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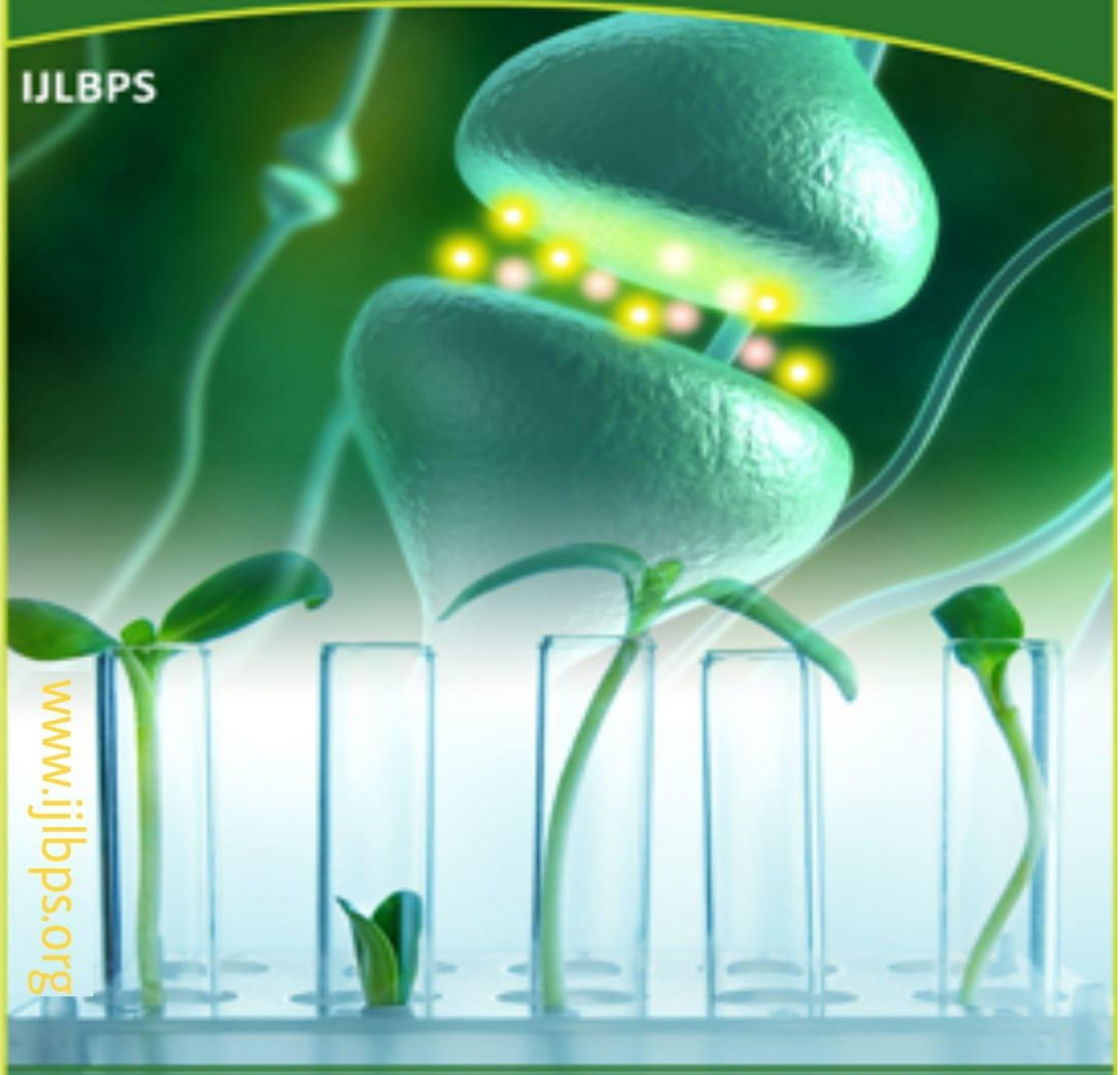

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The flower of Polygonum orientale Linn has antihyperglycemic properties. utilizing a mouse model of diabetes caused by steptozocin

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Abstract

Polygonum orientale Linn. flower aqueous extract was tested for its hypoglycemic effects in mice with diabetes caused by streptozotocin (STZ). Blood glucose, serum cholesterol, and liver glycogen levels were measured after 21 days of treatment with floral extract at dosages of 100 and 200 mg/kg b.w. The floral extract significantly decreased blood glucose level (P.0001), serum cholesterol (P.01), and increased liver glycogen (P.0001) in Streptozotocin-induced diabetic mice. The findings support the traditional use of Polygonum orientale Linn. flower extract as ethnomedicine for the treatment of diabetes by showing an anti hyperglycaemic action in Streptozotocin-induced diabetic mice.

Key-Words: *Polygonum orientale* Linn., Antihyperglycaemic, Diabetes mellitus, Streptozotocin, Oral administration

INTRODUCTION:

Diabetes mellitus is a metabolic condition that influences nutrient use. It is a heterogeneous collection of diseases characterized by hyperglycemia caused by faulty or insufficient insulin secretory response1. Many herbal treatments have been advocated for the treatment of Diabetes2, despite the fact that the presently available therapeutic alternatives for Diabetes, such as oral hypoglycemic medications and insulin, have limitations of their own. Medicinal plants include a wide range of active chemicals, many of which are assumed to exert their effects via distinct pathways. In severe diseases like diabetes and its consequences, they may prove helpful.3. The medical community is still working on a solution for side-effect-free diabetes management. As a result, there is a growing need for a plant-based, antidiabetic medicine that has fewer adverse effects. Traditional Indian medicine is a part of one of the world's most comprehensive medical traditions. North Eastern India in particular is endowed with an abundance of untapped biodiversity and traditional wisdom.Consequently, additional investigation into the region's indigenous wisdom is always called for. The World Health Organization's expert council on diabetes mellitus recommended in 1980 that traditional hypoglycemic medicines derived from plants be studied.

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Plants belonging to the genus Polygonum are native to the subtropical regions of the Himalayas, the Gangetic plain, Bihar, North Bengal, and Assam.4. The Acanthaceae family includes the medicinal plant Polygonum orientale Linn. In Hindi, it has a different name: Vasaka. The leaves of this evergreen shrub range in length from 13 to 35 centimeters and are oblanceolate, elliptic oblong, acute, or acuminate and whole. The branchlets are quadrangular. Terminal panicles of up to 30 centimeters in length are thyrsoid in shape and bear the flowers. The clavate capsule is 3.8 cm in length. The plant reaches its peak of beauty in early spring, when it produces tall, cylindrical spikes of velvety, brick red flowers. Bristly hairs cover the 6.8-millimeter calyx lobe. The length of a bract is between 6 and 12 mm. Disc-shaped seeds. The months of February through April5 are blooming months. Adhatoda vasica, another whole-plant remedy, is used to treat whooping cough and menorrhagia. Fever may be treated by burning fruits and plants. Phlogantholide A, a diterpene lactone, is said to be present in the leaves. A mixture ofleaves is also helpful for disorders of the liver and spleen[4].Polygonum orientale Linn. shows analgesic action on experimental mice7, and the ethanolic extract of the plant has antibacterial activity8. The Jaintia people of Meghalaya utilize the ash from the plant's fruit and leaves to cure fever. It has been suggested that free radical production has a role in Compounds that can scavenge free radicals have enormous promise in ameliorating disease processes including Rheumatoid Arthritis, Cancer, Diabetes, etc., the causes of which are both known and unknown. Polygonum orientale Linn. possesses strong antioxidant properties, suggesting it might be an effective therapeutic herb9.

Material and Methods

Chemicals

Streptozotocin and Glibenclamide was purchased from Sigma Chemical Co, St Louis, MO, USA. All other chemicals and reagents used were of analytical grade. Plant materialThe flowers of Polygonum orientale Linn. were collected from local market in June 2012 and herbarium was prepared. The herbarium was identified for authenticity by the experts of Dept of Botany, Sagar Institute of Research, Technology & Science- Pharmacy, Bhopal, M.P. The flowers were thoroughly washed and shade dried.Preparation of Plant extractAfter shade drying the dried flowers were powdered in mixture grinder. The powdered flower was macerated with distilled water for 72 hrs at room temperature with occasional stirring. It was then filtered through Whattman filter paper. The filtrate was air dried and stored in refrigerator for further use as PTAE (Polygonum orientale Linn. aqueous extract). The yield of the extract was 10% (w/w). During experiment the crude extract was diluted with distilled water just before administration to animals.Phytochemical screeningPhytochemical screening of the crude plant material was carried on using standard protocols for detection of flavonoid, phenol, tannin, saponin, steroid, alkaloid, carbohydrate. 10-14Experimental AnimalsHealthy adult albino mice of both sexes (20-25 g) in house bred at the Animal house of Sagar Institute of Research, Technology & Science- Pharmacy, Bhopal,M.P. India were used for the study. Mice were housed in polypropylene cages lined with husk in standard environmental conditions and 12:12 light:dark cycle. The animals were fed on a standard pellet diet ad libitum and had free access to water. The experiments were performed after approval of the protocol by the Institutional Animal Ethics Committee (IAEC) and were carried out in accordance with the current guidelines for the care of laboratory animals. Experimental Design

Antidiabetic activity of Polygonum orientale Linn. aqueous extract was assessed in normal, glucose loaded hyperglycaemic and streptozotocin induced diabeticmice. In all studies, the animals were fasted overnight for 16h with free access to water throughout the duration of the experiment.Evaluation of extract on normal healthy mice15At the end of the fasting period taken as zero time (0 h), blood was withdrawn from the tail vein. Serum was separated by centrifugation and glucose was estimated. The animals were randomly divided into four groups of six animals each. Group 1 served as control and received only distilled water. Group II, III and IV received Polygonum orientale Linn. orally at the dose of 50, 100, 200 mg/kg. Blood glucose levels were determined in 1, 2, 3h following treatment.

Evaluation of extract in Oral glucose tolerance test16 Healthy mice were divided into four groups of six animals each: Group I served as control received only vehicle (distilled water) and Groups II, III and IV received Polygonum orientale Linn .orally at the dose level of 50, 100, 200 mg/kg, respectively. All the animals were given glucose (2g/kg) 60 min after dosing. Blood samples were collected from tail vein just prior to (0h) and at 30, 60, 90 and 120 min after glucose loading and blood glucose levels were estimated.Evaluation of extract in streptozotocin induced diabetic mice17Experimental diabetes was induced by single intraperitoneal injection of 55mg/kg of Streptozotocin (STZ) freshly dissolved in distilled water. Control animals received only distilled water. After 48 hrs of Streptozotocin injection animals with fasting blood glucose above 200mg/dl were considered as diabetic and included in the study. The animals were randomly assigned into five groups of six animals each and received the following treatments: Group I: Normal control + distilled water, Group II: Diabetic control + distilled water, Group III: Diabetic + Polygonum orientale Linn.(100mg/kg), Group IV: Diabetic + Polygonum orientale Linn.(200mg/kg), Group V: Diabetic+ Glibenclamide (10mg/kg).The freshly prepared solutions were orally administered daily for 21 days. Body weights and blood glucose analysis was done weekly on overnight fasted animals. At the end of the experimental period, the animals were fasted overnight and blood was collected for various biochemical estimations. The animals were sacrificed by cervical decapitation. Liver was dissected out, immediately rinsed in ice cold saline and stored for further biochemical analysis.

Biochemical analysisSerum glucose analysis was done by GOD-POD method using Glucose Estimation kit (Crest Biosystems). Serum Cholesterol was estimatedspectrophotometrically (CHOP-PAP method, Crest Biosystems). Liver glycogen was estimated by the method of Seifter Sam et al (1950)18.

Acute oral toxicity studyAcute oral toxicity of Polygonum orientale Linn. was performed on Swiss albino mice, according to OECD Guidelines 423. Two groups of three animals in each were used for the study. Group I received distilled water. Group II received oral dose of 1000mg/kg for 3 days. The animals were observed for gross behavioural, neural, autonomic and toxic effects at short intervals of time for 24 hrs and then daily for 7 days. A food consumption and body weight was monitored daily.Statistical analysisAll results were expressed as mean ± SEM. The significance of the difference between the means of test and control studies was established by student's t-test. P value less than 0.01,.001,.0001 were considered significant.Results and DiscussionPhytochemical screeningPhytochemical screening of flower of Polygonum orientale Linn. showed the presence of flavonoid, phenol, tannin, saponin, steroid and trace amount of alkaloid.Effect of Polygonum orientale Linn.aqueous extract on normoglycaemic miceResults of the effect of

graded doses of Polygonum orientale Linn. on blood glucose level in normal healthy mice are presented in Table 1. Polygonum orientale Linn. produced peak hypoglycaemia at 2h. Dose dependent blood glucose reduction was observed in animals treated with 50, 100, 200 mg/kg. Polygonum orientale Linn. at dose 200mg/kg showed significant reduction in blood glucose (P<.001) when compared to control. Blood glucose levels were restored in all treatment groups in 3h.Effect of Polygonum orientale Linn. aqueous extract on oral glucose tolerance in normal micePolygonum orientale Linn. when administered 60 min prior to glucose loading produced significant reduction in the rise in blood glucose levels at 60 min after glucose administration which is shown in Table 2. Dose dependent blood glucose reduction was observed in animals treated with 50, 100, 200 mg/kg. All the doses showed significant reduction in blood glucose (P<.001) when compared to control.Effect of Polygonum orientale Linn. aqueous extract on fasting blood glucose and body weight in STZ induced diabetic miceThe effect of repeated oral administration of Polygonum orientale Linn. on blood glucose levels in Streptozotocin induced diabetic mice and body weightis given in Table 3 and Table 4. Polygonum orientale Linn. administered in two different doses to Streptozotocin treated diabetic mice showed significant reduction of blood glucose levels which was related to dose and duration of the treatment. Maximum reduction was observed on day 21. Polygonum orientale Linn. in both doses 200mg/kg, 100mg/kg exhibited significant glucose lowering effect in diabetic mice (P<.0001) as compared to the control. Streptozotocin produced significant loss of body weight as compared to normal animals during the study. Diabetic control continued to lose weight till the end of the study while Polygonum orientale Linn. treated group at all the two doses showed improvement in body weight compared to diabetic control.Effect of Polygonum orientale Linn. aqueous extract on serum cholesterol and Liver glycogen in STZ induced diabetic mice

Polygonum orientale Linn.treated group showed reduction in serum cholesterol compared to the diabetic control which is shown in Table 5. Polygonum orientale Linn.in both the doses 200mg/kg, 100mg/kg were effective in reducing the cholesterol levels (P<.01). Glycogen content in liver decreased in diabetic control compared to normal control. Administration of Polygonum orientale Linn.at the doses of 100 and 200 mg/kg for 21 days resulted in significant increase in the glycogen levels in liver (P<.0001) which is shown in Table 5.Acute Oral Toxicity Study:Polygonum orientale Linn. showed no mortality or behavioural change upto 1000mg/kg in the animals.The study was undertaken to evaluate the hypoglycaemic activity of Polygonum orientale Linn.in normal, glucose loaded hyperglycaemic and streptozotocin induced diabetic mice. In normoglycaemic mice Polygonum orientale Linn.showed dose dependent hypoglycaemic effect in 2h. From OGTT it could be concluded that dose 200mg/kg showed maximum improvement in glucose tolerance.Streptozotocin significantly induced hyperglycaemia. Oral administration of Polygonum orientale Linn. for 21 days caused a significant decrease in blood glucose levels. The possible mechanism by which Polygonum orientale Linn.mediated its antidiabetic effect could be by improvement of pancreatic secretion of insulin from existing β cells of islets. The hypoglycaemic effect of Polygonum orientale Linn.was compared with Glibenclamide, a standard hypoglycaemic drug. From the present study it may be suggested that the mechanism of action may Polygonum orientale Linn.be similar to glibenclamide action. So, oraladministration of Polygonum orientale Linn. has prominent hypoglycaemic effect.Hypercholesteremia is one of the primary factor involved in the development of atherosclerosis and diabetes-

related consequences, such as heart disease and stroke19. Serum cholesterol was considerably decreased in STZ diabetic mice when treated with Polygonum orientale Linn. As a result, it is plausible to assume that Polygonum orientale Linn. may help regulate irregularities in blood cholesterol levels.When a person has diabetes mellitus, their liver can't produce glycogen as efficiently as usual. Glycogen synthase activation by synthase phosphatase seems to be faulty in diabetes, leading to impaired glycogenesis. Hepatic glycogen levels were found to be lower than previously thought. After 21 days of treatment with Polygonum orientale Linn. (100 and 200mg/kg), hepatic glycogen was considerably elevated, suggesting that the extract largely addressed the poor glycogen storage associated with the diabetic condition.Therefore, the many phytoconstituents found in the phytochemical screening, each of which might impart therapeutic benefit on its own, may be responsible for Polygonum orientale Linn.'s considerable antidiabetic efficacy. This research suggests that the flower of Polygonum orientale Linn., extracted in water, may reduce blood sugar levels. It may have a beneficial impact in treating diabetes. More research is needed to determine the cellular and molecular mechanisms by which the medicinal plant exerts its effects. Researchers in our lab are now examining the impact of Polygonum orientale Linn.aqueous extract on lipid profiles and liver enzymes in Streptozotocin-induced diabetic mice..

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Table 1: Effect of *Polygonum orientale* Linn.aqueous extract in normoglycaemic mice (Mean±SEM)(n=6)

S/ No.	Groups	Doses (mg/kg)	Blood glucose level S(mg/dl) 0hr	1hr	2hr	3hr
1.	I(control)	Distilled water	71±.58	74.6±.33	71±.58	71±.58
2.	II	50	74.5±.5	70.33±.33[b]	64±.58[a]	74.5±.5
3.	III	100	74.5±.5	70.33±.33[b]	62.6±.33[b]	74.5±.5
4.	IV	200	80.5±.5	74.6±.33	62.6±.33[b]	80.5±.5

[a]P<.01 when compared with corresponding values of control group
[b]P<.001 when compared with corresponding values of control group

Table 2: Effect of *polygonum orientale* Linn.on oral glucose tolerance in normal mice (Mean±SEM)(n=6)

S/ No.	Groups	Doses (mg/kg)	blood glucose levels (mg/dl) 0hr	30min	60min	90min	120min
1.	I(control)	Distilled water	80.5±.5	140.3±.33	170.3±.33	140.3±.33	130.3±.33
2.	II	50	74.5±.5	134.6±.33[a]	140.3±.33[b]	130.3±.33[b]	121±.58[b]
3.	III	100	74.5±.5	130.33±.33[b]	140.3±.33[b]	130.3±.33[b]	121±.58[b]
4.	IV	200	83.5±.5	124.3±.33[b]	134.3±.33[b]	126±.58[b]	116.3±.33[b]

[a]P<.001 when compared with corresponding values of control group
[b]P<.001 when compared with corresponding values of control group

Table 3: Effect of *Polygonum orientale* Linn.on blood glucose in stz induced diabetic mice (Mean±SEM)(n=6)

Treatments	Blood glucose levels (mg/dl)			
Group	1th day	7th day	14th day	21st day
Control	94.7 ±5.64	96.7±5.70	94.5±5.61	95.1±5.62
Diabetic control	209.5±8.35	209.8±8.37	209.5±8.35	210.06±8.36
Treated 100mg/kg	204.06±8.24	194.6±8.05 [a,b]	164.2±7.39[a,b]	150.3±7.08[a,b]
Treated 200mg/kg	207.6±8.31	191.3±7.99[a,b]	160±7.30[a,b]	144±6.92[a,b]
Glibenclamide(10mg/kg)	207.6±8.31	189.6±7.94[a,b]	160±7.30[a,b]	140.3±6.84[a,b]

[a] P<.0001 compared to diabetic control

Table 4: Effect of *Polygonum orientale* Linn.on body weight of stz induced diabetic mice

	Body weight (gm)			
Group	**1st day**	**7th day**	**14th day**	**21st day**
Control	25.06±2.88	25.06±2.88	25.6±2.91	25.6±2.91
Diabetic control	25.06±2.88	23.6±2.80	21.06±2.64	16.6±2.34
Treated 100mg/kg	25.6±2.91	23.6±2.80	25.6±2.91	26.6±2.97
Treated200mg/kg	26.2±2.94	25.7±2.97	25.8±2.97	26.6±2.97
Glibenclamide(10mg/kg)	25.8±2.97	23.6±2.80	24.9±2.91	27.2±3.00

Table 5: Effect of *Polygonum orientale* Linn.on serum cholesterol and liver glycogen in stz induced diabetic mice

Group	Serum cholesterol (mg/dl)	Liver Glycogen (mg/g)
Control	41.6±.33	38.5±.35
Diabetic Control	82.4± 3.4207[a]	11.86±.338[c]
Treated 100mg/kg	55.6± .50[b]	29.6±.29[d]
Treated 200mg/kg	53.2± 1.41[b]	30.7±.87[d]
Glibenclamide(10mg/kg)	48.8± 2.83[b]	31.6±.27[d]

[a] P<.001 Compared to normal control
[b]P<.01 Compared to diabetic Control
[c]P<.0001 compared to the corresponding values of normal control
[d]P<.0001 compared to the corresponding values of diabetic control