



Antihyperglycaemic Activity on Flower of *Polygonum Orientale Linn.* Using Streptozotocin Induced Diabetic Mice Model.

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Research Article

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Abstract

Objective: The study was done on anti hyperglycaemic effect of aqueous extract of the flower of *Polygonum orientale Linn.* in streptozotocin (STZ) induced diabetic mice.

Methods: Experimental 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

Results: The flower extract of *Polygonum orientale Linn.* in doses 100 and 200 mg/kg b.w was administered for 21 days and blood glucose level, serum cholesterol, liver glycogen was estimated. Treatment of the Streptozotocin induced diabetic mice with the flower extract resulted in significant reduction of blood glucose level ($P < .0001$), serum cholesterol ($P < .01$) and increase in liver glycogen ($P < .0001$).

Conclusion: The results suggest that the flower extract of *Polygonum orientale Linn.* possess anti hyperglycaemic effect in Streptozotocin induced diabetic mice which justify the traditional use of this plant as ethno-medicine in treatment of diabetes.

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

INTRODUCTION

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. It represents a heterogeneous group of disorders having hyperglycemia which is due to impaired carbohydrate utilization resulting from a defective or deficient insulin secretory response^[1]. Apart from currently available therapeutic options for Diabetes like oral hypo glycaemic agents and insulin which have limitation of their own, many herbal medicines have been recommended for the treatment of Diabetes^[2]. A variety of ingredients present in medicinal plants are thought to act on variety of targets by various modes and mechanisms. They have a potential to impart therapeutic effect in complicated disorders like Diabetes and its complications^[3]. Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand of anti-diabetic medicinal plant which has comparatively less side effects. Indian traditional medicines belong to one of the richest medicinal systems are among those available in the world. Especially North Eastern part of India is blessed with a very rich biodiversity with a rich wealth of traditional knowledge which is yet to be explored. So, more and more research is required to explore the traditional knowledge of this region. According to the recommendation of the WHO expert committee on Diabetes mellitus (WHO, 1980), an investigation of hypoglycemic agents of plant origin used in traditional medicine seems important.

Polygonum orientale Linn. is found in the sub tropical Himalayas, upper Gangetic plain, Bihar, North Bengal and Assam^[4]. *Polygonum orientale Linn.* is a medicinal herb which belongs to Acanthaceae family. It is known as Vasaka in Hindi. An evergreen shrub up to 2.4 m high, branchlets quadrangular, leaves are 13-35 cm long, oblanceolate, elliptic oblong, acute or acuminate, entire. Flowers are in terminal elongated, thyrsoid panicles, up to 30cm long. Capsule is 3.8 cm long, linear clavate. In early spring the plant becomes showy with its dense cylindrical spikes of brick red velvety flower. Calyx lobe is 6.8 mm, bristly haired. Bracts are 6 to 12 mm long. Seeds are disc like.



Flowering occurs in the month of February to April^[5]. Whole plant is used like *Polygonum orientale* Linn in Whooping cough and Menorrhagia. Fruits and leaves are burnt and it is prescribed for fever. The leaves are reported to contain diterpene lactone, Phlogantholide A. A decoction of leaves is also beneficial in liver and spleen diseases^[4]. Jaintia tribe of Meghalaya uses fruit and leaf ash of *Polygonum orientale* Linn. and use it to treat fever^[6]. Ethanolic extract of *Polygonum orientale* Linn. has analgesic activity on experimental mice^[7]. *Polygonum orientale* Linn. has antimicrobial activity also^[8]. The generation of free radicals has been implicated in the causation of several diseases of known and unknown etiologies such as Rheumatoid Arthritis, Cancer, Diabetes etc., and compounds that can scavenge free radicals have great potential in ameliorating these disease processes. *Polygonum orientale* Linn. has prominent free radical scavenging property so it may prove as a very good medicinal herb^[9].

MATERIAL & 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 material:

The 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 extract:

After 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 Whatman 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 screening:

Phytochemical screening of the crude plant material was carried on using standard protocols for detection of flavonoid, phenol, tannin, saponin, steroid, alkaloid, carbohydrate.^{[10],[11],[12],[13],[14]}

Experimental Animals:

Healthy 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 diabetic mice. 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 mice^[15]:

At 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 test^[16]:

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 mice^[17]:

Experimental 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 analysis:

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

Acute oral toxicity study:

Acute 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 analysis:

All 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

Phytochemical screening:

Phytochemical screening of flower of *Polygonum orientale Linn.* showed the presence of flavonoid, phenol, tannin, saponin, steroid and trace amount of alkaloid.

Table 1: Effect of *Polygonum Orientale Linn.* Aqueous Extract in Normoglycaemic Mice (Mean±SEM) (n=6)

SL NO	GROUPS	DOSES(mg/kg)	BLOOD GLUCOSE LEVELS(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

^aP<.01 when compared with corresponding values of control group

^bP<.001 when compared with corresponding values of control group

Effect of *Polygonum orientale Linn.* aqueous extract on normoglycaemic mice:

Results 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 mice:

Polygonum 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 mice:

The effect of repeated oral administration of *Polygonum orientale Linn.* on blood glucose levels in Streptozotocin induced diabetic mice and body weight is 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.

DISCUSSION & CONCLUSION

Table 2: Effect of *Polygonum Orientale Linn.* on Oral Glucose Tolerance in Normal Mice (Mean±SEM)(n=6)

The study was undertaken to evaluate the

SL 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

^aP<.001 when compared with corresponding values of control group

^bP<.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)

^a P<.0001 compared to diabetic control

^b p<.0001 compared to day 1 of same group

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 2 h. From OGTT it could be concluded that dose 200mg/kg showed maximum improvement in glucose tolerance.

Streptozotocin significantly induced

ANIMAL GROUPING	BLOOD GLUCOSE LEVELS(mg/dl)			
	1 th day	7 th day	14 th day	21 st 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}

Table 4: Effect of *Polygonum Orientale Linn.* On Body Weight of Stz Induced Diabetic Mice

hyperglycaemia. Oral administration of *Polygonum orientale Linn.* for 21 days caused a significant decrease in blood glucose levels. The possible

ANIMAL GROUPING	BODY WEIGHT (gm)			
	1 st day	7 th day	14 th day	21 st 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
Treated 200mg/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

Acute Oral Toxicity Study:

Polygonum orientale Linn. showed no mortality or behavioural change upto 1000mg/kg in the animals.

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, oral administration of *Polygonum orientale* Linn. has prominent hypoglycaemic effect.

Hypercholesteremia is one of the primary factor involved in the development of atherosclerosis and coronary heart disease which are the secondary complications of diabetes^[19]. *Polygonum orientale* Linn. significantly reduced serum cholesterol in STZ diabetic mice. Thus it is reasonable to conclude that *Polygonum orientale* Linn. could modulate blood cholesterol abnormalities.

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

Diabetes mellitus impairs the normal capacity of the liver to synthesise glycogen. Synthase phosphatase activates glycogen synthase resulting in glycogenesis and its activation appears to be defective in diabetes^[20]. Decrease in hepatic glycogen was observed in this study. Treatment with *Polygonum orientale* Linn. (100 and 200mg/kg) for 21 days was significantly increased liver glycogen indicating that the defective glycogen storage of the diabetic state was partially corrected by the extract.

Thus, the significant antidiabetic effect of *Polygonum orientale* Linn. could be due to the presence of various phytoconstituents detected in the phytochemical screening which alone can impart therapeutic effect. From this study we can conclude that aqueous extract of *Polygonum orientale* Linn. flower has beneficial effects on blood glucose level. It has the potential to impart therapeutic effect in diabetes. Further studies are necessary to elucidate in detail the mechanism of action of the medicinal plant at the cellular and molecular levels. The studies on the effect of *Polygonum orientale* Linn. aqueous extract on lipid profiles and liver enzymes in Streptozotocin induced diabetic mice is going on in our laboratory.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.