Anti-diabetic effects of ethanol extract of *Bryonia laciniosa* seeds and its saponins rich fraction in neonatally streptozotocin-induced diabetic rats

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ABSTRACT

Context: *Bryonia laciniosa* Linn. (Cucurbitaceae) seed is used in traditional medicine for a number of ailments including metabolic disorders. **Aim:** This study evaluated the anti-diabetic action of the ethanol extract of *B. laciniosa* seeds and saponin fraction of it through its effect on hyperglycemia, dyslipidaemia and oxidative stress in neonatally streptozotocin (n-STZ)-induced diabetic rats (n-STZ diabetic rats). **Materials and Methods:** Ethanol extract (250 and 500 mg/kg; p.o.), saponin fraction (100 and 200 mg/kg; p.o.) and standard drug glibenclamide (3 mg/kg; p.o.) were administered to diabetic rats when the rats were 6 weeks old and continued for 10 consecutive weeks. Effects of ethanol extract and saponin fraction on various biochemical parameters were studied in diabetic rats. **Results:** The treatment with ethanol extract and saponin fraction for 10 weeks decrease in the levels of glucose, triglycerides, cholesterol, high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein, serum urea, serum creatinine and diminished activities of aspartate transaminase, and alanine transaminase. The anti-hyperglycemic nature of *B. laciniosa* is probably brought about by the extra- the pancreatic mechanism as evidenced from unchanged levels of plasma insulin. *B. laciniosa* modulated effect of diabetes on the liver malondialdehyde, reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activity. Administration of ethanol extract and saponin fraction to diabetic rats showed a significant reversal of disturbed antioxidant status. Significant increase in SOD, CAT, and levels of GSH was observed in treated n-STZ diabetic rats. **Conclusion:** The present study reveals the efficacy of *B. laciniosa* seed extract and its saponin fraction in the amelioration of n-STZ diabetic rats.

Key words: Diabetes mellitus, dyslipidemia, hyperglycemia, oxidative stress

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INTRODUCTION

Type 2 diabetes mellitus is one of the most common metabolic disorders with increasing prevalence during recent years. Type 2 diabetes mellitus is characterized by the progression of diminishing insulin sensitivity and impaired pancreatic function. Lifestyle modifications or appropriately prescribed drugs such as metformin, sulfonylureas or thiazolidinediones as monotherapy or add-on treatment are essential to tackle the disease; however, many patients still cannot achieve satisfactory glycemic control. Therefore, searching for alternative protective strategies is of great interest.[1]

Streptozotocin (STZ) is a chemical substance specifically toxic to pancreatic β-cells. It is taken into the pancreatic β-cells by glucose transporter-2. When injected into adult rats, STZ can cause type 1 diabetes with severely elevated blood glucose level. However, when STZ is administered to neonatal rats, the neonates experience acute hyperglycemia within the first few days, and the blood glucose gradually decreases thereafter. The surviving rats exhibit decreased β-cell mass and developed type 2 diabetes with the features described in type 2 diabetes patients (hyperglycemia, polyphagia, polydipsia, polyuria, insulin resistance and abnormal glucose tolerance) after 6 weeks. Therefore, this

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model provides an ideal platform for β-cell regeneration study and new anti-diabetic drug screening.[2-4]

Concurrently, phyto-chemicals identified from traditional medicinal plants are providing exciting opportunities for the development of the new type of therapies.[5] There are many compounds isolated from traditional medicinal plants with anti-diabetic activity[6] of which saponins are promising compounds with the potential to be developed into new drugs as anti-diabetic.[7] Many studies have demonstrated that saponins significantly decreased the plasma glucose level and plasma triglyceride level. It was previously reported that saponins possess anti-lipid peroxidation activity to protect the vascular endothelium, and to prevent diabetic complications.[8]

In the present study, we have selected a plant which is used as a hypoglycemic herb by native people of Porbandar region. *Bryonia laciniosa* Linn. syn *Bryonopsis laciniosa* Linn. (Cucurbitaceae) Syn *Diplocyclos palmatus* (Linn.) Jeffrey (Cucurbitaceae) locally known as 'Shivlingi' and 'Gargumaru' is distributed throughout India. It is an annual climber with bright red fruits and is reported to be highly medicinal.[9] Locally in India its seeds are being used for promoting conception in women. Ayurvedic literature survey indicated the use of the entire plant is a bitter tonic, hepatoprotective, anti-pyretic, laxative and used to correct the metabolic abnormalities.[10-12] From leaves, a bitter principle bryonin has been reported. From seeds, Saponin molecules are identified by thin layer chromatography and antibacterial, antifungal, anti-inflammatory and diuretic activities were investigated.[13] Occurrence of punicic acid, goniothalamine, and glucomannan has been reported in this plant.[14-16] Antimicrobial, larvicidal, anti-inflammatory, cytotoxic, analgesic, anti-pyretic and anti-diabetic activities have been reported for various extracts of this plant.[16-18] There are other two species of *Bryonia* reported earlier in the literature. One of the species *Bryonia alba* possess antidiabetic activity.[19] We also investigated the anti-diabetic potential of seeds of *B. laciniosa* in STZ induced type-1 diabetic rats.[20] Therefore, the present study was designed to evaluate the anti-diabetic effects of *B. laciniosa* seeds in neonatally streptozotocin (n-STZ) induced diabetic rat (n-STZ diabetic rats).

MATERIALS AND METHODS

Materials

Streptozotocin was purchased from Sisco Research Laboratory Pvt. Ltd., India. All other standard chemicals were obtained from common commercial suppliers.

Collection of plant material and extraction

The fruits of *B. laciniosa* were collected in October 2009 to December 2009 from Anand and Jamnagar region of Gujarat, India. The plant was identified and authenticated by Dr. A.S. Reddy of department of Bioscience by comparison with voucher specimen No. VSM502 and ARM 2174 at the Prof. G.L. Shah Herbarium of Sardar Patel. University, Vallabh Vidyanagar, Anand, Gujarat, India. The fruits were macerated in a large amount of water and passed through large pore size sieve to separate seeds. The seeds were grinded mechanically to make powder. The powdered seeds were extracted with ethanol by Soxhlet to give *B. laciniosa* ethanol extract (BLEE). The alcohol extract was concentrated, suspended in distilled water and then partitioned with *n*-butanol saturated with water. The upper *n*-butanol layer was collected in a glass flask, and lower aqueous extract was collected and further extracted with *n*-butanol two more times to increase the yield. The *n*-butanol extract was pooled and evaporated using a rotary evaporator at 60°C to yield *B. laciniosa* saponin rich fraction (BLSF).

Determination of total saponins

The total saponins content of each extract was determined approximately using the method described by Hiai *et al*.[21] Briefly, the extracts (50 μL) were mixed with the vanillin (8% w/v, 0.5 mL) and sulfuric acid (72% w/v, 5 mL). The mixture was incubated at 60°C for 10 min, cooled in an ice water bath for 15 min and the absorbance read at 538 nm. Oleanolic acid was used as a reference standard, and the content of total saponins was expressed as oleanolic acid equivalents (μg/mg extract).

Animals

Sprague Dawley rats from an inbred colony were bred under well-controlled conditions of temperature, humidity and 12 h/12 h light-dark cycle with diet and water provided *ad libitum*. 2-day-old neonates of either sex were injected intraperitoneally with 90 mg/kg STZ (Sisco, Mumbai, USA) in a 0.1 M citrate buffer (pH 4.5). Control neonates received an equivalent amount of isotonic saline alone. Site of injection was controlled to be under the inguinal fat of the neonates to prevent any leakage of the liquids. All procedures were performed on ice and in darkness to avoid the degradation of STZ. The pups were weaned when 4 weeks old and 6 weeks after the injection of STZ, animals were checked for fasting glucose levels using a commercially available kit (Span Diagnostics Ltd., India). The animals with fasting glucose levels >140 mg/dl were considered diabetic.[1]

Experimental design

In the experiment, a total of 54 rats (42 diabetic rats, 12 normal rats) were used. Study of the effect of BLEE

and BLSF on type 2 diabetic rats involved 2 sets of experiments. The groups of animals in two different set were as follows:

- Set 1: Effect of BLEE on n-STZ diabetic rats ($n = 6$ animals per group)
  - Group 1: Normal control rats were administered vehicle
  - Group 2: Diabetic control rats were administered vehicle
  - Group 3: Diabetic rats were administered BLEE (250 mg/kg)
  - Group 4: Diabetic rats were administered BLEE (500 mg/kg).
- Set 2: Effect of BLSF on n-STZ diabetic rats ($n = 6$ animals per group)
  - Group 1: Normal control rats were administered vehicle
  - Group 2: Diabetic control rats were administered vehicle
  - Group 3: Diabetic rats were administered BLSF (100 mg/kg)
  - Group 4: Diabetic rats were administered BLSF (200 mg/kg)
  - Group 5: Diabetic rats were administered glipizide (10 mg/kg).

All the treatments were administered and given orally (once a day) by gavage syringe at 8:30 a.m. when the rats were 6 weeks of age and continued for 10 consecutive weeks. The rats in control and diabetic groups were administered the same volume of vehicle.

The protocol of this experiment was approved by our institutional animal ethical committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals, the Ministry of Social Justice and Empowerment, Government of India (Protocol No.: IICP/PH/2009-06/02).

Oral glucose tolerance test

At the end of the 10 weeks study, rats were subjected to an oral glucose tolerance test (OGTT). Glucose (1.5 g/kg) was administered to 18 h fasted rats. Blood samples were collected from retro-orbital plexus vein at 0, 30, 60 and 120 min. Serum was analyzed for glucose and insulin. The results were expressed as an integrated area under the curve (AUC) for glucose that was calculated by the trapezoid rule (AUC = C1 + C2/2 × [t1 - t2]) and changes in glucose concentrations during OGTT were expressed as AUCglucose (mg/dl/min).

Blood collection and serum analysis

At the end of 10 weeks of treatment, body weight, food intake and water intake were determined. Thereafter, animals were fasted for 12 h, and blood samples were collected from the retro-orbital plexuses of each rat under light ether anesthesia. The serum was separated and analyzed spectrophotometrically for glucose, cholesterol, triglycerides, high-density cholesterol, creatinine, urea, aspartate amino transferase (AST) and alanine amino transferase (ALT) (Shimadzu, UV-1701E, Japan) using commercially available biochemical diagnostic kits (Bayer Diagnostics Ltd., India). Serum insulin was estimated by radioimmunoassay using kits from Rat Insulin RIA Kit, Linco Research, Inc., St. Charles, MO and γ-counter (Packard, USA). Low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and atherogenic index were calculated.

At the end of the experiment, Liver tissue was excised, rinsed in ice-cold saline and then homogenized in Tris-HCl buffer (pH 7.4) using a Teflon homogenizer. The liver homogenate was then centrifuged in a cooling centrifuge at 5000 × *g* to remove the debris, and the supernatant was used for the analysis of nonenzymatic antioxidants according to the method of Mihara and Uchiyama for malondialdehyde (MDA)[22] and Sedlak and Lindsay for reduced glutathione (GSH).[23] The enzymatic antioxidants such as superoxide dismutase (SOD) and catalase (CAT) were assayed.[24,25]

Statistical analysis

Results are presented as the mean ± standard error of the mean statistical differences between the means of the various groups were evaluated using one-way analysis of variance, followed by Tukey's test. Data were considered statistically significant at $P < 0.05$.

RESULTS

General parameters

Rats which received STZ showed a significant reduction in body weight, increase in food intake, and water intake at the end of 10 weeks when compared with nondiabetic control rats. Noninsulin-dependent diabetes mellitus diabetic rats when treated with BLEE (500 mg/kg, p.o.) and BLSF (200 mg/kg, p.o.) significantly ($P < 0.05$) reduced the food intake and the water intake when compared with diabetic control rats. Further, Treatment with BLEE and BLSF failed to show any changes in the body weight [Tables 1 and 2].

Serum glucose, insulin and oral glucose tolerance test

Rats which received STZ showed a significant hyperglycemia associated with hyperinsulinemia in rats. Treatment with BLEE (250 and 500 mg/kg, p.o.) and BLSF (100 and

200 mg/kg, p.o.) significantly decreased fasting glucose, but not the insulin levels when compared with diabetic control groups. The result of OGTT revealed that AUCglucose significantly increased in diabetic control rats when compared with normal control rats. Treatment with BLEE (250 and 500 mg/kg, p.o.) and BLSF (100 and 200 mg/kg, p.o.) significantly reduced elevated AUCglucose levels [Tables 3 and 4].

Lipid parameters

Diabetic control rats showed significant rise in serum lipid levels when compared with normal control rats. Diabetic control rats produced significant rise in serum cholesterol, triglycerides, VLDL, LDL, decrease in high-density lipoprotein (HDL) levels when compared with control rats. Treatment of diabetic rats with BLEE (250 and 500 mg/kg, p.o.) and BLSF (100 and 200 mg/kg, p.o.) produced significant decrease in serum cholesterol, triglycerides, and VLDL and LDL while no significant change in serum HDL levels when compared with diabetic control group [Tables 3 and 4].

Kidney and liver function tests

Diabetic control rats exhibited significant rise in serum creatinine and urea levels when compared with normal control rats. Treatment of diabetic rats with BLEE (250 and 500 mg/kg, p.o.) and BLSF (100 and 200 mg/kg, p.o.) produced significant decrease in urea and creatinine levels when compared with diabetic control group. Diabetic control rats exhibited significantly higher AST and ALT levels when compared with normal control rats. Treatment of diabetic rats with

Table 1: Effect of BLEE on general parameters of n-STZ diabetic rats

Parameters	Normal control	Diabetic control	Diabetic control treated with BLEE (mg/kg)	
			250	500
Body weight (g/rat)	194.16±7.87	160.83±11.37*	167.83±8.01	177.66±14.81
Food intake (g/rat/day)	13.25±0.26	25.45±0.57	21.39±1.12	18.34±0.97**
Water intake (ml/rat/day)	21.36±1.29	37.45±1.43*	29.23±0.95**	27.56±1.27**

Values are given as mean±SEM for groups of six rats in each. Values are statistically significant at *P<0.05. Statistical significance was compared within the groups as follows: *Diabetic rats were compared with control rats, **Treated diabetic rats were compared with diabetic rats. SEM: Standard error of the mean, BLEE: *Bryonia laciniosa* ethanol extract, n-STZ: Neonatally streptozotocin

Table 2: Effect of BLSF on general parameters of n-STZ diabetic rats

Parameters	Normal control	Diabetic control	Diabetic control treated with BLSF (mg/kg)		Diabetic treated with glipizide (10 mg/kg)
			100	200	
Body weight (g/rat)	197.5±8.82	156.66±5.11*	165.83±624	177.5±7.15	169.16±6.50
Food intake (g/rat/day)	17.49±1.02	29.39±1.74*	25.32±0.74	22.13±1.43**	23.15±1.23**
Water intake (ml/rat/day)	26.29±1.01	47.29±0.89*	31.21±2.19**	29.19±1.39**	24.39±1.75**

Values are given as mean±SEM for groups of six rats in each. Values are statistically significant at *P<0.05. Statistical significance was compared within the groups as follows: *Diabetic rats were compared with control rats, **Treated diabetic rats were compared with diabetic rats. SEM: Standard error of the mean, n-STZ: Neonatally streptozotocin, BLSF: *Bryonia laciniosa* saponin rich fraction

Table 3: Effect of BLEE on glucose, serum insulin, AUCglucose lipid profiles, renal function tests and liver function tests in n-STZ diabetic rats

Parameters	Normal control	Diabetic control	Diabetic control treated with BLEE (mg/kg)	
			250	500
Glucose (mg/dl)	118.83±6.62	246.33±14.36*	204.5±8.43**	158.33±5.74**
Insulin (µU/ml)	17.66±2.91	83.33±6.33*	81.33±4.99	72.16±4.20
OGTT (AUC mg/dl/min)	5.73±0.23	10.12±0.32*	7.71±0.42**	7.62±0.18**
Triglycerides (mg/dl)	43.83±2.57	100.83±8.09*	72.01±6.29**	60.66±3.84**
Cholesterol (mg/dl)	60.83±3.46	114.5±11.58*	78.33±2.62**	70.16±4.08**
HDL-C (mg/dl)	24.66±2.26	14.00±2.06*	20.00±2.80	22.83±1.99
VLDL-C (mg/dl)	8.76±0.51	0.16±1.61*	14.4±1.25**	12.13±0.76**
LDL-C (mg/dl)	27.4±4.63	80.33±11.03*	43.93±4.07**	35.20±5.48**
Creatinine (mg/dl)	0.44±0.03	0.93±0.08*	0.63±0.03**	0.55±0.06**
Urea (mg/dl)	36.83±4.18	64.66±3.08*	50.50±4.24	49.00±4.48**
AST (U/ml)	34.33±2.27	75.5±8.80*	51.33±7.63**	38.16±5.29**
ALT (U/ml)	24.16±2.92	41.16±3.91*	34.33±4.20	27.33±2.81**

Values are given as mean±SEM for groups of six rats in each. Values are statistically significant at *P<0.05. Statistical significance was compared within the groups as follows: *Diabetic rats were compared with control rats, **Treated diabetic rats were compared with diabetic rats. SEM: Standard error of the mean, AUC: Area under the curve, BLEE: *Bryonia laciniosa* ethanol extract, n-STZ: Neonatally streptozotocin, OGTT: Oral glucose tolerance test, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, VLDL: Very low-density lipoprotein cholesterol, AST: Aspartate amino transferase, ALT: Alanine amino transferase

BLEE (250 and 500 mg/kg, p.o.) and BLSF (100 and 200 mg/kg, p.o.) failed to exhibit significant changes in elevated AST and ALT levels when compared with diabetic control group [Tables 3 and 4].

Lipid peroxidation

Streptozotocin-induced type 2 diabetic rats exhibited higher lipid peroxidation in liver tissue when compared with normal control rats. Treatments with BLEE (250 and 500 mg/kg, p.o.) and BLSF (100 and 200 mg/kg, p.o.) decreased lipid peroxidation at significantly extent when compared with diabetic control animals [Tables 5 and 6].

Effect on antioxidant parameters

Streptozotocin-induced type 2 diabetic rats showed significant reduction in SOD, CAT and GSH content in liver tissue as compared to normal control rats. Treatment with BLEE (250 and 500 mg/kg, p.o.) and BLSF (100 and 200 mg/kg, p.o.) elevates the SOD and CAT level to a significant extent when compared with diabetic control rats. However, treatment with extract (500 mg/kg), significantly

Table 4: Effect of BLSF on glucose, serum insulin, AUCglucose lipid profiles, renal function tests and liver function tests in n-STZ diabetic rats

Parameters	Normal control	Diabetic control	Diabetic control treated with BLBF (mg/kg)		Diabetic treated with glipizide (10 mg/kg)
			100	200	
Glucose (mg/dl)	83.69±6.17	210.71±8.21*	165.10±6.25**	140.48±7.46**	153.70±11.06**
Insulin (µU/ml)	36.16±2.61	71.50±5.75*	71.00±4.76	73.33±8.36	38.83±3.22
OGTT ($AUC_{mg/dl\ min}$)	3.36±0.15	7.92±0.28*	6.01±0.341**	5.34±0.32**	5.25±0.35**
Triglycerides (mg/dl)	86.07±5.49	189.81±13.82*	149.61±10.71**	121.93±4.92**	92.77±13.92**
Cholesterol (mg/dl)	91.90±5.94	166.28±9.62*	126.56±9.83**	125.74±11.19**	121.56±6.19**
HDL-C (mg/dl)	37.82±2.48	25.01±1.70*	29.55±2.08	32.80±4.65	27.11±2.73
VLDL-C (mg/dl)	17.21±1.10	37.96±2.76*	29.92±2.03**	24.38±0.98**	18.55±2.78**
LDL-C (mg/dl)	36.86±8.19	103.32±7.61*	67.08±8.24**	68.56±12.96**	75.85±9.59**
Creatinine (mg/dl)	0.58±0.07	1.23±0.17*	0.86±0.09**	0.78±0.06**	1.29±0.08
Urea (mg/dl)	31.72±2.46	51.17±4.82*	37.81±2.94**	33.43±2.38**	33.83±3.65**
AST (U/ml)	14.83±5.89	78.33±7.82*	43.16±6.01**	37.16±4.88**	45.66±4.45**
ALT (U/ml)	24.16±3.37	62.00±3.55*	39.50±6.46**	31.66±2.96**	32.50±5.89**

Values are given as mean±SEM for groups of six rats in each. Values are statistically significant at *P<0.05. Statistical significance was compared within the groups as follows: *Diabetic rats were compared with control rats, **Treated diabetic rats were compared with diabetic rats. SEM: Standard error of the mean, AUC: Area under the curve, BLEE: *Bryonia laciniosa* ethanol extract, n-STZ: Neonatally streptozotocin, OGTT: Oral glucose tolerance test, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, VLDL: Very low-density lipoprotein cholesterol, AST: Aspartate amino transferase, ALT: Alanine amino transferase, BLBF: Bryonia laciniosa saponin rich fraction

Table 5: Effect of BLEE on oxidative stress parameters in n-STZ diabetic rats

Parameters	Normal control	Diabetic control	Diabetic control treated with BLEE (mg/kg)	
			250	500
Malondialdehye (ng/proteins)	3.71±0.40	13.09±1.22*	5.36±0.74**	4.03±0.86**
SOD (U/mg of proteins)	0.48±0.04	0.23±0.04*	0.45±0.06**	0.43±0.05**
Catalase (U/mg of proteins)	44.36±1.84	23.29±5.06*	32.60±4.55**	40.35±4.09**
Reduced glutathione (µg/mg of proteins)	6.06±0.67	2.35±0.86*	3.53±0.44	5.07±0.89**

Values are given as mean±SEM for groups of six rats in each. Values are statistically significant at *P<0.05. Statistical significance was compared within the groups as follows: *Diabetic rats were compared with control rats, **Treated diabetic rats were compared with diabetic rats. BLEE: *Bryonia laciniosa* ethanol extract, n-STZ: Neonatally streptozotocin, SOD: Superoxide dismutase, SEM: Standard error of the mean

Table 6: Effect of BLSF on oxidative stress parameters in n-STZ diabetic rats

Parameters	Normal control	Diabetic control	Diabetic control treated with BLSF(mg/kg)		Diabetic treated with glipizide (10 mg/kg)
			100	200	
Malondialdehye (ng/proteins)	6.66±0.46	13.51±1.20*	7.63±0.96**	7.38±0.41**	7.10±0.99**
SOD (U/mg of proteins)	0.56±0.05	0.32±0.09*	0.41±0.05	0.45±0.10**	0.49±0.04**
Catalase (U/mg of proteins)	40.79±3.38	20.42±3.98*	29.49±6.32**	20.54±2.54	38.34±5.99**
Reduced glutathione (µg/mg of proteins)	9.98±2.26	4.34±0.73*	7.30±0.41	8.81±0.65**	8.91±0.88**

Values are given as mean±SEM for groups of six rats in each. Values are statistically significant at *P<0.05. Statistical significance was compared within the groups as follows: *Diabetic rats were compared with control rats, **Treated diabetic rats were compared with diabetic rats. SEM: Standard error of the mean, SOD: Superoxide dismutase, BLSF: *Bryonia laciniosa* saponin rich fraction, n-STZ: Neonatally streptozotocin, BLSF: *Bryonia laciniosa* saponin rich fraction

raise the level of GSH when compared with diabetic control rats [Tables 5 and 6].

DISCUSSION

In the present study, we investigated the anti-diabetic effects of ethanol extract of seeds of *B. laciniosa* and its saponin fraction in n-STZ diabetic rats. It has been established previously that the anti-diabetic activity of *B. laciniosa* in STZ-induced type 1 diabetic rats.[20] Herein, we showed for the 1st time that, ethanol extract of seeds of *B. laciniosa* and its saponin fraction significantly attenuated hyperglycemia and improved glucose homeostasis in experimentally induced n-STZ induced diabetic rats.

The development and progression of type 2 diabetes first manifests itself as glucose intolerance and postprandial hyperglycemia, and eventually fasting hepatic glucose production increases,[26] leading to the elevated fasting glucose levels characteristic of symptomatic diabetes. Furthermore, dyslipidemia is often associated with hyperglycemia in type 2 diabetes due to the close relationship between glucose and lipid metabolism. The n-STZ diabetic rat model experienced the classical pathogenesis of type 2 diabetes from merely glucose intolerance to elevated fasting glucose levels in the current study, along with a significant increase in lipid levels. Chronic administration of BLEE and BLSF dose-dependently lowered postprandial glucose and lipid levels, attenuated the glucose intolerance and prevented the onset of overt diabetes in n-STZ diabetic rats.

The n-STZ diabetic rats show significant glucose intolerance when 1.5 g/kg glucose is orally administered, and they also have high levels of insulin.[27] Hyperinsulinemia with low hepatic excretion and the hypersecretion of β-cells are also reported in mildly glucose intolerant obese subjects.[28] In the present study, STZ produced a significant increase in glucose levels that was associated with a significant increase in insulin levels in diabetic rats. Treatment with BLEE and BLSF significantly reduced the serum glucose and but failed to alter the insulin levels of the diabetic rats. Although we did not determine the insulin sensitivity of the cells, the unchanged insulin profile and improved glucose response during OGTT suggest that the extract may have improved glucose tolerance via increased insulin sensitivity.[29] In considering this, the anti-hyperglycemic activity of the *B. laciniosa* may be due to an improved glucose uptake, insulin resistance in cells or inhibiting the glucose absorption through gastrointestinal tract. Our study suggests that the hypoglycemic activity of *B. laciniosa* is not related to the insulin secretion and that post prandial glucose reduction is probably effected without any extra load on pancreatic β-cells and/or stimulation of glucose uptake by peripheral tissues. Hence, the hypoglycemic effect may be probably brought about by an extra pancreatic mechanism. There are medicinal plants, which are known to exert antidiabetic activity without the stimulation of insulin secretion.[30-32] *In vitro* studies on Hep G2 cell lines have shown that berberine is able to exert an insulin independent glucose lowering effect glucose lowering effect in hepatocytes similar to that of metformin.[33]

Circulating levels of free fatty acids are elevated in diabetes.[34] Free fatty acids may impair endothelial function through several mechanisms, including the increased production of oxygen-derived free radicals, the activation of protein kinase c, and the exacerbation of dyslipidemia.[35] In the present study, diabetic animals showed elevated triglyceride, cholesterol, LDL, and VLDL levels and decreased HDL levels, compared with control animals. Serum cholesterol, triglyceride, LDL, and VLDL levels were significantly decreased by treatment with BLEE and BLSF in diabetic rats. The reduction in cholesterol and triglyceride levels by extract and its saponin fraction suggests a beneficial effect on coronary heart diseases.

Chronic metabolic disorders in type 2 diabetes are reported to cause varied forms of complications, including liver or kidney diseases.[36-38] Blood ALT and AST levels are considered to be liver function markers, and ALT value has been reported to increase significantly in STZ-induced diabetic rats.[36,38] Moreover, in n-STZ diabetic rats, chronic hyperglycemia and polyuria symptoms lead to further deterioration of kidney function and the ultimate occurrence of diabetic nephropathy. Renal hypertrophy and abnormal extracellular matrix deposition were reported to be the hallmarks of diabetic nephropathy, and glomerular hyperfiltration occurred at the early phase of diabetic nephropathy.[39] In the present study, n-STZ diabetic rats exhibited significantly elevated creatinine and urea levels, and chronic BLEE and BLSF administration dose-dependently reversed the renal and liver function parameters induced by diabetes, suggesting that *B. laciniosa* is capable of protecting the peripheral target organs from damages induced by chronic hyperglycemia in type 2 diabetes.

The accumulation of reactive oxygen species is a characteristic feature of oxidative stress, and a relationship between oxidative stress and the vascular complications in diabetes was suggested previously.[40] Oxidative stress is associated with complications of diabetes and has been linked to insulin resistance *in vitro* and *in vivo*.[41] When glucose and free fatty acids increase; they cause oxidative stress and the activation of stress sensitive signaling pathways. The activation of these pathways, in

turn, worsens both insulin action and secretion, which leads to overt type 2 diabetes.[42] In our study, there was a high level of serum MDA and a low level of SOD, CAT, GSH in the diabetic group, compared with levels in normal animals. Chronic BLEE and BLSF treatment reduced the elevated level of MDA and increased the levels of SOD, CAT, GSH, which indicates a decrease in oxidative stress.

CONCLUSION

Taken together, we conclude that *B. laciniosa* treatment is effective to significantly enhance glucose homeostasis in n-STZ diabetic rats. As such, *B. laciniosa* may be considered as a candidate anti-diabetic drug in diabetic patients. However, further studies are needed to address more detailed information regarding the action mechanisms for *B. laciniosa* in treating diabetes.

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