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Research Article

Screening of Antidiabetic Activity of Extract and Fraction of *Biophytum sensitivum* in Rats Using STZ-Nicotinamide Induced Diabetes Model

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ABSTRACT

Objective: *Biophytum sensitivum* DC. (Family – Oxalidaceae) has long tradition of use in the treatment of diabetes but no systematic work has been carried out to explore its potential thus it was envisaged to evaluate antidiabetic activity of methanol extract of *B. sensitivum* and its ethylacetate fraction in rats using STZ-Nicotinamide-induced type 2 diabetes model. Methods*:* The methanol extract of *B. sensitivum* whole plant was prepared by exhaustive extraction of defatted plant material with methanol in a Soxhlet apparatus. It was then partitioned with ethyl acetate using standardized procedure to get ethyl acetae fraction. The methanol extract was subjected to acute toxicity at a dose of 2000 mg/kg, p.o. in rats. The methanol extract (200 or 400 mg/kg, p.o.) and ethyl acetate fraction (15 or 30 mg/kg, p.o.) were screened for their antidiabetic potential using STZ-Nicotinamide-induced type 2 diabetes model. The antidiabetic effects of test drugs were statistically compared with the standard drug metformin (150 mg/kg, p.o.). Results: In acute toxicity studies, methanol extract did not show lethality in rats. The methanol extract and ethyl acetate fraction significantly reduced the blood glucose level in diabetic rats at 200 mg/kg, p.o. and 15 mg/kg, p.o. respectively with respect to control and statistically equivalent to the standard drug. The phytochemical screening reveals the presence of flavonoids as the major class of phytoconstituents. Conclusion: The traditional claims for the antidiabetic effect of *B. sensitivum* are validated.

Keywords: Antidiabetic, *Biophytum sensitivum*, Ethyl acetate fraction, Phytochemical screening.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycaemia with disturbances in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or may be both. It is the world's largest endocrine disease. According to W.H.O. projection, the prevalence of diabetes is likely to increase by 35%. Recent estimates indicate there were 171 million diabetics across the world in the year 2000 and this would increase to 366 million by the year 2030[1-2]. India leads the world with largest number of diabetic subjects and also known as "diabetes capital of the world". According to statistical projections about India, it has been estimated that the number of diabetics will rise to 70 million in the year 2030 making the country with the highest number of diabetics in the world followed by China and then USA.

Many drugs and intervention like sulfonylureas and related compounds, biguanides, thiazolidenediones, α-glucosidase inhibitors and insulin, etc. are available to manage diabetes. In most cases they are expensive, produce serious side effects, and are not considered to be safe for use during pregnancy. Therefore it is necessary to look for new solution to manage this health problem. The use of herbal medicines for the treatment of diabetes mellitus has been popular throughout the world. The W.H.O. also recommended and encouraged this practice especially in countries where access to the conventional treatment of diabetes is not adequate[3]. The use of medicinal plants for treatment of diabetes mellitus dates back from the Ebres papyrus about 1550 B.C[4-5]. Before the introduction of insulin and other pharmaceutical preparations, traditional medicine mainly derived from plants was used to treat diabetes mellitus. Indeed, the widely prescribed insulin-sensitizer, metformin was derived from guanidine, a molecule isolated from *Galega officinalis* L[6]. Around eight hundred plant species have been reported to possess antidiabetic properties. A limited number of medicinal plant species have been studied and validated for their antidiabetic potential by clinical and preclinical studies[7]. However, search for newer, efficacious and safer antidiabetic drugs still continues.

Biophytum sensitivum DC. (Family – Oxalidaceae) commonly known as "Lajjalu" in Northern India, is an annual herb that grows at the foothills of the Himalayas. It is an indigenous medicine, used against "*Madhumeha*" (Diabetes mellitus) apart from being used as a tonic, stimulant and in the treatment of stomach ache, asthma, insomnia, convulsions, cramps, chest complaints, inflammations, tumours and chronic skin diseases[8]. *B. sensitivum* has been reported to contain phenolic and polyphenolic compounds, saponin, essential oil,

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polysaccharides, pectin, amentoflavone with minute amount of cupressoflavone, luteolin 7-methyl ether, isoorientin, 3-methoxyluteolin 7-*O*-glucoside, 4-caffeoylquinic acid and 5-caffeoylquinic acid[9-11]. It has been reported to possess anti-tumour, antipyretic, immunomodulatory, antidiabetic, antiulcer, radio-protective, larvicidal, antibacterial and antioxidant[12]. Previous studies carried out on *B. sensitivum* are too preliminary to validate antidiabetic potential of *B. sensitivum.* Thus, it was planned to investigate antidiabetic activity of the plant in rats using STZ-nicotinamide induced type 2 diabetes model.

MATERIALS AND METHODS

Chemicals and reagents: STZ was purchased from Sigma-Aldrich (St Louis, MO, USA). Metformin HCl was obtained as a gift sample from Sun Pharmaceutical Industries Limited, India. All the chemicals used in the experiment were, of analytical grade, purchased from S.D. Fine Chemicals, Mumbai.

Procurement of plant material: The whole plant of *B. sensitivum* was procured from Tamilnadu (District, Thoothukudi) in January 2012. The identity of the plant was confirmed by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, NISCAIR, New-Delhi, (specimen No.-NISCAIR/RHMD/Consult/2012-11/1528/126).

Preparation of methanol extract and ethyl acetate fraction: The plant material was dried at room temperature under well-ventilated shade by spreading uniformly. The dried material was powdered, weighed (1kg) and filled in a Soxhlet apparatus for successive solvent extraction. First the powdered drug was extracted with 4 litre petroleum ether (60-80°C) until drug was completely defatted (Intermittent filter paper stain test was done to ensure complete defatting). Defatted plant material was then dried and extracted exhaustively with methanol (4L) in a Soxhlet apparatus. The methanol extract was concentrated using rotary vacuum evaporator. It was further fractionated using ethyl acetate solvent. The methanol extract (100 g) was suspended in 500 ml distilled water and refluxed with 100 ml of ethyl acetate for 30 min, the contents were taken in a separating funnel and ethyl acetate layer was separated. Residual aqueous layer was partitioned with ethyl acetate five times adopting the similar procedure as mentioned above. The collected ethyl acetate layers were pooled, concentrated using rotator vacuum evaporator and dried under vacuum to obtain solid dried powder. Percentage yield of the ethyl acetate fraction was calculated and preliminary phytochemical screening of methanol extract as well the ethyl acetate fraction was carried out for the identification of various constituents like alkaloids, glycosides, flavonoids, steroids, proteins, tannins and phenolic compounds[13-14].

Animals: S.D. rats of either sex (200-300 g) were used in pharmacological studies. Throughout the experiment the animals were housed in colony cages. Animals were maintained at a temperature of 23 ± 2°C and relative humidity of 45-55% under 12 h light: 12 h dark cycle. The animals were provided with food (Golden feed, Delhi) and water *ad libitum*. The studies were conducted after obtaining ethical committee clearance from the institutional animal ethics committee of Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India (Approval No. 107/99/CPCSEA/2012-48).

Acute toxicity studies: Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development (OECD) guidelines-423[15]. After oral administration of the methanol extract of *B. sensitivum*, animals were observed individually for behavioural profile (alertness, restlessness, irritability and fearfulness), neurological profile (spontaneous activity, reactivity, touch response and pain response) and autonomic profile (defecation and urination) for at least once during the first 30 min and periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 days. A total of six rats sex were used and each received a single oral-dose of 2000 mg/kg/p.o. Animals were kept overnight fasting prior to drug administration and food was withheld for further 3-4 h.

Induction of Diabetes: Non-insulin dependent type 2 diabetes in rats was induced by STZ-NA. A freshly prepared solution of streptozotocin (55 mg/kg, i.p.) in 0.1 M citrate buffer (pH 4.5) was administered intraperitoneally. The nicotinamide (100 mg/kg) was given i.p. 15 min before STZ administration[16-17]. After 48 h of administration, rats with hyperglycemia (>200 mg/dl) were taken for experimental studies. The doses of standard drug and test substances were prepared using vehicle [Distilled water + Tween 80 (2%)].

Experimental Design: 42 rats (36 diabetic surviving rats, 6 normal rats) were used for evaluation of antidiabetic activity of methanol extract and ethyl acetate fraction. The rats were divided into seven groups after the induction of STZ-nicotinamide induced type 2 diabetes. In the experiment, six rats were used in each group:

Group I: Normal control (Vehicle, p.o.)
Group II: Diabetic control (STZ/NA; 55/100 mg/kg, i.p.)
Group III: Diabetic + Metformin (50 mg/kg, p.o.)
Group IV: Diabetic + Methanol extract (200 mg/kg, p.o.)
Group V: Diabetic + Methanol extract (400 mg/kg, p.o.)
Group VI: Diabetic + Ethyl acetate fraction (15 mg/kg, p.o.)
Group VII: Diabetic + Ethyl acetate fraction (30 mg/kg, p.o.)

The standard drug and test samples were administered orally via a standard orogastric cannula once daily for 21 days to diabetic rats. The blood glucose concentration was determined in all the groups on 0 day, 7th day, 14th day and 21th day. Blood glucose level was estimated using glucose oxidase-peroxidase reactive strips and a glucometer (Contour, Bayer Healthcare, Japan). Antidiabetic activity in diabetic rats was assessed by fall in blood glucose.

Statistical analyses

Table 1: Effect on the blood glucose level after treatment with methanol extract and ethyl acetate fraction of *B. sensitivum*.

S. No.	Group	Dose (mg/Kg)	Blood Glucose Levels (mg/dl)			
			Day 0	Day 7	Day 14	Day 21
1	Normal control	Vehicle	81.50±5.54[a]	82.67±6.40[a]	84.50±4.55[a]	86.00±5.25
2	Diabetic control (STZ-NA)	55/100	233.00±18.44*	240.67±18.60*[a]	246.67±16.63*[a]	254.33±14.30*[a]
3	Standard (Metformin)	150	249.17±12.90*	142.83±9.57*	124.33±3.66*	93.33±6.65
4	Methanol extract	200	218.33±28.09*	123.67±17.47*[a]	99.67±11.23*[a]	90.00±11.53
5	Methanol extract	400	222.33±21.07*	130.33±20.50*	102.00±2.00*[a]	100.00±3.60
6	Ethyl acetate fraction	15	225.00±20.74*	136.00±4.18*	94.00±6.52*[a]	95.67±3.78
7	Ethyl acetate fraction	30	237.50±12.55*	131.67±9.31*	92.83±4.40*[a]	87.33±2.33

The data is expressed as Mean ± S.D.; n = 6; *P<0.05 vs. Normal control; [a]P<0.05 vs. Standard (Metformin); One-way ANOVA followed by Student-Newman-Keul's test.

The data are expressed as the mean ± S.D. The statistical analysis was carried out by using Sigma Stat version 3.5. The obtained results are analyzed by one-way ANOVA followed by Student Newman Keul's test.

RESULTS AND DISCUSSION

Phytochemical screening of methanol extract and ethyl acetate fraction

The percentage yield of methanol extract was found to be 23.29 % w/w. Methanol extract showed the presence of flavonoids, tannins, phenolic compounds, proteins and amino acids. Percentage yield of ethyl acetate fraction was found to be 14%, which is in relation to methanol extract and the fraction showed the presence of flavonoids and phenolic compounds.

Acute Toxicity Studies

Acute toxicity studies revealed the non-toxic nature of the methanol extract of *B. sensitivum* at the dose of 2000 mg/kg, p.o. as it did not show any mortality in rats. There was no lethality or any toxic reactions observed at dose selected until the end of the study period. As per OECD-423 guidelines, the dose is said to be "unclassified" under the toxicity scale. Hence further study with lower doses was not performed.

Antidiabetic activity studies

The rats were made diabetic by administering streptozotocin (55 mg/kg, p.o.) and nicotinamide (100 mg/kg, p.o.) was administered to rats to avoid induction of severe hyperglycemia in rats. The standard drug metformin and test drugs were administered for 21 days to the diabetic rats with a view to assess their antidiabetic potential in terms of reduction in blood glucose level, which was compared with the blood glucose level of normal rats. After 21 days treatment blood glucose level of rat was recorded. It is clearly evident from table 1 that the methanol extract and ethyl acetate fraction exhibited significant antidiabetic activity at the dose of 200 and 15 mg/kg, respectively. On the day 21, methanol extract and ethyl acetate fraction significantly reduced diabetes in rats statistically equivalent to normal control and standard drug.

Diabetes mellitus is one of the most common chronic diseases and is associated with hyper lipidemia and co-morbidities such as obesity and hypertension. *Biophytum sensitivum* is traditionally used in the treatment of diabetes. In order to establish the scientific basis for the utility of the plant in the treatment of diabetes, evaluation of the antidiabetic activity of the methanol extract and ethyl acetate fraction of *B. sensitivum* was carried out. It is reported that Streptozotocin causes diabetes mellitus due to destruction of the β-cells of the islets of Langerhans of the pancreas. It has been reported that the rats administered with nicotinamide (230 mg/kg, i.p.) 15 min before STZ (65 mg/kg, i.v.) develop moderate and stable non-fasting hyperglycaemia without any significant change in plasma insulin level.[18] As nicotinamide is an antioxidant which exerts protective effect on the cytotoxic action of STZ by scavenging free radicals and causes only minor damage to pancreatic beta cell mass producing type 2 diabetes. The present studies have validated traditional claims of *B. sensitivum* for antidiabetic activity. As crude extract of the plant did not show any toxic signs in animals during acute toxicity studies, the plant can be developed as safer and efficacious antidiabetic drug.

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