

## Antidiabetic activity of *Plumeria rubra L.* in normal and alloxan induced diabetic mice

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**Received:** 11 March 2016

**Accepted:** 15 April 2016

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### ABSTRACT

**Background:** Diabetes mellitus is a major source of morbidity in developed countries. In spite of the introduction of hypoglycemic agents, diabetes and related complications continue to be a major medical problem. Our present study aims to investigate the antidiabetic activity of aqueous extract of plumeria rubra (PR) in experimental animals.

**Methods:** PR extract was subjected to antidiabetic study in alloxan induced diabetic model at three-dose levels 100,200 and 400 mg/kg respectively. It was also tested for hypoglycemic activity at same dose levels. Diabetes was induced by alloxan monohydrate (150 mg/kg, i.p.). PR extracts and standard drug glibenclamide (10 mg/kg, p.o.) was administered to animals for 28 days. The blood glucose, serum cholesterol, serum triglyceride, high density lipoprotein, low density lipoprotein, glycosylated hemoglobin. Body weights were assessed in the experimental animals. Histopathological observations during 28 days treatment were also evaluated.

**Results:** PR extract induced significant reduction ( $P < 0.001$ ) in fasting blood glucose levels in normal and alloxan-induced diabetic mice. Significant differences were observed in serum lipid profiles, glycosylated haemoglobin by extract treated-diabetic animals, when compared with the diabetic control and normal animals. The protective effect of PR was also evident from the histopathological examination on pancreas, liver and kidney. It reduced the injuries induced by alloxan.

**Conclusions:** PR exhibited significant antihyperglycemic activity in normal and alloxan-induced diabetic mice. The results of the present study provide support to the traditional usage of the plant in diabetes.

**Keywords:** Alloxan, Antidiabetic activity, Plumeria rubra

### INTRODUCTION

Diabetes mellitus is a group of syndromes characterized by hyperglycaemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular diseases.<sup>1</sup> It is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin.<sup>2</sup> Such a deficiency results in

increased concentrations of glucose in the blood, which in turn damage many of the body systems, in particular the blood vessels and nerves.<sup>3</sup> It is currently growing at a rapid rate throughout the world, and it is the 16th leading cause of global mortality.<sup>4</sup>

Different types of oral hypoglycaemic agents are available along with insulin for the treatment of diabetes; there is an increased demand by patients to use natural

products with antidiabetic activity. Since time immemorial, patients with non-insulin dependent diabetes have been treated orally in folk medicine, with a variety of plant extracts. In India, a number of plants are mentioned in ancient literature for the treatment of diabetic conditions.<sup>5</sup>

In the present study we have investigated the antidiabetic activity of aqueous extract of flowers of plumeria rubra (PR) in laboratory animals. The decoction of the flowers has been traditionally used by the Mexican people for treating diabetes mellitus.<sup>6,7</sup> Phytochemical investigation of PR reveals the presence of flavonoids, tannins.<sup>8</sup> These principles are known to be bioactive for the management of diabetes.<sup>9,10</sup> Flavonoids exhibit hypoglycaemic activity and are also known for their ability of beta cell regeneration of pancreas.<sup>11-13</sup>

## METHODS

### *Collection and authentication of plant material*

PR flowers were collected fresh from the garden of Pune University in the month of October. Authentication of plant material was done at the Botanical Survey of India, Pune. The vouchure Specimen No. given was AMRPLR1.

### *Extraction and preliminary phytochemical evaluation*

The flowers were separated, dried and powdered in grinder. The 60 g powder was extracted with 1000 ml of boiling water for 30 min. The resulting extract was dried under room temperature. The percentage yield obtained was 16.6%. Resulted extract was collected and stored at room temperature. The extract was screened for the presence or absence of secondary metabolites such as alkaloids, flavonoids, tannins, glycosides using standard procedures.<sup>14</sup>

### *Animals*

Albino mice of either sex weighing 18-22 gm were used for the study. The animals were procured from the animal house of Seth Govind Raghunath Sable College of Pharmacy, Saswad, Maharashtra, India. Protocol of the experiments and animal usage were discussed in the institutional ethical committee meeting and permission was obtained to carry out the parameters selected for the study. Animals were housed individually in polypropylene cages, maintained under standard conditions (12-hours light and 12-hours dark cycle; 25±5°C; 35-60% humidity), the animals were fed with standard pellet diet and provided water ad libitum.

### *Acute toxicity study*

Acute oral toxicity was carried out according to OECD guidelines 425. Albino mice (female) weighing between

25 to 40 g was used. Above study was done at dose 2000 mg/kg and 5000 mg/kg body weight.<sup>15</sup>

### *Assessment of PR extract in normal fasted mice*

For the normoglycemic study, mice were divided into five groups (n=6) and were administered distilled water, glibenclamide (10 mg/kg), PR extract 100, 200, 400 mg/kg respectively. The blood glucose levels were measured at 0, 1, 2, 4, 8, 12 hours after drug administration.

### *Assessment of PR extract in alloxan induced diabetic mice*

Diabetes was induced by a single i.p. injection of freshly prepared 150 mg/kg of alloxan monohydrate in sterile saline. After 48 h of alloxan injection, the diabetic mice (glucose level >250 mg/dl) were used for the study. The animals are divided into following groups, each containing 6 mice.<sup>16-18</sup>

### *Experimental design*

Group I- received distilled water (D.W.)  
Group II- received glibenclamide (10 mg/kg)  
Group III- received PR extract (100 mg/kg p.o.)  
Group IV- received PR extract (200 mg/kg p.o.)  
Group V- received PR extract (400 mg/kg p.o.)

Blood samples were withdrawn from all animals on 0, 3<sup>rd</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, 28<sup>th</sup> day in animals by tail vein puncture method and BG was estimated by one touch horizon glucometer strips. Change in blood glucose, mean and SEM were calculated and tabulated.

### *Histopathological study*

Pancreas, liver, kidney were removed, stored in 10% formalin and then processed with varying grades of alcohol followed by xylene and paraffin. Tissues were embedded in paraffin and cut at 5 micron using microtome and stained after deparrination using hematoxylin and eosin stains to verify morphological assessment. Microscopic analysis was done.

### *Statistical analysis*

The results were expressed as mean±SEM. Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by "Dunnett's test". P<0.05 was considered to be significant.

## RESULTS

### *Effect of PR extract on acute toxicity study*

Acute toxicity studies revealed the nontoxic nature of the aqueous extract of PR. There were no lethality or toxic reactions found till dose of 5000mg/kg until the end of the

study period. All the animals were alive, healthy, and active during the observation period.

### Preliminary phytochemical evaluation

The preliminary phytochemical screening of the aqueous extract showed the presence of alkaloids, flavonoids, tannins.

### Effect of PR extract on blood glucose level in normal and diabetic mice, body weight and lipid profile of diabetic mice

Table 1 shows the mean±SEM blood glucose values at the intervals of 0, 1, 2, 4, 8, 12 hours respectively. In normal animals, significant (P<0.001) reduction in the blood glucose level was observed by the aqueous extract as compared to the control.

**Table 1: Effect of PR extract on blood glucose level in normal mice.**

Groups	Dose mg/kg	0 hour (mg/dl)	1 hour (mg/dl)	2 hours (mg/dl)	4 hours (mg/dl)	8 hours (mg/dl)	12 hours (mg/dl)
Normal control	vehicle	90.83±1.515	91.33±1.726	103.8±1.014	96.00±1.23	95.50±1.28	97.67±1.52
Glibenclamide	10	90.67±1.38	77.53±0.87***	71.50±1.17***	76.50±1.43***	77.67±1.22***	76.8±1.44***
PR	100	90.17±1.60	86.00±0.73**	85.87±2.00***	81.67±1.52***	82.67±1.22***	85.00±0.96***
PR	200	91.91±2.07	83.67±0.61***	80.83±2.34***	75.83±1.44***	73.17±1.13***	71.83±1.01***
PR	400	93.4±1.63	85.67±0.55**	84.00±1.39***	80.67±1.52***	82.50±0.99***	80.67±1.96***

Values are expressed as mean±S.E.M (n=6). \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 when compared with control (ANOVA followed by Dunnet's test).

**Table 2: Effect of PR extract on blood glucose level in diabetic mice.**

Groups	Dose (mg/kg)	Day 0 (mg/dl)	Day 3 (mg/dl)	Day 7(mg/dl)	Day 14 (mg/dl)	Day 21 (mg/dl)	Day 28 (mg/dl)
Diabetic control	vehicle	94.17±2.56	320.5±3.83	325.5±3.67	326.3±3.92	318.0±3.05	317.7±2.89
Glibenclamide	10	95.67±2.66	315.7±22.24	280.±2.01***	181.0±4.48***	142.0±4.84***	100.7±3.61***
PR	100	96.33±2.57	316.8±2.38	291.3±2.12***	230.7±2.40***	166.3±2.60***	129.7±3.77***
PR	200	95.67±2.84	317.0±2.17	288.0±2.09***	200.5±2.48***	156.0±6.87***	118.2±3.39***
PR	400	94.83±2.89	317.3±2.18	290.7±1.97***	221.2±2.35***	161.7±4.60***	128.0±2.53***

Values are expressed as mean±S.E.M (n = 6). \*P< 0.05 \*\*P<0.01 \*\*\*P<0.001 when compared with diabetic group (ANOVA followed by Dunnet's test).

Table 2 shows the mean±SEM blood glucose values at the intervals of 0, 3, 7, 14, 21, 28 day respectively. Glibenclamide and PR extract treated group (100,200,400 mg/kg), showed significant reduction (P<0.001) from 7<sup>th</sup>

to 28<sup>th</sup> day. In DC group, the elevated blood glucose levels were maintained throughout the 30 days of treatment period indicating that the mice were rendered diabetic.

**Table 3: Effect of PR extract on body weight in alloxan induced diabetic mice.**

Groups	Dose(mg/kg)	Day 0 (mg)	Day 7 (mg)	Day 14 (mg)	Day 21 (mg)	Day 28 (mg)
NC	Vehicle	29.83±1.57	30.00±1.50	30.67±1.35	31.00±1.21	32.00±1.12***
DC	Vehicle	28.17±1.30	28.00±1.82	27.00±1.82	25.50±2.32	23.83±1.27
Glibenclamide	10	28.83±1.44	28.17±1.30	29.50±1.28	29.33±1.80	30.50±1.43**
PR	100	28.67±1.22	27.67±1.22	27.17±1.74	27.83±1.44	27.67±1.08
PR	200	29.00±1.39	27.50±1.47	28.00±1.65	27.83±1.44	28.50±0.99
PR	400	28.83±1.30	27.67±1.35	27.50±1.43	27.83±2.02	27.50±1.60

Values are expressed as mean±S.E.M (n = 6). \*P< 0.05 \*\*P<0.01 \*\*\*P<0.001 when compared with diabetic group (ANOVA followed by Dunnet's test).

Table 3 shows the changes in body weight of diabetic mice. In the control treated group, significant increase (P<0.001) was seen on day 28, while Glibenclamide (10

mg/kg), treated group showed increase (P<0.01) on day 28. No significant changes were observed in the test group.

**Table 4: Effect of PR extract on lipid profile.**

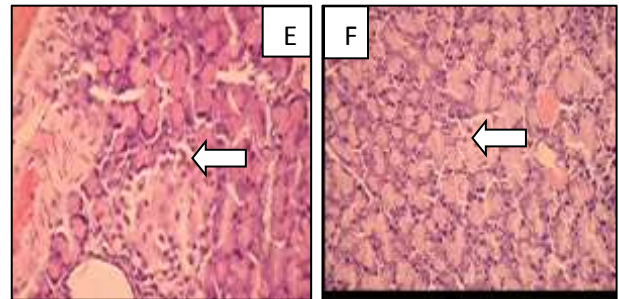
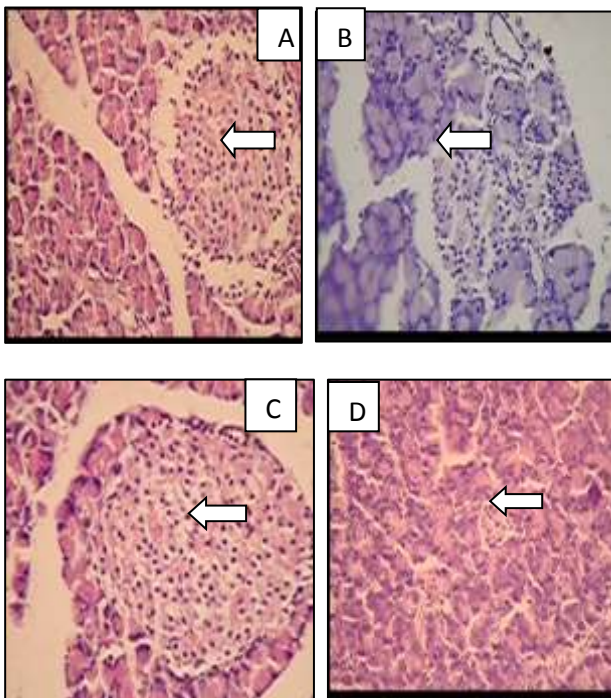
Groups	Dose mg/kg	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
NC	vehicle	34.66±1.58**	50.83±2.16**	23.33±0.66***	20.57±2.84**	6.93±0.31***
DC	vehicle	45.00±2.32	61.83 ±1.01	15.30±0.60	33.83±1.40	9 ±0.46
Glibenclamide	10	31.83±1.40***	50.66±1.82**	22.33±0.61***	21.97±1.73**	6.36±0.28***
PR	100	34.5±1.38**	53.33±2.36*	19.83±0.47***	26.60±2.70	6.90±0.27***
PR	200	33.17±1.16***	52.5±2.34*	21.16±0.60***	24.70±2.52*	6.63±0.23*
PR	400	34.16±1.27**	52.33±2.18*	20.16±0.70***	25.33±1.79*	6.83±0.25***

Values are expressed as mean±S.E.M (n = 6). \*P< 0.05 \*\*P<0.01 \*\*\*P<0.001 when compared with diabetic group (ANOVA followed by Dunnet's test)

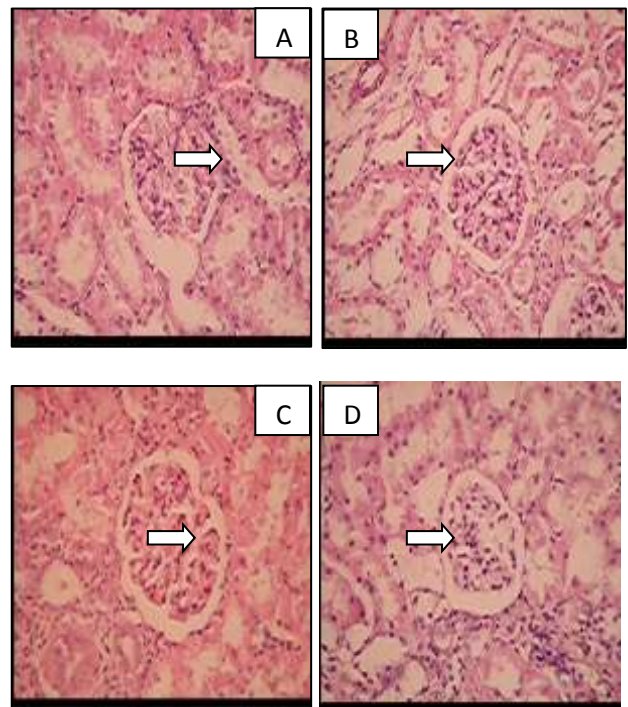
Table 4 shows the changes in lipid profile of diabetic mice. In glibenclamide (10 mg/kg) treated groups, significant decrease (P<0.001) was seen in TG, HDL and VLDL. TC, LDL also showed significant decrease (P<0.01) as compared to diabetic control.

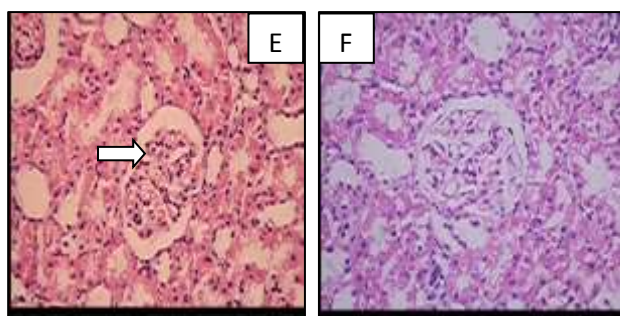
PR (100mg/kg) showed reduction in TG (P<0.01), TC (P< 0.05), HDL (P<0.001) and VLDL (P<0.001) levels as compared to DC. PR (200 mg/kg) showed reduction in TG (P<0.001), TC (P<0.05), HDL (P<0.001), LDL (P<0.05), VLDL (P<0.001). PR (400 mg/kg) showed reduction in TG (P<0.01), TC (P<0.05), HDL (P<0.001), LDL (P<0.05), VLDL (P<0.001).

**Histopathology of pancreas**

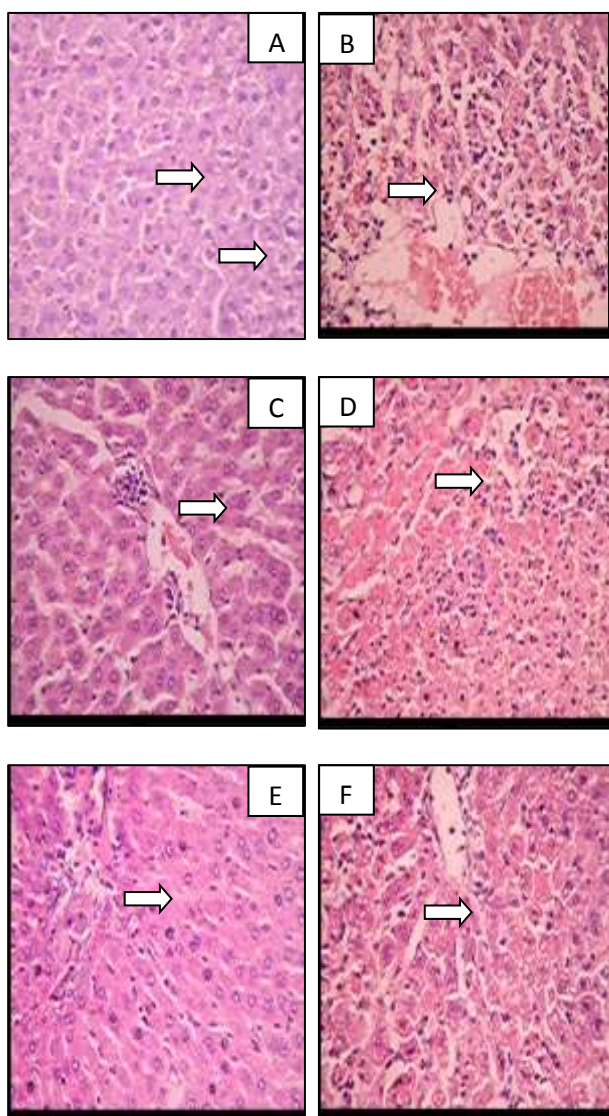


**Figure 1: Effect of PR extract on histopathological studies; (A) NC; (B) DC; (C) glibenclamide (10 mg/kg); (D) PR (100 mg/kg); (E) PR (200 mg/kg); (F) PR (400 mg/kg).**





**Figure 2: Effect of PR extract on histopathology of kidney; (A) NC; (B) DC; (C) glibenclamide (10 mg/kg); (D) PR (100 mg/kg); (E) PR (200 mg/kg); (F) PR (400 mg/kg).**



**Figure 3: Effect of PR extract on histopathology of kidney; (A) NC; (B) DC; (C) glibenclamide (10 mg/kg); (D) PR (100 mg/kg); (E) PR (200 mg/kg); (F) PR (400 mg/kg).**

## DISCUSSION

In light of the results, our study indicates that aqueous extract of PR exhibited significant anti-hyperglycaemic activity in normal and alloxan-induced diabetic mice. Effective blood glucose control is the key for preventing or reversing diabetic complications and improving the quality of life in patients with diabetes. Thus, sustained reduction in hyperglycaemia will decrease the risk of developing microvascular complications and most likely reduce the risk of macro vascular complications. Alloxan induces diabetes by destroying B cells of pancreas, through production of reactive oxygen species. Our investigations indicate the efficiency of the plant extract in the maintenance of blood glucose levels in normal and alloxan induced diabetic mice.

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease. This abnormally high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots, mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state, lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglyceridemia and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities.<sup>19</sup> Regular administration of the aqueous extract of PR nearly normalized lipid profile in diabetic animals. It not only lowered TC, TG and LDL but also enhanced the cardio protective lipid HDL. Decrease in the body weight was arrested by PR which was precise in alloxan induced diabetic mice.

The protective effect of PR is also evident from the histopathological examination on pancreas, liver and kidney. It evidently reduced the injuries of pancreas, kidney, liver induced by alloxan. From this study we can conclusively state that PR aqueous extract has shown remarkable effects on blood glucose level and marked improvement on hyperlipidemia due to diabetes. Its specific effect on HDL has additional advantage in checking coronary risks. The extract seems to have no toxicity as no death is reported up to 10 times of effective dose. This anti-hyperglycaemic effect of PR extract suggests that its main mechanism may be mediated through the interaction between insulin and gluconeogenesis, which further affects glucose metabolism in the liver and other tissues in the body, and introduces abnormal or disordered glucose metabolism to normal or ordered. Further pharmacological and biochemical investigations are underway to elucidate the detailed mechanism of the antidiabetic and hypolipidemic effect of the plant.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

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**Cite this article as:** Yadav AV, Undale VR, Bhosale AV. Antidiabetic activity of *Plumeria rubra* L. in normal and alloxan induced diabetic mice. Int J Basic Clin Pharmacol 2016;5:884-9.