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### Research Article

## Anti-diabetic Potential of Extract Obtained from *Parkia biglandulosa* (mimosaceae) Stems Bark using Alloxan Induced Diabetes Model

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### ABSTRACT

Diabetes mellitus is a chronic metabolic disorder that places a huge health and economic burden on societies. Since currently available medications have many drawbacks, it is important to seek alternative therapies. Medicinal plants used in traditional medicine are ideal candidates. Therefore, this study was carried out to investigate the anti-diabetic activity of the stem bark extract of *Parkia biglandulosa* (mimosaceae) in wister rats. The phytochemical composition of the stem bark extract was qualitatively evaluated using standard procedures. In vivo anti-diabetic activity was performed by using the alloxan-induced diabetes method by orally administering methanolic extract of stem bark at doses of 200 and 400 mg/kg of body weight and profiling blood glucose levels at 0, 7, 14, 21 days using a glucometer. Biochemical parameters such as cholesterol, high-density lipoproteins, low-density lipoproteins, creatinine, urea and alkaline phosphatase were also evaluated. *P. biglandulosa* stem bark extract at the tested dose levels of 200 and 400 mg/kg showed a significant reduction in blood glucose level with  $175.34 \pm 9.357$  and  $171.69 \pm 6.248$  mg/dL respectively as compared with diabetic control group. The observed anti-diabetic activity could be associated with the phytochemicals present in this plant extract. The results suggest that *P. biglandulosa* bark extract possess anti-diabetic activity and this validates its folkloric use.

### INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Metabolic abnormalities in carbohydrates, lipids, and proteins are consequences from the importance of insulin as an anabolic hormone.<sup>[1]</sup> The classification of diabetes mellitus is based on its etiology and clinical presentation. There are four types or classes of diabetes mellitus: type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific types.<sup>[2]</sup> Diabetes mellitus is a growing health problem worldwide that causes severe morbidity and mortality. The prevalence of diabetes is increasing day by day.<sup>[3]</sup> Diabetes is already recognized as a public health problem of pandemic magnitudes. There are currently more than 150 million people with diabetes worldwide, which appears to reach 300 million by 2025.<sup>[4,5]</sup> The

control of diabetes is a global problem so far and the success of the complete cure has not yet been discovered. There are many synthetic drugs developed for patients, but it is the fact that someone has never been reported to have fully recovered from diabetes. Despite the presence of known anti-diabetic drugs on the pharmaceutical market, diabetes and related complications remained a serious medical problem.<sup>[6]</sup> Given the side effects of insulin therapy and currently available synthetic medications, the search for effective and safer anti-diabetic herbal medications continues worldwide. Herbal medicines play a vital role in this part to prevent side effects.<sup>[7]</sup> Currently, the main and effective treatment for diabetes is the use of insulin and hypoglycemic drugs, but these compounds also have many negative side effects. Medicinal plants have a long history of use and today they are widely used for various diseases.<sup>[8]</sup> For many decades, medicinal plants

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have been useful resources to treat various diseases, including diabetes. Medicinal plants have always been an important source for finding new remedies for human health problems. Traditionally, numerous herbs have been recommended for treating diabetes. Furthermore, many researchers have reported anti-diabetic effects of so many plants.<sup>[9]</sup>

*P. biglandulosa*, belonging to the mimosaceae family, is commonly known as the Badminton Ball Tree. Its various parts are used against a wide range of ailments.<sup>[10]</sup> It is a well-known and traditionally used medicinal plant in the world. Different parts of the plant possess antiulcer, antibacterial activity, antifungal activity and anti-inflammatory activity.<sup>[11]</sup> Although the extensive research on the different species from the genus parkia such as *P. biglobosa*, *P. speciosa*, *P. javanica*, *P. bicolor*, *P. biglandulosa*, *P. filicoidea*, and *P. clappertoniana* with properties of medicinal use has been made till date,<sup>[12]</sup> the anti-diabetic potential of *P. biglandulosa* bark is yet unexplored. Hence, this study has been carried out to determine the *in vivo* anti-diabetic activity of the methanolic extract of stem bark using the alloxan-induced diabetes model in wister rats.

## MATERIAL AND METHODS

**Study Location:** This study was conducted in the Pharmacology Department of T.V.E.S. Hon. Loksevak Madhukarrao Chaudhari College of Pharmacy, District-Jalgaon, Maharashtra-425503, India.

**Plant Material:** *P. biglandulosa* stem barks were collected from Chinchwad, Pune. Botanist D.L. Shirodkar authenticated the plant specimen at the India Botanical Survey Herbarium, Western Regional Center in Pune, Maharashtra, where sample No. RCK01 with voucher reference no. BSI / WRC / IDEN.CER / 2019 / H3 has been deposited. The clean stem bark was air-dried in the shade and ground to a coarse powder using an electric grinder, and stored in an airtight container at room temperature until the extraction time.

**Preparation of the Plant Extract:** 500 g of coarse pulverized stem bark packed in a Soxhlet apparatus sequentially extracted with petroleum ether, chloroform and methanol as solvent. After the extraction process, the extracts were concentrated to dryness using a rotary evaporator. The obtained extracts were stored in the refrigerator at 4°C until the next use; however only methanolic extract of stem bark was selected for anti-diabetic evaluation.

**Experimental Animals:** This study used wister rats of both sexes weighing between 180–250 g. The animals were housed individually in polypropylene cages in the Pharmacology Department, kept under standard cycle conditions of 12 hours of light and 12 hours of darkness, 25 ± 30°C, 35–60% relative humidity to minimize physiological effects, cardiovascular and immunological

induced by the environment, changes in the central nervous system and endocrine due to stress associated with transport.<sup>[13]</sup> The experimental animals were rigorously observed for the first four hours after administration and once a day for the next 14 days. Neurological, behavioral, and autonomic changes, physical changes such as alertness to motor activity, seizures, coma, restlessness, diarrhea, lacrimation, and animal appearance, changes in respiratory circulation, eyes and sleep critically observed every day.<sup>[14]</sup> The experimental animals provided standard rodent pellets and water. The animal experimentation protocol (IAEC/01/18-19/PN01) has been approved by the Institutional Animal Ethics Committee (IAEC) at T.V.E.S. Hon. Loksevak Madhukarrao Chaudhari College of Pharmacy, District- Jalgaon, Maharashtra-425503, India.

## Acute Toxicity Test

Acute toxicity studies were performed with albino mice. Mice in one group were given 2000 mg/kg body weight of *P. biglandulosa* stem bark extract. One group was kept as a normal control and was administered vehicle alone. The animals were observed individually for symptoms of toxicity and mortality, if any, and then periodically for the next 24 hours, and then every 24 hours for any signs of acute toxicity over 14 days. The acute toxicity study was carried out according to the OECD-425 guideline.<sup>[15]</sup> No morbidity or profound toxic reactions were observed at a dose of 2000 mg/kg which indirectly pronounces the safety profile of the plant extract doses of 200 and 400 mg/kg of body weight were selected to evaluate anti-diabetic activity.

## Anti-diabetic Activity

Diabetes was induced in overnight fasted animals by a single intraperitoneal injection of alloxan monohydrate 150 mg/kg body weight dissolved in normal saline. The solution was freshly prepared just before administration. After 72 hours of alloxan injection, the rats with elevated glucose levels in plasma 200 mg/dL were confirmed as diabetic and used for the experiment.

The animals were randomly divided into five groups of six rats in each.

Group I rats served as normal control received vehicle (Tween 80, 3% v/v in normal saline) alone.

Group II rats served as positive control were administered with alloxan (150 mg/kg body weight) in vehicle.

Group III rats received the standard drug, Glibenclamide (5 mg/kg body weight), in normal saline.

Rats of groups IV and V were treated with plant extract at 200 mg/kg of body weight and 400 mg/kg of body weight in the vehicle, respectively.

The plant extract prepared for administration was stored in the refrigerator, and the doses of the treatments were administered orally using a cannula once daily for 21 days. Blood was drawn each time to determine glucose levels at 0, 7, 14, and 21 days. Blood glucose levels were measured with a blood glucometer.



### Determination of Body Weight

All animal body weight groups were documented before treatment (day 0) and throughout the treatment period (days 7, 14 and 21). A suitably adjusted electronic balance was used to measure the bodyweight of the rats.

### Analysis of biochemical Parameters

For estimation of biochemical parameters, blood was collected from retro-orbital plexus of the rats under light ether anesthesia using capillary tubes. The plasma was separated by centrifugation and then analyzed for cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL), creatinine, urea, and alkaline phosphatase (ALP). These biochemical parameters were analyzed by colorimetric method using specific commercial biochemistry test kits.

### Statistical Analysis

All the data are expressed as Mean  $\pm$  SEM, and Statistical differences at 5% probability level ( $p < 0.05$ ) between groups were analyzed by one-way ANOVA followed by Dunnett's test.

## RESULT

The current study evaluated the anti-diabetic activity of methanolic extract of *P. biglandulosa* stem bark in alloxan-induced diabetes rats. The yield of methanolic extract obtained was 19.1% dark reddish-brown powder.

### Phytochemical Screening

The results of preliminary phytochemical screening revealed the presence of phyto-constituents in methanolic extract of stem bark obtained from *P. biglandulosa* (Table 1).

### Acute Toxicity Study

No signs and symptoms of acute toxicity and mortality were observed up to a dose of 2000 mg/kg of body weight of experimental animals during the entire experimental period. Body weight and food consumption were normal compared to a vehicle-treated animal. For further studies, the doses were set at 200 and 400 mg/kg of body weight.

### Anti-diabetic Activity

The effect of plant extract on blood glucose level is depicted in Table 2. Treatment with *P. biglandulosa* methanolic extracts of 200 and 400 mg/kg showed lowering of blood glucose level with  $175.34 \pm 9.357$  mg/dL and  $171.69 \pm 6.248$  mg/dL, respectively. The treatment with Glibenclamide 5 mg/kg showed a gradual reduction in the blood glucose in diabetic rats. The treatment with 200 and 400 mg/kg extracts showed a similar type of gradual reduction in the blood glucose level in the diabetic rats for 21 days treatments (Fig. 1).

### Determination of Body Weight

The results of body weight elevation in rats are shown in Table 3. Alloxan caused weight reduction during 21 days, which was reversed by plant extract 200 mg/kg and 400 mg/kg and standard drug group rats (Fig. 2).

**Table 1:** Preliminary phytochemical screening of stem bark extract of *P. biglandulosa*.

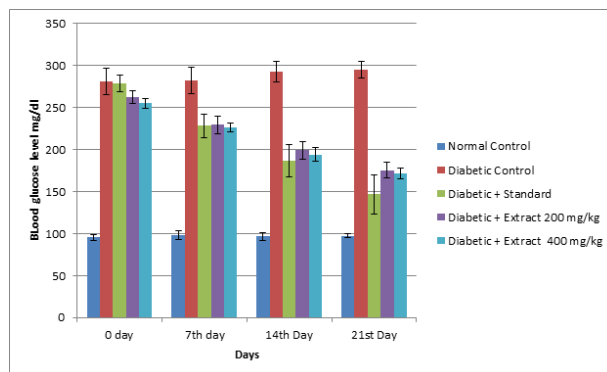
Constituents	Inference
Alkaloids	+
Flavonoids	++
Glycosides	++
Cardiac glycoside	+
Anthraquinone glycoside	-
Saponin	++
Steroids	+
Tannins	++

+: Present in Trace, ++: Abundant, -: Absent

**Table 2:** Effect of *P. biglandulosa* methanolic extract on blood glucose level in rats

Groups	Blood glucose level (mg/dL)			
	0 day	7 <sup>th</sup> day	14 <sup>th</sup> Day	21 <sup>st</sup> Day
Normal Control	95.33 $\pm$ 3.630	98.33 $\pm$ 5.16	96.5 $\pm$ 4.288	97.21 $\pm$ 2.380
Diabetic Control	281.17 $\pm$ 15.814	282.33 $\pm$ 15.54	292.67 $\pm$ 11.935	295.01 $\pm$ 10.25
Diabetic + Standard	279.17 $\pm$ 9.96 <sup>ns</sup>	228.17 $\pm$ 13.81**	186.83 $\pm$ 19.321**	146.7 $\pm$ 23.46**
Diabetic + extract 200 mg/kg	262.5 $\pm$ 7.53 <sup>ns</sup>	229.33 $\pm$ 10.16**	199 $\pm$ 10.279**	175.34 $\pm$ 9.357**
Diabetic + extract 400 mg/kg	255 $\pm$ 6.16 <sup>ns</sup>	226.33 $\pm$ 5.68**	194.17 $\pm$ 8.542**	171.69 $\pm$ 6.248**

Values are expressed as Mean  $\pm$  S.E.M. n=6, <sup>ns</sup>p > 0.05, \*\*p < 0.01 respective Diabetic control (One way ANOVA followed by Dunnett's test)

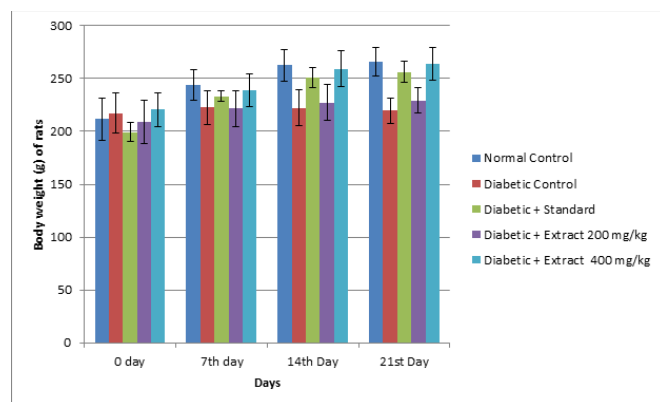


**Fig. 1:** Effect of methanolic extracts of *P. biglandulosa* on blood glucose level of rats

## Biochemical Parameters

Alloxan administration raised the serum level of cholesterol and LDL while reduced the HDL level. The serum level of HDL appeared significantly (\* $p < 0.05$ , \*\* $p < 0.01$ ) raised by both the extracts and standard group in rats (Table 4). The renal function markers like creatinine and urea were elevated in the alloxan-induced diabetic rats compared

with the normal rats. Whereas the 200 and 400 mg extract significantly (\*\* $p < 0.01$ ) reduces the elevated levels in a dose-dependent manner. The liver function marker ALP level was elevated in alloxan-induced diabetes. The rat treated with 200 and 400 mg extract exhibited significant (\*\* $p < 0.01$ ) reduction in the elevated level of liver enzyme in a dose-dependent manner (Table 5).



**Fig. 2:** Effect of methanolic extracts of *P. biglandulosa* on body weight of rats

**Table 3:** Effect of *P. biglandulosa* methanolic extract on body weight in rats

Groups	Body weight (g)			
	0 day	7 <sup>th</sup> day	14 <sup>th</sup> Day	21 <sup>st</sup> Day
Normal Control	211.67 ± 19.861	244.17 ± 14.270	262.5 ± 15.259	266.10 ± 13.300
Diabetic Control	217.33 ± 19.237	222.5 ± 16.099	222.33 ± 16.848	219.46 ± 11.854
Diabetic + Standard	199.17 ± 9.035 <sup>ns</sup>	233.16 ± 5.115 <sup>ns</sup>	250.83 ± 9.867 <sup>ns</sup>	256.13 ± 9.778 <sup>ns</sup>
Diabetic + Extract 200 mg/kg	208.67 ± 20.381 <sup>ns</sup>	221.5 ± 16.986 <sup>ns</sup>	227.16 ± 17.050 <sup>ns</sup>	229.23 ± 12.004 <sup>ns</sup>
Diabetic + Extract 400 mg/kg	220.67 ± 16.037 <sup>ns</sup>	239.67 ± 15.444 <sup>ns</sup>	259.17 ± 16.885 <sup>ns</sup>	263.65 ± 15.218 <sup>ns</sup>

Values are expressed as Mean ± S.E.M. n=6, <sup>ns</sup> $p > 0.05$  respective Diabetic control (One way ANOVA followed by Dunnett's test)

**Table 4:** Effect of *P. biglandulosa* methanolic extract on Cholesterol, HDL and LDL in rats

Groups	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Normal Control	70.5 ± 3.160	78.18 ± 2.151	22.17 ± 1.520
Diabetic Control	97.33 ± 7.940	55.36 ± 3.621	41.72 ± 2.775
Diabetic + Standard	72.17 ± 1.939**	76.625 ± 1.315**	22.15 ± 1.071**
Diabetic + Extract 200 mg/kg	73.33 ± 2.667**	67.025 ± 2.193*	28.98 ± 1.648**
Diabetic + Extract 400 mg/kg	71.83 ± 1.537**	73.775 ± 3.371**	24.93 ± 1.731**

Values are expressed as Mean ± S.E.M. n=6, \* $p < 0.05$ , \*\* $p < 0.01$  respective Diabetic control (One-way ANOVA followed by Dunnett's test)

**Table 5:** Effect of *P. biglandulosa* methanolic extract on creatinine, urea and alkaline phosphatase

Groups	Creatinine (mg/dL)	Urea (mg/dL)	ALP (u/L)
Normal Control	0.71 ± 0.03130	44.8 ± 2.962	0.450 ± 0.01257
Diabetic Control	0.86 ± 0.04240	82.73 ± 3.100	0.720 ± 0.05176
Diabetic + Standard	0.588 ± 0.020**	47.175 ± 1.441**	0.463 ± 0.008**
Diabetic + Extract 200 mg/kg	0.618 ± 0.01973**	62.03 ± 1.657**	0.596 ± 0.019**
Diabetic + Extract 400 mg/kg	0.590833 ± 0.01254**	46.57 ± 1.369**	0.524 ± 0.0068**

Values are expressed as Mean ± S.E.M. n=6, \*\* $p < 0.01$  respective Diabetic control (One-way ANOVA followed by Dunnett's test)

## DISCUSSION

The phytochemical screening confirmed the presence of alkaloids, glycosides, flavonoids, saponins, steroids and tannins. The anti-diabetic activity of the *P. biglandulosa* stem bark may be associated with the existence of numerous secondary metabolites in the plant.<sup>[16,17]</sup> The presence of alkaloids in this extract could be responsible for the activity. The alkaloids of the herbs *Ephedra distachya* induced an increase in insulin secretion, causing the regeneration and restoration of the atrophied pancreatic islets.<sup>[18]</sup> The alkaloids isolated from the leaves of *Acanthus montanus* showed hypoglycemic action in diabetic rats induced by alloxan.<sup>[19]</sup> The therapeutic impact of alkaloids against the pathogenesis of blood glucose is mediated through various cascades and signaling pathways,





through inhibition or stimulation of system diversity, such as inhibition of the enzyme  $\alpha$ -glucosidase, increasing insulin sensitivity and modulating oxidative stress.<sup>[20]</sup> O-glycoside, C-glycoside, and N-glycoside like *N*-( $\beta$ -D-glucopyranosyl)-amides, *N*-( $\beta$ -D-glucopyranosyl)-ureas, and 1,2,3-triazolyl derivatives possess good anti-diabetic potential.<sup>[21]</sup> Dimethoxy derivative of perlargonidin 3-O- $\alpha$ -L rhamnoside, Glycoside isolated from the bark of *Ficus bengalensis* showed anti-diabetic effect by increasing insulin secretion by beta-cells.<sup>[22]</sup> Triterpene glycoside isolated from *Gymnema sylvestre* exhibits anti-diabetic activity, enhancing glucose transporter 2 protein levels and ameliorating impaired insulin secretion.<sup>[23]</sup> Flavonoids have multiple positive health effects in metabolic disorders, such as cardiovascular disease, cancer, obesity, and diabetes.<sup>[24,25]</sup> Apigenin showed an antihyperglycemic effect and a protective effect on the destruction of pancreatic  $\beta$  cells in streptozotocin-induced diabetes.<sup>[26]</sup> The flavonoid content of *Cuminum nigrum* seeds caused a significant decrease in blood sugar in a dose range of 0.5 to 1.5 g/kg, both in normoglycemic and alloxan-induced diabetic rabbits.<sup>[27]</sup> Flavonoids act on various molecular targets and regulate different signaling pathways in pancreatic  $\beta$ -cells, hepatocytes, adipocytes, and skeletal myofibers.<sup>[28]</sup> The anti-diabetic activity of flavonoids supports the regulation of carbohydrate digestion, insulin signaling, insulin secretion, glucose absorption, and fat deposition.<sup>[29]</sup> Saponins have been reported to possess a wide range of biological activities against ailments; saponins were known to be bioactive against diabetes.<sup>[30]</sup> Saponin extracted from *Holothuria thomasi*, sea cucumber, has a potent hypoglycemic effect in streptozotocin diabetic rats.<sup>[31]</sup> *Momordica charantia* saponin increases glucose utilization by the liver, decreases gluconeogenesis by inhibiting the two key enzymes glucose-6-phosphatase and fructose-1,6 biphosphatase, and enhances glucose oxidation by activating glucose-6-phosphate dehydrogenase through the pathway<sup>[32]</sup>. The saponin-rich fraction of *Commiphora kerstingii* reduced the elevated blood glucose level in both alloxane-induced and fructose-induced hyperglycemia.<sup>[33]</sup> All forms of tannins have been reported to be involved in blood glucose management. Tannin stimulates receptor cells to use carbohydrates. The presence of tannins in *Rhoicissus tridentata* could have determined a hypoglycemic effect.<sup>[34]</sup> The condensed tannins extracted from some Kenyan foods have shown an antihyperglycemic action due to the inhibition of the enzymes  $\alpha$ -amylase and  $\alpha$ -glucosidase.<sup>[35]</sup>

Therapeutic uses of *P. biglandulosa* in treating several ailments like ulcer and inflammation have been reported in the literature alongside numerous other traditional claims.<sup>[11]</sup> Phytoconstituents reported from *P. biglandulosa* such as triterpenoid like Lupeol, sterols like  $\beta$ -Sitosterol and Campesterol, tannins like gallic acid, may be contributed for the therapeutic activity of the plant.<sup>[36]</sup> In order to form the scientific evidence for the

utility of *P. biglandulosa* in the management of diabetes, experimental study of the stem bark methanol extracts was performed in alloxan-induced diabetic rats.

## CONCLUSION

This research indicates that *P. biglandulosa* stem bark has a beneficial effect on elevated blood glucose level in alloxan-induced diabetic rats, therefore justifying its use as an anti-diabetic plant ethno-pharmacologically. However, the phytoconstituents responsible and the exact mechanism of the anti-diabetic effects of *P. biglandulosa* stem bark are unknown. Hence, future investigation is required to study phytoconstituents and their mechanism of action targeting the anti-diabetic potential of *P. biglandulosa* plant.

## ABBREVIATIONS

OECD- Organisation for Economic Co-operation and Development, SEM- Standard error means, ANOVA- Analysis of variance, HDL- High density lipoprotein, LDL- Low level lipoprotein, ALP- Alkaline phosphatase,

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