Bangladesh J. Bot. **53**(3): 503-510, 2024 (September) DOI: https://doi.org/10.3329/bjb.v53i3.76264

EVALUATION OF ANTIDIABETIC ACTIVITIES OF LINSEED (*LINUM USITATISSIMUM* L.) SPROUT EXTRACTS IN ADULT STREPTOZOTOCIN-INDUCED (A-STZ) LONG-EVAN RATS

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Keywords: Streptozotocin, Long-Evan rats, Antidiabetic Activity, *Linum usitatissimum* L.

Abstract

The study investigates the anti-diabetic properties of linsseed (*Linum usitatissimum* L**.**) sprout extracts in adult streptozotocin (STZ)-induced Long-Evan rats, addressing the limitations of existing treatment strategies in Bangladesh. The diabetic rats were administered orally with 400 mg/kg body weight of methanolic extracts of linseed sprout with a lab diet and glibenclamide as a positive control**.** Blood sugar levels were measured before, after, and after STZ injection in Long-Evan rats. The lipid profiles and liver function tests were performed. Administration of extracts of linseed sprouts and a standard anti-diabetic drug reduced the glucose level from 9.1 mmol/l to 6.0 mmol/l and 10.5 mmol/l to 6.6 mmol/l , respectively. The study also showed a healthy level of liver function tests and lipid profiles. The *in vivo* analysis of the potential antidiabetic activity of linseed sprouts in the STZ-induced diabetic mouse model might be used to treat and manage diabetes.

Introduction

Diabetes mellitus (DM) is becoming a pandemic worldwide. The highest percentages of increases in disease prevalence are likely to be in developing nations (Chowdhury *et al*. 2022). Various extensive screenings have been performed on the use of traditional medicinal plants for treating DM in many ethnomedical systems within the subcontinent (Ocvirk *et al*. 2013).

The number of diabetic patients has been increasing over the years in Bangladesh. The existing treatment strategy can benefit to some extent but is limited to efficacy and side effects. Therefore, food supplements may be one of the important approaches to combat diabetes and its associated complexities. Sprouts have been studied and considered to protect against various chronic illnesses (Ruiz Hernández *et al.* 2021). Sprouts possess several properties that help keep blood sugar levels low (Rehman *et al*. 2021). However, in Bangladesh, the traditional medicinal plants that are used for the treatment of DM need more investigation for better understanting the mechanism of this plants. Therefore, these herbal remedies are important objects of research, especially in the context of the virtually exploding prevalence of DM in Bangladesh.

Linum usitatissimum L. is a blue or purple blooming crop of the Linaceae family, commonly known as lin seed in English or tisi in Bangla, and is found in tropical areas (Jhala and Hall, 2010). Linseed is important in food and illness studies because of its medicinal properties linked with its high amount of linolenic acid and a significant lignan, secoisolariciresinol di-glucoside (SDG). According to earlier studies, linseed has antioxidant, anticancer, antiviral, bactericidal, anti-inflammatory, and antiatherosclerotic properties (Halligudi 2012), a reduction in blood

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glucose and cholesterol levels (Kristensen *et al.* 2012), and a reduction in the chance of dyslipidemia, overweight, and insulin resistance (Saxena *et al.* 2014). Sprouts possess several properties that help keep glucose levels low (Yao *et al.* 2013). Hence, the present study evaluated the potential pharmacological activity of linseed sprouts in diabetic mice models.

Materials and Methods

The ethical approval was approved by the institutional ethical committee of Khwaja Yunus Ali University (Ref: KYAU/IRB/08-2021) and Jahangirnagar University (Ref:BBEC,JU/ M2022/11(1)). First, the seeds were disinfected with mercuric chloride ($HgCl_2$, 0.1%) three times, and then washed (three times) using distilled water (Ramakrishna *et al.* 1991). For 8-10 days, sprouts were dried at room temperature in the shade, then powdered with a crushing machine and sieved

Linseed Linseed sprouts

Fig. 1. Production, and harvest of linseed sprouts for antidiabetic activity.

In 200 ml of methanol and ethyl acetate, 100 gm of powders were added separately. Then it was shaken several times to form a fine mixture of solution. To obtain extracts, powders were dried in the rotary flash evaporator at 40°C until proper dehydration, and then pure extracts were stored in discrete impenetrable holders at a steady temperature of 4°C for additional examination.

Long-Evan rats 180-250 gm body weight) were obtained and selected as experimental animals to do this study. Rats were collected from the Animal Resource Division of ICDDR,B Mohakhali, Dhaka. It was used for the assurance of an unusually high concentration of blood glucose. Before the experiment, rats need to be adapted to the lab condition for 7 days at 22°C (±5°C) with 40-70% relative humidity. These procedures were approved by the Khwaja Yunus Ali University (KYAU) Animal Ethical Committee and Jahangirnagar University Ethical Committee.

Fig. 2. Photograph of immature Long Evan rat.

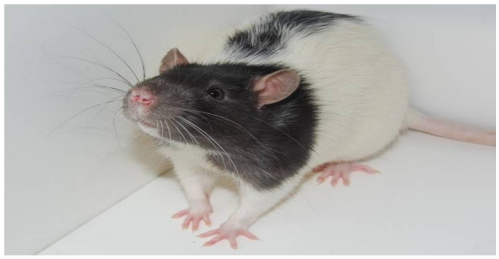

Fig. 3. Photograph of mature Long Evan rat.

In the experiment, Long Evan rats were divided into four groups consisting of at least 3 rats in each group (Group I: non-diabetic control (just a laboratory diet was used); Group II: diabetes control (STZ-induced and a laboratory diet was used); Group III: Standard control (STZ-induced and glibenclamide at a dose of 5 mg/5ml/kg body weight with lab diet). Group IV: diabetic rats were given a methanol extract of linseed sprout at 400 mg/kg body weight). There is a total of twelve Long Evan rats were taken for this study (including 9 diabetic surviving rats and 3 normal rats). Before starting an experiment, the rats were weighed and carefully marked.

Table 1. The experimental design of antidiabetic activity of linseed sprout extracts in adult streptozotocin-induced (A-STZ) Long-Evan Rats.

Group of Long Evan Rats	Treatment	Dose mg/kg body weight
Group-I (Normal control)	Normal	Normal diet
Group-II (Diabetic control)	Diabetes-induced rats but non-treated with standard drugs and extracts	Normal diet
Group-III (Standard control)	Diabetes-induced rats were treated with standard drug at a dose of 5mg/5ml WFI/kg of body weight	Glibenclamide + Normal diet
Group-IV (Experimental Extract)	Diabetes-induced rats were treated with sprout extracts of methanol on a lab diet.	Methanol extracts (400mg/ kg.b. wt.) + Normal diet

Before induction of diabetes in the rats, fasting blood glucose and weight were measured and noted. Normally, Long-Evan rats were permitted to fast for 12 hrs and then 55 mg/10ml WFI/kg of body weight were intravenously given with freshly prepared streptozotocin (STZ) (Shibly *et al.* 2018). This dose permanently destroys the beta cells of the pancreas and increases the glucose level in the blood which is diabetes mellitus. After seven days, all enduring rats' blood sugars and weight were determined using BioHermes Limpid Blood Glucose Meter (China) and AND Gulf EK600H (China). Long Evan rats with fasting blood glucose levels of more than 7.2 mmol/l were considered diabetic and selected for further analysis.

Sprout extracts were given to the laboratory diet for 18 hours at a dose of daily 400 mg/kg.b.wt of methanol for Long Evan rats- Group-III, and Group-IV respectively. The drug glibenclamide was given as a medication control at a dose of 5 mg/5ml/kg body weight for pharmacological efficacy investigation. The extracts of sprouts were administered orally with food for four weeks. During the administration of extracts, the experimental rat's blood glucose from the tail of its edge and weight were measured on the 1^{st}, 7^{th}, 14^{th}, $21^{st,}$ and 28^{th} day after treatment by using the BioHermes Limpid Blood Glucose Meter and weight machine, respectively, in both diabetic and control rats, following the manufacturer's guidelines.

After the completion of the treatment schedule (28^{th} day), rats of all groups I-V were exposed to chloroform and sacrificed by cervical dislocation. Blood samples and organs were collected for biochemical and histopathological investigation respectively.

Results and Discussion

The current study looks at the anti-diabetic efficacy of linseed sprout extract on blood glucose levels in STZ-induced Long-Evan diabetic rats. STZ-induced hyperglycemia has been reported as a helpful experimental paradigm for studying hypoglycemic drug activity. Streptozotocin kills pancreatic beta cells and causes hyperglycemia.

After being stimulated with STZ within the 7th day of the study, experimental rats had significantly higher blood glucose levels than normal rats (Tables 2, 3, and 4). linseed sprout extracts were efficacious in a dose-dependent manner. Injection of streptozotocin intraperitoneally in Long-Evan rats considerably raised blood sugar levels of 10.9 mmol/l , 10.5 mmol/l , and 9.1 mmol/l in Group-II Diabetic control group, Group-III Standard and Group IV: Experimental group respectively but normal blood glucose levels in Group I: Normal control (5.8 mmol/l). The standard drug of glibenclamide caused a remarkable (p0.05) drop in blood glucose levels (from 10.5 mmol/l to 6.6 mmol/l) in 28 days. The linseed sprout extract-treated group showed a notable diminution of glucose level in the blood (from 9.1 mmol/l to 6.0 mmol/l in methanol extract-treated groups on the 28th day).

Table 2. Effect of sprout extracts (methanolic extract) on weight and serum glucose levels (Mean) in experimental STZ-induced diabetic Long-Evan rats.

Groups		Group-I:		Group-II:		Group-III:		Group-IV	
Weight-Sugar (W-S)		W	S	W	S	W	S	W	S
Before injecting with STZ (Baseline)		115	5.7	140	6.3	110	6.0	110.0	6.6
After being injected with STZ (on 7th Day)		128	5.8	161	10.9	133	10.5	123.8	9.1
Treated	14th day	142	6.2	193	11.3	155	7.3	181	7.3
	21st day	165	5.9	205	10.0	162	6.4	154.3	6.1
	28th day	171	6.0	205	9.5	163	6.6	155.0	6.0
	Average	144	6.0	181	10.0	145	7.0	144.8	7.0

Table 3. Effect of blood glucose levels (Mean ± SE) treated with sprout extracts in experimental STZ-induced diabetic Long-Evan rats

Groups		Group-I	Group-II	Group-III	Group-IV
Before injecting with STZ (Baseline, 1st)		5.7 ± 0.26	6.3 ± 0.13	6.0 ± 0.31	5.3 ± 0.21
After being injected with STZ (7th day)		5.8 ± 0.15	10.9 ± 0.17	10.5 ± 0.20	9.1 ± 0.31
Treated	14th day	6.2 ± 0.32	11.3 ± 0.09	7.3 ± 0.19	6.9 ± 0.35
	21st day	5.9 ± 0.28	10.0 ± 0.30	6.4 ± 0.26	6.1 ± 0.19
	28th day	6.0 ± 0.24	9.5 ± 0.50	6.6 ± 0.29	6.0 ± 0.27
	Average	6.0 ± 0.04	10.0 ± 0.38	7.0 ± 0.19	7.0 ± 0.20

In the current research, Streptozotocin intraperitoneal injections were used to produce diabetes (Karim *et al.* 2020). Streptozotocin's methylnitrosourea moiety's ability to alkylate DNA dictates its toxicity (Sharma *et al*. 2014). DNA methylation ultimately causes beta-cell death, even though streptozotocin also methylates proteins (Eleazu *et al.* 2013). This study showed the antihyperglycemic effect of alcoholic extract of sprouts of linseed. Here, the standard antidiabetic drug glibenclamide was used in diabetes-induced rats and treated as standard control. In all experiments, compared to standard medicine, the extract both by itself and in combination with glibenclamide significantly lowered blood sugar levels.

The current study's findings largely reflect previous research that found a considerable rise in body weight after treatment with herbal remedies in hyperglycemic mice (Pang *et al*. 2019). Another finding revealed that diabetic rats that were given the methanol component of vegetables twice daily acquired body weight (Omoboyowa *et al.* 2016). It is now known that beta-cell function gradually declines with age, which may raise the chance of developing type II diabetes (Shibly *et al.* 2015).

Table 4. Effect of Blood glucose levels (mean ± percentage) treated with linseed sprout extracts and glibenclamide in experimental STZ-induced diabetic Long-Evan rats.

Groups		Group-I	Group-II	Group-III	Group-IV
Before injecting with STZ (Baseline, 1st)		5.7	6.3	6.0	5.3
After being injected with STZ (on 7th Day)		5.8	10.9	10.5 (100%)	9.1 (100%)
Treated	14th day	6.2	11.3	7.3 (69.5%)	6.9 (75.8%)
	21st day	5.9	10.0	6.4 (60.9%)	6.1 (67.0%)
	28th day	6.0	9.5	6.6 (62.8%)	6.0 (65.9%)
	Average	6.0	10.0	7.0 (66.7%)	7.0 (71.4%)

STZ was utilized to induce diabetes since it was demonstrated to produce pancreatic beta-cell degranulation. Following a total of 28 days of therapy, only one dose (400 mg/kg.b.wt) of the methanolic extract of sprouts and glibenclamide medication showed substantial changes in fasting blood sugar levels when compared to the untreated control group and standards. The current study found that linseed extract effectively reduced blood sugar levels in STZ diabetic Long-Evan rats. Both extracts significantly reduced hyperglycemia without inducing hypoglycemia.

Daily oral administration of sprout extracts of linseed at the dose of 400 mg/kg in diabetic rats for 28 days were normal lipid profile levels in all rats as depicted in Fig. 3. Lipids are critical in the development of diabetes. When people take STZ, their plasma cholesterol, triglyceride, and HDL levels frequently rise, as do their blood glucose levels (Hirano 2018). Diabetes is associated with higher levels of serum lipids, which is an increased risk factor for coronary artery diseases (Bhowmik *et al*. 2018). When STZ induces diabetes, a high level of fatty acids in the plasma drives the liver to produce more phospholipids and cholesterol from unutilized fatty acids. Both of these molecules and excess triglycerides produced in the liver may be transported into the bloodstream as lipoproteins (Levant *et al.* 2013). HDL is a lipoprotein that is beneficial to our

hearts. It transports cholesterol from various regions of the body to the liver, which aids in the prevention of coronary heart disease. In these trials, sprout extracts were found to lower serum lipids and boost HDL-cholesterol levels in diabetic rats (Fig. 4).

Liver is an important insulin-dependent organ that regulates glucose and cholesterol levels. The most prevalent enzymes produced by the liver in experimental rat liver function tests (LFTs) treated with sprouts were SGOT (Serum glutamic oxaloacetic transaminase), SGPT (Serum glutamate pyruvate transaminase), and alkaline phosphatase (SALP). All of the pamperers of liver function tests namely serum creatinine, serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), serum alkaline phosphatase (SALP) were normal as compared to diabetic control rats as depicted in Table 5.

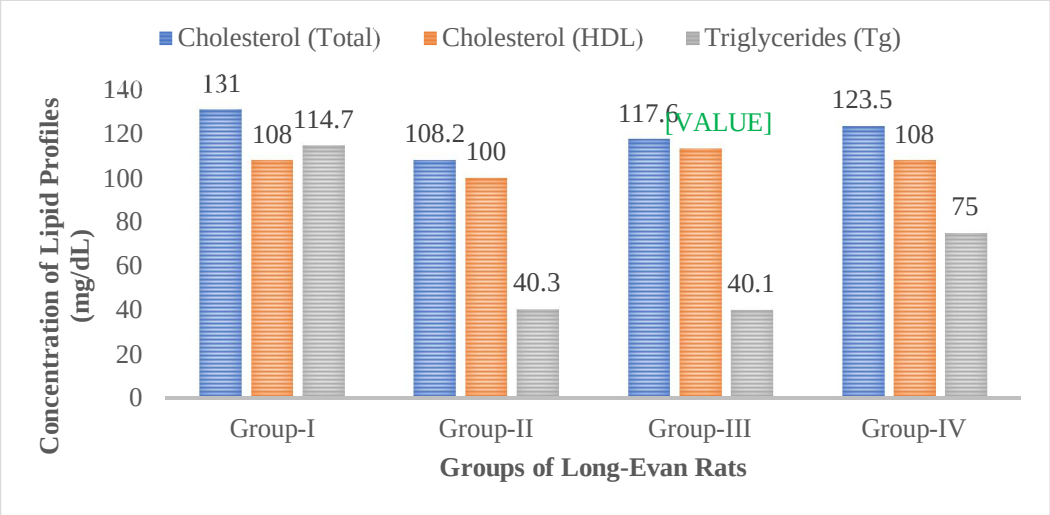

Fig. 4. Estimation of experimental Rat's Lipid profiles treated with linseed sprout sprouts. Healthy values for rats: cholesterol (Total) = 140–240 mg/dL, cholesterol (HDL) = >45 mg/dL, and triglycerides (Tg) = 40–140 mg/dl (www.janvier-labs.com)

Table 5. Effect of linseed sprout extracts and gilbenclamide therapy at repeated doses for four weeks on liver function tests (serum Creatinine, SGPT, SGOT, and SALP) in STZiDLERs. Healthy values for rats: Creatinine: 0.4–1.4 mg/dL; SGPT: up to 63 U/L; SGOT: up to 58 U/L; and S. ALP: 30–300 IU/L (www.janvier-labs.com)

Groups	Creatinine (mg/dL)	SGPT (U/L)	SGOT (U/L)	SALP (IU/L)
Group-I	0.95	37	33	290
Group-II	0.52	22	19	209
Group-III	0.43	25	26	189
Group-IV	0.72	25	23	224

In conclusion, it was shown that linseed sprout extracts containing methanol can help diabetic rats by lowering their glucose levels and maintaining normal lipid profiles and liver functions. This study found that linseed sprout extracts of methanol have powerful anti-diabetic and anti-lipidemic effects in normal and diabetic rats. In the future, more research is needed to figure out what chemical components of linseed sprouts do these things. In this study, the combination of sprouts and STZ at 400 mg/kg b.wt. and 5 mg/5 ml water for injection/kg dosage, respectively showed improvement in the declination of blood sugar levels and unchanged of their SGOT, SGPT, and SALP levels. While these results are encouraging, further research is warranted to elucidate the precise mechanisms involved and to assess the long-term effects of sprout consumption on diabetes management. Additionally, clinical trials with larger sample sizes and diverse populations are needed to validate the general applicability of these findings.

Acknowledgment

The authors express their sincere gratitude to the authorities of the Department of Botany, Jahangirnagar University, and the Department of Microbiology and Pharmacy at Khwaja Yunus Ali University, Sirajganj, for providing the necessary laboratory facilities and resources that made this research possible. We would also like to extend our appreciation to the technical staff for their support during the experimental work. Special thanks go to the Institutional Ethical Review Boards for their approval and guidance throughout the study. Lastly, we acknowledge the valuable assistance and encouragement from colleagues and fellow researchers during the research process.

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(Manuscript received on 10 March, 2024; revised on 10 September, 2024)