ORIGINAL ARTICLE

Antidiabetic potential of the combination of fermented soy milk and flaxseed milk in alloxan-induced diabetic rats

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Abstract

Background: Type-1 diabetics are an autoimmune disease first appears at childhood or early adolescence. The prevalence of this disease is increasing around the world and no preventive procedure exists. The naturally effective flaxseed and soybeans have various health benefits. The study was carried out to evaluate the effects of probiotic soy and flaxseed milk in alloxan-induced rats. **Methods:** Fermented soy and flaxseed milk (FSFM) was screened for their antidiabetic potential in alloxan-induced diabetic rats using the oral route. Fermented milk with probiotics increases the efficacy of isoflavones in the treatment of diabetic mellitus. Single and multiple dose (28 days) studies were conducted to evaluate the efficacy of the fermented milk through blood glucose level, body weight, hematological profile, estimation of insulin level, biochemistry parameters, as well as homeostatic model assessment for insulin resistance. **Results:** Intraperitoneal administration of alloxan into the rats caused significant diabetogenic response with increase in the levels of blood sugar as compared with normal rats. As a control, metformin was used to compare the potential of FSFM in rats. Oral administration of low dose and high dose of FSFM significantly reduced blood glucose level in normoglycemic and alloxan-induced hyperglycemic rats with comparative increase in the insulin production as that of metformin dose. There were no evident hematological changes in all the groups. The body weight and feed intake of the diabetes induced rats were increased after treatment with FSFM throughout the study period as compared with the normal and diabetic control group. **Conclusion:** The observed data showed the antidiabetic potential of FSFM against Type-1 diabetes of alloxan-induced rats and can be used as an antidiabetic supplement. **Clinical Impact:** At present, there is no effective medication to cure Type 1 diabetics. The available treatments are causing number of side effects in long-term usage. The present study on flaxseed and soy milk shows that it is effective in reducing Type 1 diabetics without any after effects.

Key words: Antidiabetic activity, diabetic rats, flaxseed milk, homeostatic assessment, hyperglycemic, probiotics, soy milk

INTRODUCTION

Diabetes mellitus (DM) is a predominant metabolic disorder found associated with the cardiovascular, nephropathy, ophthalmic, obesity, and hepatic diseases. The immune-mediated depletion of β-cells leads to the lifelong dependence on external insulin.[1] It is estimated that 75% of the world population is affected by this disease.[2] Impaired functioning of the metabolism of macronutrients encounters long-term health complications.[3] The continuous medication of diabetes treatments such as pharmacological hypoglycemic agents and insulin causes various side effects. To prevent these hazards, pharmacognosy and alternative therapies have come into limelight and have proven effective as hypoglycemic drug.[4,5] Thus, naturally available bioactive compounds have to be identified to overcome these metabolic disorders.

Many epidemiological examination and human clinical studies suggest that soy products are essential for lowering

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diabetes risk and improved insulin secretion. Soy milk is enriched with calcium, vitamins, high-quality proteins, saturated fatty acids, and certain bioactive components.[6] They are suitable for the people who are lactose intolerant and are effective as antidiabetic and antiobesity.[7] The isoflavone contents in soy are genistein, daidzein. and glycitein. They are helpful in the treatment of cardiovascular disease and osteoporosis.[8]

Flaxseed, a supplement, may be effective in reducing complications associated with diabetes. It is enriched with high content of alpha-linolenic acid,[9] dietary fiber, and high amount of omega-3 fatty acid.[10] It is void of cholesterol and naturally lactose free, thus it is suitable for lactose intolerant people. The phenolic compounds are potent for antioxidative and hepatoprotective properties. The flaxseeds are rich in phytoestrogen (PE) lignans which have been shown to have tumor inhibitory properties in humans and rodents of previous studies.[11] Fermentation of soy and flaxseed milk increases the efficacy of isoflavones and peptides in the treatment of DM.[12] Probiotics containing *Bifidobacterium* and *Streptococcus thermophilus* are essential in reducing total cholesterol (TC) and low-density lipoprotein and increase the level of high-density lipoprotein.[13] The isoflavone glycosides are changed into isoflavone aglycones during fermentation, thus it reduces the genistein efficacy of soy milk.[14]

The fermentation process is an efficient way to produce bioactive peptides. Apart from the increase in bioavailability of isoflavones, it promotes protein digestion and solubilization of calcium and improves health and immune system.[7] The supplement flaxseeds are rich in PE lignans which have been shown to have tumor inhibitory properties in humans and rodents of previous studies. Thus, to suppress the PE of soy milk, flaxseeds can be added for the better result due to its various health benefits. The consumption of omega-3 fatty acid from flaxseed can potentiate the probiotic effects in the small intestine by altering fatty acid exhaustion.[10]

Streptozotocin reflects toxin-induced reflection of beta-cell, whereas alloxan induces ROS-mediated beta-cell toxicity.[15] The present study encounters whether the combined products of probiotics, soy milk, and flaxseed milk had an antidiabetic effect on blood glucose levels, hematological, insulin resistance (IR), and biochemical parameters with alloxan-induced diabetic insulin-dependent model.

MATERIALS AND METHODS

Preparation of Soy and Flaxseed milk

Soy and flaxseed milk were prepared by following the method described in previous studies.[16] First, the soybeans and flaxseeds were soaked separately in distilled water overnight and the soaked beans were blended individually with distilled water 10 times their weight. The mixture was then filtered to produce milk.[17] The obtained soy and flaxseed milk were combined for fermentation with probiotics. The fermented milk was then stored in refrigerator. The samples FSM samples were prepared once in a week in a hygienic conditions. The Fermented Soy and Flaxseed Milk (FSFM) samples were prepared in Low Dose(1%) and High Dose (5%) once in a week under hygienic conditions.

Experimental Animals

Initially, male Sprague Dawley rats weighing 100–140 g were selected and housed in stainless steel cages (4/cage) in groups of six animals per cage and kept in the animal house for 1 week for proper acclimatization before starting the experiment under controlled conditions of illumination (12 h light/12 h darkness) with temperature ranging 20–25°C. They were housed under the above laboratory conditions, maintained on standard pellet diet and water. In our study, animal studies were conducted as per the norms of the committee for the purpose of supervision of experiments on animals. All the studies were conducted in compliance with guidelines and approved by the Institute of Animal Ethics commission no. IAEC-P.No.14/PPK/2016;02/16.

Diabetes Induction

After 1 week of the acclimatization, male Sprague Dawley rats were injected intraperitoneally once with low dose of alloxan dissolved in saline (80–150 mg/kg), to induce partial insulin deficiency. The glucose value was noted before alloxan injection. This is considered as basal value. After 48–96 h of alloxan injection, the rat's fasting blood glucose value was noted using tail flick method. A glucometer (Accu-Check, Roche, Germany) was used to obtain blood glucose levels. The animals would display hyperglycemia and glucose intolerance.

Study Design

The treatment period for the study was 28 days. Animals with similar degrees of hyperglycemia (mostly >95 mg/dl) were considered, and according to their blood glucose value [Table 1], animals were randomized and divided into groups.[18]

Group 1 included normal control (G1), Group 2 included diabetic control (G2), Group 3 included diabetic rats given 1 ml/day metformin (350 mg/kg, p.o) (G3), Group 4 included diabetic rats given low dose of FSFM (G4), and Group 5 included diabetic rats given high dose of FSFM (G5).

Biochemical Parameter Analysis

Body weight and blood glucose levels of the rats were monitored periodically. The rats were fasted for 12 h on

Table 1: Body weight

Groups	Body weight in grams		
	Initial	Final	Mean
Normal control (G1)	114.83±18.422	144.67±17.705	130.52±0.82
Diabetic control (G2)	110.5±25.018	112.0±11.454	117.46±3.48
Metformin 350 mg/kg (G3)	119.83±15.575	147.33±10.309	133.15±2.877
FSFM (L) (G4)	114.5±12.57	145.67±15.029	130.51±1.99
FSFM (H) (G5)	111.17±10.53	147.67±2.42	132.14±1.83

FSFM: Fermented soy and flaxseed milk

Table 2: Feed intake in grams

Groups	Feed intake in grams				
	Week 1	Week 2	Week 3	Week 4	Mean±SD
Normal control (G1)	8.43	9.86	13.00	15.00	11.57±2.98
Diabetic control (G2)	8.86	6.14	5.71	4.57	6.32±1.82
Metformin (350 mg/kg G3)	8.57	8.29	9.86	10.29	9.25±0.97
FSFM (L) (G4)	7.71	7.43	8.71	10.14	8.5±1.23
FSFM (H) (G5)	8.57	7.86	8.71	12.14	9.32±1.92

FSFM: Fermented soy and flaxseed milk, SD: Standard deviation

Table 3: Blood glucose value of each group

Groups	Pre-intrusion	Post-intrusion				Mean±SD
		Week 1	Week 2	Week 3	Week 4	
Normal control	75.33	77.33	79.50	79.67	78.50	78.75±0.65
Diabetic control (G2)	149.33	151.17	152.33	154.17	150.33	152.00±4.34
Metformin (350 mg/kg)	149.5	137.50	128.67	125.83	120.50	128.13±1.22
FSFM (L)	149.5	144.33	138.00	133.50	130.67	136.63±5.00
FSFM (H)	150.33	142.83	135.50	127.33	119.17	131.21±1.20

FSFM: Fermented soy and flaxseed milk, SD: Standard deviation

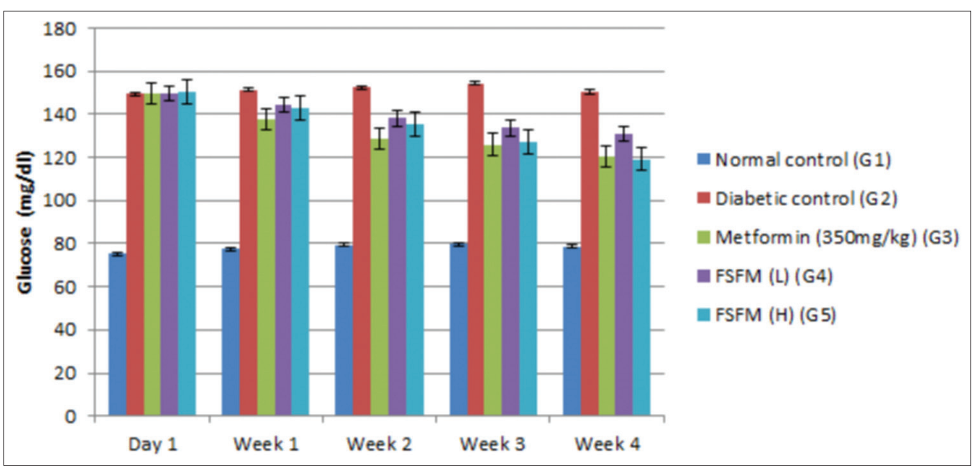

Figure 1: Estimation of glucose in mg/dl. **P* < 0.05 is the statistically significant data *P* < 0.0001

the day 29, with animal restrainer (e.g., Broom restraint, Plas Labs), the rats were anesthetized using isoflurane, approximately 5 ml of blood was collected by tail flick method, 1–2 ml of blood was collected in a tube containing heparin for analyzing biochemical parameters. Blood plasma was separated by centrifuging at 3500 rpm for 10 min. The remaining whole blood was used for measuring hematological parameters.

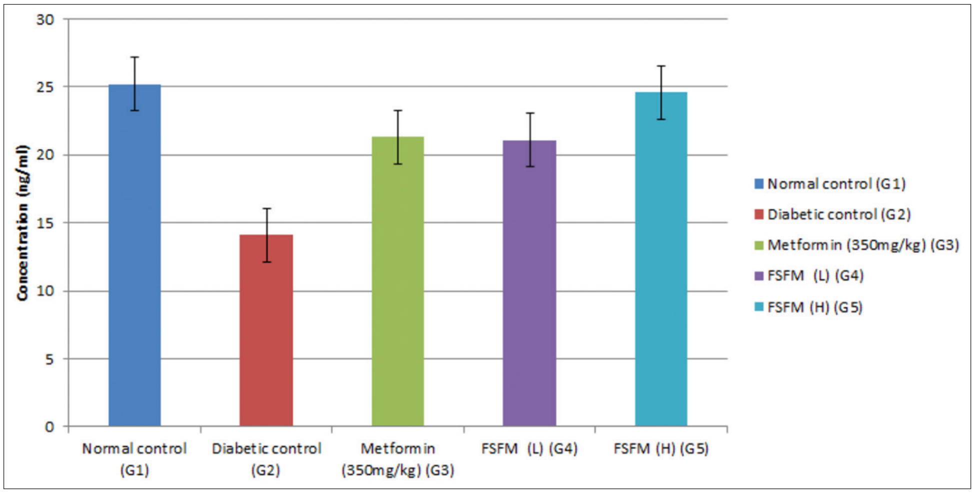

Figure 2: Estimation of insulin. Each value is in mg/ml. *$P < 0.05$ is the statistically significant data. $P < 0.0001$

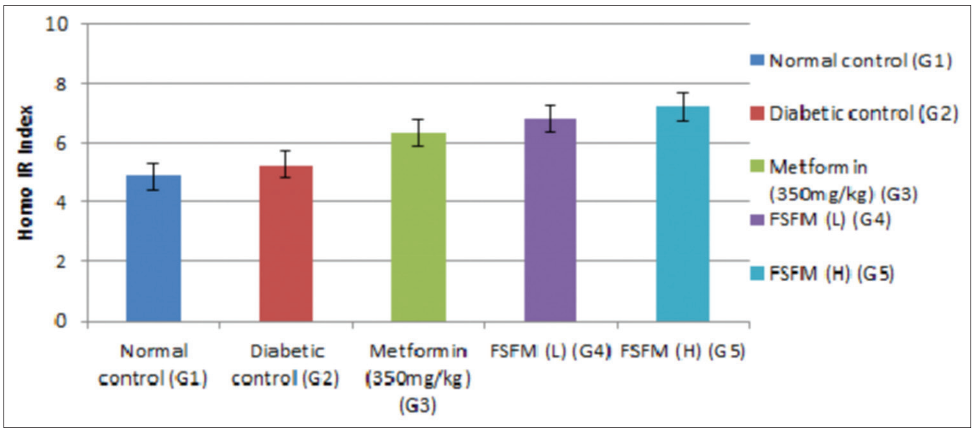

Figure 3: Evaluation of homeostatic model assessment for insulin resistance. *$P < 0.05$ is the statistically significant data $P = 0.0001$

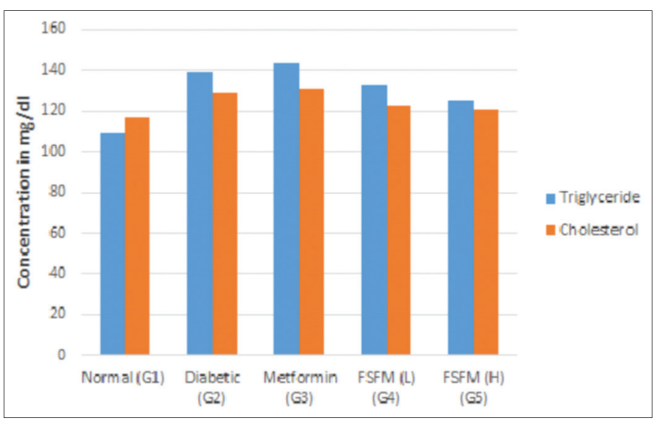

Figure 4: Effect of fermented soy and flaxseed milk on triglyceride and cholesterol. Each value is in mg/ml. *$P < 0.05$ is the statistically significant data. Cholesterol $P = 0.42$, Triglyceride $P = 0.003$

Homeostatic Assessment (HOMA)-IR

The major function of insulin is to counter the concerted action of a number of hyperglycemia-generating hormones and to maintain low blood glucose levels. Since there are numerous hyperglycemic hormones, untreated disorders associated with insulin generally lead to severe hyperglycemia and shortened life span. The estimation of insulin is done using ELISA method; insulin resistance was determined using the HOMA-IR (Mathews *et al.*, 1985) using the following formula:

HOMA-IR index = [fasting glucose (mmol/L) × fasting insulin (μU/ml)]/22.5

Table 4: Estimation of insulin and HOMO-IR index

Groups	HOMO-IR index (Mean±S.D)	Insulin (Mean±S.D)
Normal control (G1)	4.88±0.35	25.15±0.68
Diabetic control (G2)	5.25±0.68	14.11±0.48
Metformin (350 mg/kg)	6.33±0.59	21.28±1.74
FSFM (L)	6.82±1.13	21.12±0.76
FSFM (H)	7.21±1.07	24.56±1.13

FSFM: Fermented soy and flaxseed milk, SD: Standard deviation, HOMO-IR: Homeostatic model assessment for insulin resistance

Table 5: Estimation of biochemical parameters

Parameter	Normal	Diabetic	Metformin	FSFM (L)	FSFM (H)
Triglyceride	109	139	143	133	125
Cholesterol	116.83	128.33	130.83	122	120.67

FSFM: Fermented soy and flaxseed milk

RESULTS

Body Weight

The body weight and feed intake of the diabetes induced rats were increased after treatment with FSFM throughout the study period as compared with the normal and diabetic control group [Tables 1 and 2]. Results of ANOVA with repeated measures showed significant changes in the body weight of diabetic rats from the 1st week until the end of the experiments [Table 2].

Blood Glucose

The blood sugar levels of both normal and experimental rats before and after 28 days of treatment are shown in Table 3 and Figure 1. Intraperitoneal administration of alloxan into the rats caused significant diabetogenic response in rats with significant increase in the levels of blood sugar as compared with normal rats. The blood glucose level was increased, decreasing the insulin secretion with variation in calculated HOMO-IR index [Figures 2 and 3, Table 4]. Following oral administration of soya milk low and high dose, the blood glucose level was significantly reduced, with comparative increase in insulin production in comparison with the values of metformin dose (350 mg/kg).

Hematological Parameters

No apparent hematological changes, but some parameters such as red blood cells, hemoglobin, and white blood cells reduced but not significantly. This may be due to arose of diabetes due to alloxan induction as the treatment group shows no or very less changes. Effects of treatments on serum lipids are shown in Table 5 and Figure 4. TC and triglyceride concentrations of all treated diabetic rats were slightly decreased ($P< 0.05$) compared to the control group and metformin-treated group, with maximum reduction seen in the FSFM high dose.

CONCLUSION

Fermented soy milk and flaxseed milk preparation are active and can be used as antidiabetic supplement. It is beneficial in reducing the implications of diabetes such as weight loss, high blood glucose, and HOMO-IR index. Further, pathological studies will reveal the beta-cell revival and sensitivity to FSFM treatment.

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