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Effect of *Biophytum sensitivum* on streptozotocin and nicotinamide-induced diabetic rats

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ABSTRACT

Objective: To investigate the effect of aqueous solution of *Biophytum sensitivum* leaf extract (BSEt) on normal and streptozotocin (STZ)-nicotinamide-induced diabetic rats. **Methods:** Diabetes was induced in adult male Wistar rats by the administration of STZ-nicotinamide (40, 110 mg/kg b.w., respectively) intraperitoneally. BSEt (200 mg/kg) was administered to diabetic rats for 28 days. The effect of extract on blood glucose, plasma insulin, total haemoglobin, glycosylated haemoglobin, liver glycogen and carbohydrate metabolism regulating enzymes of liver was studied in diabetic rats. **Results:** BSEt significantly reduced the blood glucose and glycosylated haemoglobin levels and significantly increased the total haemoglobin, plasma insulin and liver glycogen levels in diabetic rats. It also increased the hexokinase activity and decreased glucose-6-phosphatase, fructose-1, 6-bisphosphatase activities in diabetic rats. **Conclusions:** The results of our study suggest that BSEt possesses a promising effect on STZ-nicotinamide-induced diabetes.

1. Introduction

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both[1]. Globally, the estimated incidence of diabetes and projection for year 2030, as given by International Diabetes Federation (IDF) is 350 million[2]. Defects in carbohydrate machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism pose an over exertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing deterioration of endocrine control exacerbates the metabolic disturbances by altering carbohydrate metabolic enzymes and leads primarily to hyperglycemia[3]. Diabetes can be managed by diet, exercise, and chemotherapy. However, the pharmacological drugs are either too expensive or have undesirable side effects or contraindications[4]. Throughout the world, many traditional plant treatments for diabetes exist, and therein lies a hidden wealth of potentially useful natural products for the control of diabetes[5]. Natural plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones[6].

Biophytum sensitivum (*B. sensitivum*) D. C. belonging to the family of Oxalidaceae and commonly known as 'Nagbeli', is a folk medicine against diabetes. Powdered dry leaves of the plant are a known traditional remedy for the treatment of 'Madhumeha' (diabetes)[7]. It is an annual herb that grows at the foothills of the Himalayas, around the inner Tarai region (east of Koshi river) in Eastern Nepal. Pharmacologically the plant has been investigated for its hypoglycemic[8], anti-inflammatory[9], hypocholesterolemic[10], anti-cancer effect[11]. In addition, the active ingredient examination of this plant has indicated the presence of two biflavones, (cupressuflavone and amentoflavone) three flavonoids, (luteolin 7-Methyl ether, isoorinentin and 3-methoxyluteolin 7-O-glucoside) as well as two acids (4-caffeoylquinic acid and 5-caffeoylquinic acid)[12]. However, despite the various bioactive phytochemical and diverse medicinal activities attributed to this plant, no biochemical studies have been carried out to shed light on the role of this plant in diabetes. In the light of the above, the current study was undertaken to investigate its role on blood glucose, body weight, urine sugar, plasma insulin, total hemoglobin (Hb), glycosylated

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hemoglobin (HbA1c), liver glycogen, and carbohydrate metabolic enzymes in normal and streptozotocin (STZ)-nicotinamide-induced diabetic rats.

2. Materials and methods

2.1. Animals

Male albino (9 weeks old) rats of the Wistar strain, weighing (180-200 g), were obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University, and maintained in an air-conditioned room (25±1) ℃ with a 12-h light:12-h dark cycle. Feed and water were provided *ad libitum* to all the animals. All studies were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and the study was approved by the Ethical Committee of Rajah Muthiah College and Hospital (Reg. No. 157/2007/CPCSEA), Annamalai University, Annamalainagar.

2.2. Chemicals

STZ was purchased from Sigma-Aldrich (St Louis, MO, USA). Boehringer Mannheim GmbH Kit (ELISA-Principle) was used for insulin assay. All the biochemicals and chemicals used in the experiment were of analytical grade obtained from E. Merck and HIMEDIA (Mumbai India).

2.3. Preparation of extract

B. sensitivum leaves were collected during the month of June from Neythaloore, Thanjavur district, South India. The plant was identified at the Herbarium of Botany Directorate in Annamalai University. A voucher specimen (No. 5089) was deposited in the Botany Department of Annamalai University. 500 g of *B. sensitivum* leaves were chopped into small pieces extracted with 1 500 mL water by the method of continuous hot extraction at 60 ℃ for 6 h and it evaporated. The residual extract was dissolved in water and used in this study. A dark semi-solid (greenish-black) material was obtained (30.5 g). It was stored at 4 ℃ until use.

2.4. Experimental induction of diabetes

STZ was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline. Non-insulin-dependent diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of STZ (45 mg/kg b.w.). 15 min later, the rats were given the intraperitoneal administration of nicotinamide (110 mg/kg b.w.)[13]. These animals exhibited massive glycosuria (determined by Benedict's qualitative test) and hyperglycemia (by glucose oxidase method) within a few days. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h. The animals with blood glucose concentration more than 250 mg/dL were considered to be diabetes and used for the experiment.

2.5. Experimental design

A total of 30 rats (18 diabetic surviving rats, 12 normal rats) were used. The rats were divided into five groups after the induction of STZ-nicotinamide diabetes. In the experiment six rats were used in each group: group I, normal untreated rats; group II, normal + *B. sensitivum* leaf extract (BSEt) (200 mg/kg b.w.), BSEt dose was fixed from earlier report of Puri[29]; group III, diabetic control; group IV, diabetic + BSEt (200 mg/kg b.w.); group V, diabetic + glibenclamide (600 μg/kg b.w.)[4].

The plant extract and the drug glibenclamide were given in aqueous solution daily using an intragastric tube for 28 days. At the end of the experimental period, the animals were deprived of food overnight and then killed by decapitation. Blood was collected in fresh vials containing potassium oxalate and sodium fluoride (1:3 vol/vol) for the estimation of blood glucose and in tubes with EDTA for the estimation of Hb, HbA1c. Plasma was separated for the estimation of insulin. Liver was dissected out, washed in ice-cold saline, patted dry and weighed. The liver homogenate was used for biochemical investigations.

2.6. Biochemical estimations

Glucose was estimated by O-toluidine method of Sasaki *et al*[14]. Hb was estimated by cyanmethaemoglobin method of Drabkin and Austin[15]. HbA1c was estimated by the method of Sudhakar and Pattabiraman[16] with modification by Bannon[17]. The plasma insulin level was assayed by enzyme linked immuno sorbent assay (ELISA) kit (Boerhringer Mannheim kit). Hexokinase, glucose-6-phosphatase and fructose-1,6-bis phosphatase were assayed according to the method of Brandstrup, Baginsky and Gancedo *et al*[18-20], respectively and the inorganic phosphate (Pi) liberated was estimated by the method of Fiske *et al*[21]. Glycogen content was determined as described by Morales *et al*[22].

2.7. Statistical analysis

Values were given as means±SD for six rats in each group. Data were analyzed by one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) using SPSS version 10 (SPSS, Chicago, IL). The limit of statistical significance was set at $P<0.05$.

3. Results

The effect of BSEt on blood glucose in normal and

Table 1
Effect of BSEt on blood glucose level in normal and diabetic rats (mean±SD) (mg/dL).

Groups	Blood glucose			
	Day 0	Day 14	Day 21	Day 28
Normal	72.51±6.96	79.40±6.87	76.57±7.06	74.50±6.30
Normal + BSEt (200 mg/kg)	80.48±7.06	76.21±6.44	70.25±6.42	67.48±6.67
Diabetic	310.24±27.07	323.35±29.15[a]	360.60±30.66[a]	394.19±32.67[a]
Diabetic + BSEt (200 mg/kg)	279.58±23.66	223.20±19.37[b]	176.30±14.86[b]	121.50±10.84[b]
Diabetic + glibenclamide (600 μg/kg)	288.49±24.52	248.02±21.99[b]	194.58±18.72[b]	140.87±12.09[b]

a: $P<0.05$ *vs* normal control group; b: $P<0.05$ *vs* diabetic control group.

Table 2
Effect of BSEt on body weight, plasma insulin, Hb, HbA1C, and urine sugar of normal and diabetic rats (mean±SD).

Groups	Body weight (g)		Insulin (μU/mL)	Hb (g/dL)	HbA1c (mg/g Hb)	Urine sugar
	Initial	Final				
Normal	187.00±11.13	221.49±13.29	16.73±1.15	12.20±0.93	0.26±0.01	Nil
Normal + BSEt (200 mg/kg)	195.48±13.12	225.41±15.16	17.33±1.45	12.80±0.99	0.25±0.01	Nil
Diabetic	212.83±15.64	191.33±10.65[a]	6.29±0.47[a]	9.04±0.54[a]	0.78±0.01[a]	+++
Diabetic + BSEt (200 mg/kg)	204.15±16.64	211.70±12.15[b]	14.54±1.53[b]	11.70±0.82[b]	0.42±0.02[b]	Nil
Diabetic + glibenclamide (600 μg/kg)	195.33±12.22	205.25±14.43[b]	15.60±0.90[b]	12.10±0.85[b]	0.43±0.03[b]	Trace

a: $P<0.05$ *vs* normal control group; b: $P<0.05$ *vs* diabetic control group; +++: indicating more than 2% sugar.

Table 3
Effect of BSEt on carbohydrate metabolic enzymes and glycogen in the liver of normal and diabetic rats (mean±SD).

Groups	Hexokinase (U/g protein)^2	Glucose-6-phosphatase (U/g protein)^2	Fructose-1,6-bisphosphatase (U/g protein)^3	Glycogen (mg/100 g tissue)
Normal	150.30±11.34	0.18±0.02	0.35±0.03	54.14±1.47
Normal + BSEt (200 mg/kg)	153.45±11.68	0.17±0.01	0.33±0.03	59.33±1.21
Diabetic	106.88±8.09[a]	0.27±0.02[a]	0.60±0.04[a]	16.00±1.54[a]
Diabetic + BSEt (200 mg/kg)	136.51±10.39[b]	0.21±0.02[b]	0.38±0.03[b]	46.83±1.60[b]
Diabetic + glibenclamide (600 μg/kg)	134.37±8.14[b]	0.19±0.10[b]	0.45±0.03[b]	50.50±1.87[b]

a: $P<0.05$ *vs* normal control group; b: $P<0.05$ *vs* diabetic control group.

experimental rats on day 0, 14, 21 and 28 was depicted in Table 1. The blood glucose was elevated significantly in diabetic rats as compared with normal control rats. In diabetic rats, treatment with glibenclamide and BSEt (200 mg/kg b.w.) lowered the blood glucose significantly as compared with diabetic control rats.

The levels of plasma insulin, Hb, HbA1c, change in body weight and urine sugar of normal and experimental rats were given in Table 2. Body weight, plasma insulin and Hb decreased, urine sugar and HbA1c increased significantly in diabetic control rats, and these values were reversed by treatment with BSEt (200 mg/kg b.w.) and glibenclamide. A significant elevation in plasma insulin and the level of haemoglobin and glycosylated haemoglobin remained unaltered were also observed in normal rats treated with BSEt as compared with normal control rats.

The activities of carbohydrate metabolic enzymes and level of glycogen in the liver of normal and diabetic rats were given in Table 3. The decreased activities of hexokinase and the level of glycogen, increased activities of glucose-6-phosphatase and fructose-1,6-bisphophatase were observed in the liver of diabetic rats as compared with normal control rats, and theses values were reversed by treatment with BSEt (200 mg/kg b.w.) and glibenclamide. Normal rats treated with BSEt also showed a significant elevation in the activities of hexokinase and level of glycogen and non-significant decrease in the activities of glucose-6-phosphatase and fructose-1,6-bisphophatase were observed as compared with normal control rats.

4. Discussion

Diabetes mellitus is a worldwide problem, and type 2 diabetes is found to be more prevalent. Patients in this group range from those with insulin deficiency and insulin resistance to a predominantly secretory defect with some insulin resitance[23]. To the best of our knowledge, this is the first report that analyzes BSEt on hepatic enzymes in experimental diabetes. STZ-nicotinamide injection caused diabetes mellitus, which may be due to destruction of β-cells of the islet of langerhans of the pancreas[13]. Over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues are the fundamental basis of hyperglycemia in diabetes mellitus[24]. This study has revealed that BSEt produced a marked decrease in blood glucose at 200 mg/kg b.w. in normal as well as in diabetic rats after 28 days of treatment. The reduction of blood glucose was also reflected in urine sugar level. These findings are in agreement with

those reported by Pari and Satheesh[25]. The antidiabetic effect of BSEt may be due to increased release of insulin from the existing β-cells of pancreas similar to that observed after sulphonylurea administration. Previous studies have shown that daily administration of *B. sensitium* for one week produced significant glucose lowering with insulin release stimulatory effect in hyperglycemic rabbits[8,26]. The extract of *B. sensitium* contains biflavones (cupressoflavone and amentoflavone) and flavonoids (luteolin 7-methyl ether, isoorientine, 3′-methoxyluteolin 7-o-glucoside)[12] and these may be responsible for the antidiabetic effect[27,28].

STZ-nicotinamide-induced diabetes is characterized by a severe loss in body weight[29]. The decrease in body weight in diabetic rats shows that the loss or degradation of structural proteins is due to diabetes, and structural proteins are known to contribute to the body weight[30]. When diabetic rats were treated with BSEt, the weight loss was reversed. The capability of BSEt to protect body weight loss seems to be as a result of its ability to reduce hyperglycemia.

In BSEt treated diabetic rats, the significant elevation of plasma insulin may be due to the stimulation of insulin secretion from the existing β-cells of pancreas. Insulin generally has an anabolic effect on protein metabolism in that it stimulates protein synthesis and retards protein degradation which may be responsible for the decreased level of Hb in diabetic rats. In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of protein including hemoglobin and β-crystalline of lens[31]. Glycosylated hemoglobin (HbA1c) was significantly increased in diabetic animals, and this increase was found directly proportional to the fasting blood glucose level. During diabetes, the excess glucose present in blood reacts with hemoglobin. Therefore, the total hemoglobin level is decreased in diabetic rats[32]. In this study a decrease in total haemoglobin during diabetes has been observed and this may be due to the formation of glycosylated haemoglobin. Administration of aqueous extract prevents a significant elevation in glycosylated hemoglobin thereby increasing the level of total hemoglobin in diabetic rats. This could be due to the improved glycemic control produced by BSEt.

In experimental diabetes enzymes of glucose metabolism are markedly altered. Persistent hyperglycemia is a major contributor to such metabolic alterations that lead to the pathogenesis of diabetic complications, especially, neuropathy and micro vascular diseases. One of the key enzymes in the catabolism of glucose is hexokinase, which phosphorylates glucose and converts it into glucose-6-phosphate[33]. The activity of this enzyme was decreased in the liver of STZ-nicotinamide-induced diabetic rats. Administration of BSEt to STZ-nicotinamide-induced rats resulted in an increased activity of liver hexokinase. The increased activity of hexokinase can cause increased glycolysis and increased utilization of glucose for energy production. BSEt has been observed to reduce the levels of glucose in the blood[34]. The decrease in the concentration of blood glucose in STZ-nicotinamide-treated rats treated with BSEt may be due to increased glycolysis (increased liver hexokinase activity).

The gluconeogenic enzyme glucose-6-phosphatase is a crucial enzyme of glucose homeostasis because it catalyses the ultimate biochemical reaction of both glycogenolysis and gluconeogenesis[35]. In addition, glucose-6-phosphatase plays an important role in glucose release in liver and kidney through a mechanism involving gene expression or biochemical inhibition of its enzymatic activity[36]. Increased glucose-6-phosphatase activity in diabetic rats provides hydrogen, which binds with $NADP^+$ in the form of NADPH and enhances the synthesis of fats from carbohydrates (*i.e.* lipogenesis)[37] and finally contributes to increased levels of glucose in blood. Increased hepatic glucose production in diabetes mellitus is associated with impaired suppression of the gluconeogenic enzyme fructose-1,6-bisphosphatase. Activation of gluconeogenic enzymes is due to the state of insulin deficiency, because under normal conditions, insulin functions as a suppressor of gluconeogenic enzymes.

Liver plays an important role in buffering the postprandial hyperglycemia and is involved in synthesis of glycogen. Diabetes mellitus is known to impair the normal capacity of the liver to synthesize glycogen[38]. Synthase phosphatase activates glycogen synthase, resulting in glycogenesis, and this activation appears to be defective in STZ-nicotinamide-induced diabetic rats[39]. Diabetic rats treated with BSEt had liver glycogen brought back to near normal levels, which could be due to increased secretion of insulin, which enhances glycogenesis.

Administration of BSEt and glibenclamide significantly reduced the activities of gluconeogenic enzymes in diabetic rats. The levels of plasma insulin were found to increase significantly in diabetic rats treated with BSEt, which may be a consequence of the significant reduction in the level of gluconeogenic enzymes. The reduction in the activities of gluconeogenic enzymes can result in the decreased concentration of glucose in blood.

The present investigation confirms the enhanced effect of *B. sensitium* on STZ-nicotinamide-induced diabetes.

Conflict of interest statement

We declare that we have no conflict of interest.

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