Vol 5/1 (2005) 31 - 34

JOURNAL OF NATURAL REMEDIES

A study on the anti-hyperglycaemic effect of *Premna corymbosa* Rottl. roots

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Abstract

Objective: To study the antihyperglycaemic activity of the ethanol (50% v/v) root extract of *Premna corymbosa* Rottl. on albino rats. Materials and methods: Antihyperglycaemic activity of the root extract was evaluated by using both normoglycaemic and alloxan induced hyperglycaemic rats at dose levels of 200 and 400 mg/kg respectively through oral route. Results: The extract was found to produce marked reduction in blood glucose concentration at tested dose levels in a dose dependant manner in both the models. However, in normoglycaemic animals, the extract at 400 mg/kg dose level produced significant reduction of blood glucose at the 8th hour of administration. Conclusion: The results of the present study justify the use of the roots of the plant for treating diabetes as suggested in folklore remedies.

Key words: *Premna corymbosa* Rottl, Alloxan, Antihyperglycaemic, Normoglycaemic, Glibenclamide.

1. Introduction

Premna corymbosa Rottl. (Fam-Verbenaceae) is a large, thorny deciduous shrub or a small tree up to 9 m in height with yellowish lenticellate bark, aromatic and elliptic-ovate leaves, flowers in terminal corymbs, fruits globose drupes, common along the Indian and Andaman coasts [1,2]. The roots and leaves are reported to be useful in the traditional system of medicine. The roots are astringent, anodyne, anti-inflammatory, expectorant, laxative, antibacterial and tonic while the leaves are useful in dyspepsia, flatulence, neuralgia, haemorrhoids and tumors [2]. The roots form official part in Ayurveda. The roots are light brown or yellowish brown, woody and aromatic. Traditionally, the drug is highly valued for its anti-inflammatory property. Some of the important Ayurvedic formulations containing the drug include Dasamularistam, Dhanvantaram kasayam, Agastyarasayanam Sukumaraghrtam etc. [3]. In Orissa, the tribes and the local herbal practitioners use and prescribe the paste of roots (about 10 g) along with rice washed water orally twice daily to reduce blood sugar in diabetic patients [4]. The present paper deals with the anti-

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hyperglycaemic activity of the hydroalcoholic (ethanol 50% v/v) extract of the roots on both normoglycaemic and alloxan induced hyperglycaemic rats.

2. Materials and methods

2.1 Plant material

Fresh roots were collected during early summer from young matured plants from the rural belt of Salipur in Cuttack district of Orissa after due authentication of the plant by the taxonomists of Department of Botany, Utkal University, Bhubaneswar. The collected roots were washed under running tap water to remove adhering dirt, dried under shade, pulverised, amd passed through sieve no. 40. The resulting coarse powder was used for further studies.

2.2 Preparation of extract

The powdered roots were extracted with ethanol-water (1:1) by maceration for 7 days. The liquid extract was concentrated under vacuum to yield a dry extract (7.32% w/w with respect to dry material) and preserved in a desiccator till further experiments. Glibenclamide was used as reference standard. The test substances were suspended in 0.5% w/v sodium carboxy methylcellulose in distilled water.

2.3 Animals used

Adult Wister albino rats (130-160g) of either sex were used in the studies. The animals were kept in standard polypropylene cages at room temperature of 27 ± 2°C.

3. Experimental

3.1 Antidiabetic evaluation [4,5,6,7]

3.1.1 Using hyperglycaemic rats

The acclimatized animals were kept fasting for 24 h with water *ad libitum* and injected intraperitoneally a dose of 120mg/kg of alloxan monohydrate in normal saline. After one hour, the animals were provided feed *ad libitum*. The blood glucose level was checked before alloxanisation and 48 h after alloxanisation by withdrawing blood from the tip of the tail of each rat under mild ether anaesthesia. The blood glucose level was measured with haemoglucostrips supplied by M/s Lifescan, Inc. USA with the help of a ONE TOUCH blood glucose monitor.

Animals were considered diabetic when the blood glucose level was raised beyond 200 mg/ 100 ml of blood. This condition was observed at the end of 48 h after alloxanisation. The animals were segregated into four groups of six rats in each. Group-I served as solvent control and received only vehicle (2ml/kg) through oral route. Group-II received glibenclamide (2.5mg/ kg). Group-III and IV received the test extract at doses of 200 and 400 mg/kg in a similar manner. Blood samples were collected from each rat by cutting the tip of the tail under mild ether anaesthesia. Blood glucose level was estimated at 0 h,1 h, 2 h, 4 h and 8 h respectively.

3.1.2 Using normoglycaemic rats

The animals were fasted for 18 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn from the tip of the tail of each rat under mild ether anaesthesia and the blood glucose was estimated as above. The normal rats were then divided into three groups of six animals each. Group I and II received the test extract at a dose of 200 and 400mg/kg respectively through oral route. Group-III received glibenclamide (2.5mg/kg) and served as reference control. All the test samples were administered in a similar manner. Blood glucose levels were monitored after 1, 2, 4 and 8 h of administration of single dose of test samples.

3.2 Statistical analysis

Results were analysed by student's *t* - test. The minimum level of significance was fixed at $p < 0.01$.

Table 1
Effect of root extract of *Premna corymbosa* on the blood glucose concentration in alloxan induced hyperglycaemic rats.

Group	Treatment	Dose	Blood glucose conc. (mg/dl.)				
			0h	1h	2h	4h	8h
I	0.5% w/v Sodium CMC (Vehicle)	2 ml/kg	172.41±10.81	179.46±9.81	182.26±11.26	186.54±11.31	190.54±8.58
II	Glibenclamide	2.5mg/kg	167.23±9.74	121.38±11.23* (27.42%)	86.38±10.48** (48.35%)	63.49±9.72** (62.03%)	58.21±6.27** (65.19%)
III	Extract	200 mg/kg	183.49±14.23	174.43±12.39 (4.94%)	146.54±11.69 (20.14%)	138.26±10.38* (24.65%)	128.42±8.46** (30.01%)
IV	Extract	400 mg/kg	187.62±16.67	166.84±15.26 (11.08%)	128.23±9.64* (31.65%)	101.86±11.72** (45.71%)	98.36±7.39** (47.57%)

Results expressed as Mean ± SEM from six observations. * $p < 0.01$, ** $p < 0.001$ when compared with controls.
Figures in parenthesis denote percentage reduction in the blood glucose concentration.

Table 2
Effect of root extract of *Premna corymbosa* on the blood glucose concentration in normoglycaemic rats.

Group	Treatment	Dose	Blood glucose conc. (mg/dl.)				
			0h	1h	2h	4h	8h
I	0.5% w/v Sodium CMC (Vehicle)	2 ml/kg	69.83±1.95	68.17±2.68	70.0±2.24	68.67±2.19	70.17±2.02
II	Glibenclamide	2.5 mg/kg	65.33±2.05	52.83±2.52*	48.17±1.86**	44.33±1.41**	42.83±1.91**
III	Extract	200 mg/kg	68.0±1.45	67.12±1.76	65.0±1.9	63.21±2.26	60.5±2.39
IV	Extract	400 mg/kg	66.33±1.77	63.16±2.12	61.35±1.98	58.33±2.69	54.0±3.16*

Results expressed as Mean ± SEM from six observations. *$p < 0.01$, **$p < 0.001$ when compared with controls.

4. Results and discussion

The studies on the root extract of the plant *Premna corymbosa* revealed significant reduction in the blood glucose levels in the alloxan induced hyperglycaemic rats. The extract was found to produce marked reduction in blood glucose concentration at tested dose levels in a dose dependant manner (Table 1).

However, in normoglycaemic animals, the extract at 400 mg/kg dose level produced significant reduction of blood glucose at the 8^{th} hour of administration (Table 2) and no effect was observed with 200 mg/kg dose.

Alloxan is widely used to induce experimental diabetes in animals. The mechanism of action in β-cells of the pancreas has been intensively investigated and now is quite well understood. The cytotoxic action of alloxan is mediated by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals [8].

These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells [9].

The comparable effect of the extract with glibenclamide may suggest similar mode of action, since alloxan permanently destroys the pancreatic β-cells and the extract lowered blood sugar level in alloxanized rats to a significant level, indicating that the extract possesses extrapancreatic effects. The exact mode of action and the biologically active constituents responsible for the said effect are not reported earlier. The only observation was that it is used in the folklore diabetic treatments.

5. Conclusion

The present studies on antihyperglycaemic activity of *Premna corymbosa* roots reveal that the extract of the roots showed significant antihyperglycaemic activity against alloxan induced diabetic rats, when compared with the controls. However, in normoglycaemic animals, there was not much marked decrease observed in the blood sugar level at tested dose levels. The results of the present study justify the use of the roots of the plant for treating diabetes as suggested in folklore remedies.

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