 10(3):116-23.
- Makni M, Fetoui H, Gargouri NK, Garoui EM, Zeghal N. Antidiabetic effect of flax and pumpkin seed mixture powder: effect on hyperlipidemia and antioxidant status in alloxan diabetic rats. *Journal of Diabetes and its Complications*. 2011; 25(5):339-45.
- Rezaei A, Heidarian E. Co-administration of trientine and flaxseed oil on oxidative stress, serum lipids and heart structure in diabetic rats. *Indian journal of Experimental Biology*. 2013; 51:646-52
- Haran WH, AL-Saeed MH, AL-Masoudi EA. Study the Effect of Flax Lignan Extract of *Linum usitatissimum* and Conjugated Estrogen on Physiological Parameters in Female Rats. 2017; 16(1):20-49
- Elimam H, Ramadan K. Comparative Study of the

Possible Prophylactic and Curative Effects of Flaxseed Oil on the Lipid Profile and Antioxidant Status of Hyperlipidaemic Rats. *Journal of Applied Pharmacy*. 2018; 10(1):1-6

23. Dusane MB, Joshi BN. Beneficial effect of flax seeds in streptozotocin (STZ) induced diabetic mice: isolation of active fraction having islet regenerative and glucosidase inhibitory properties. *Canadian journal of physiology and pharmacology*. 2013; 91(5):325-31.