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Antioxidant and Hypolipidemic Effect of *Plumeria Rubra* L. in Alloxan Induced Hyperglycemic Rats

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Abstract: Antioxidant and hypolipidemic activity of the flovone glycoside isolated from *Plumeria rubra* L. was carried in alloxan induced hyperglycemic rats. The flavonoid treatment produced a significant reduction in the level of serum triglycerides, while there was no reduction in the serum cholesterol and glucose. Antioxidant activity of the drug was also confirmed through *in vitro* studies.

Keywords: *Plumeria rubra* L., Flavone glycoside, Hypolipidemic effect, Antioxidant activity.

Introduction

Hyperlipidemia, one of the common complications of diabetes mellitus is found in about 40% of diabetic[1-4]. The elevation in lipoproteins was shown to accelerate athrogenesis in diabetes mellitus[5,6] and contribute to increased susceptibility to vascular complications[7]. Free radical and lipid peroxides are generated under various pathological conditions including hyperglycemia. Alloxan produces oxygen radicals in the body, which causes pancreatic injury and should be responsible for the increase in the blood sugar level. However it is found that the action of alloxan is not only specific to pancreas, but other organs such as the kidney and liver are also affected[8,9] which shows a negative nitrogen balance results in increased urea nitrogen production along with the createnine in the serum of the diabetic groups.

Natural products like flavonoids and β-carotenoids are reported for their free radical scavenging action[10,11] and antioxidant property by donating hydrogen or reacting with superoxide anions thus eliciting free radical scavenging activity. *Plumeria rubra L.* belongs to the family apocynaceae is come to know that it contains a flavonoidal glycoside. Hence the present study was designed to determine the *in vivo* hypolipidemic effect on alloxan induced hyperglycemic rats and *in vitro* antioxidant property of the flavone glycoside of *Plumeria rubra L.*

Experimental

Alloxan monohydrate and thiobarbituric acid were purchased form Sigma Chemicals Company. All other chemicals and reagents used were of analytical grade with high purity.

Plant material and drug preparation

Fresh flowers of *P.rubra* were collected from Thanjavur District during the month of October 2005, and were extracted with 80% methanol. The methanolic extract was concentrated *in vacou*. The aqueous concentrate was successively fractionated with petroleum ether at 60-80 ^{o}C, peroxide free ether and ethyl acetate. The ethyl acetate fraction was concentrated *in vacou* and left in ice chest for a week. A yellow solid separated was filtered and identity of the compound was confirmed by colouring reactions, chromatographic methods and was found to have flavone glycoside, which was used as drug in the present study.

Animals

Male albino rats (wistar) weighing about 150-200 g, used in the present study were maintained under normal temperature (25 ^{o}C) and relative humidity (50-60%). Care was taken in maintaining the animals as per the rules and regulations given by the animal ethical committee. The animals were fed with normal pelleted diet manufactured by Amruth India Ltd., and had free access to water. After an acclitimization period of one week they were used for the study.

Experimental design

The animals were divided into four groups of each containing six.
Group 1: Normal animals- Received normal feed and water throughout the study.
Group 2: Hyperglycemic control animals
Group 3: Drug treated hyperglycemic animals.
Group 4: Standard drug- glibenclamide treated hyperglycemic animals.

All the animals were made to fast for 18 hours and the animals in the groups 2,3&4 were injected with a single dose of alloxan monohydrate (150 mg/kg body weight) in physiological saline[12] and 5% glucose solution was kept for 24 hours to prevent fatal hypoglycemia. After 24 hours of the alloxan injection the animals in group 3 were treated with the flavonoidal drug with a daily dose of 250 mg/kg body weight orally. The animals in group 4 were treated with the standard drug glibenclamide with a daily oral dose of 10 mg/kg body weight. The treatment was continued for a period of 6 days continuously. After the last dose of drug treatment, the animals were made to fast overnight and were sacrificed by decapitation under mild ether anesthesia. The blood samples were collected for serum separation (without any anticoagulant) and a set of samples in tubes rinsed with anticoagulant.

The whole blood samples collected with the anticoagulant rinsed tubes were precipitated with 10% trichloroacetic acid. After centrifugation the supernatant was used for the estimation of glucose [13] urea[14] and createnine[15]. The serum samples after separation were used for the estimation of total cholesterol[16], triglycerides[17], the activities of aspartate transaminase and alanine transaminase[18].

In vitro antioxidant effect of the flavonoidal extract was tested by following the method established by Kunal Roy *et al.,*[19]. Results were expressed as mean ± SD and student's *t*-test was used to assess the statistical significance.

Results and Discussion

Table 1 shows the levels of blood glucose, serum total cholesterol and triglycerides of the normal and experimental animals. Significant elevations in the levels of blood glucose (P<0.1) serum total cholesterol (P<0.1) and triglycerides (P<0.1) were observed in the alloxan injected hyperglycemic animals. On treatment with the drug to the hyperglycemic animals, there was no significant alteration in the blood glucose and serum total cholesterol while a significant reduction in the level of serum triglycerides (P<0.1) was observed when compared with the alloxan injected hyperglycemic control animals.

Table 1. Levels of glucose, total cholesterol and triglycerides in control and experimental rats.

Groups	Glucose, mg/dL	Total Cholesterol, mg/dL	Triglycerides, mg/dL
Group I	92.03±8.02	64.18±4.38	19.93±1.12
Group II	285.92±19.32*	88.85± 7.49 *	37.35±2.08 *
Group III	265.0± 21.82NS	80.74±6.68NS	23.56±0.99*
Group IV	208.34±18.68*	53.73±3.82*	20.50±1.08*

*Values are expressed as Mean ± SD (n=6). Group I: normal control; Group II: hyperglycemic control; Group III: Drug treated hyperglycemic animals; Group IV: Standard drug Glibenclamide treated hyperglycemic animals. Comparisons were made: (a) Group I vs. Groups II, III and IV; (b) Group II vs. Group III. Statistical significant: *p<0.1, NS- Not Significant.*

The liver and kidney cell damage caused by alloxan injection was clearly observed through the significant elevations in the levels of blood urea (P<0.1), createnine (P<0.1) and the activities of SGOT (P<0.1) and SGPT (P<0.05) in the alloxan injected hyperglycemic animals when compared with the normal animals (Table 2). Administration of the drug to the hyperglycemic animals showed a significant reduction in the levels of blood urea (P<0.1), createnine (P<0.1) and the activities of SGOT (P<0.1) and SGPT (P<0.01) when compared with the hyperglycemic control animals.

Table 2. Levels of Urea, Createnine, SGOT and SGPT in control and experimental rats.

Groups	Urea, mg/dL	Createnine, mg/dL	SGOT, IU/L	SGPT, IU/L
Group I	19.93±1.51	0.93±0.052	25.39±1.98	41.47±3.96
Group II	37.33±2.61*	1.3±0.073*	33.34±2.23*	46.49± 2.98**
Group III	23.26±2.21*	0.79±0.023*	25.1±2.16*	40.4±2.26*
Group IV	20.48±1.91*	0.93±0.051*	25.1±1.78*	42.0±3.78***

*Values are expressed as Mean ± SD (n=6). Group I: Normal control; Group II: Hyperglycemic control; Group III : Drug treated hyperglycemic animals ; Group IV : Standard drug Glibenclamide treated hyperglycemic animals. Comparisons were made: (a) Group I vs. Groups II, III and IV; (b) Group II vs. Group III. Statistical significant: *p<0.1, **p <0.05, ***p < 0.01.*

Figure 1 shows *in vitro* antioxidant effect of the drug against CFZ induced lipid peroxidation in goat RBC. Inhibition in the formation of MDA was observed in both the concentrations of drug used (37.8% at 50µg and 26.77% 100 µg) in the present study. From the results a dose dependent inhibition in the rate of formation of MDA was observed.

In diabetic rats significant elevation of both the total cholesterol and triglycerides was observed. Treatment with the flavone glycoside of *P. rubra* produced a significant reduction in the serum triglycerides, where there was no change in the total cholesterol. The hyperlipidemia in diabetic rats was shown to be, due to both enhanced synthesis and slow clearance of the lipoproteins from the circulation[20]. The significant reduction in the serum

triglyceride on drug treatment definitely has prognostic significance as triglycerides are atherogenic in diabetes[21]. In the present study there was also no change in blood glucose level following the administration of the drug. The beneficial effect of the flavone glycoside treatment on triglycerides observed, assumes greater significance as a useful drug to decrease hyperlipidemic risks in diabetes. This finding is in consistent with the effect of felodipime (dihydro pyrimidine compound) a calcium channel modulator[22].

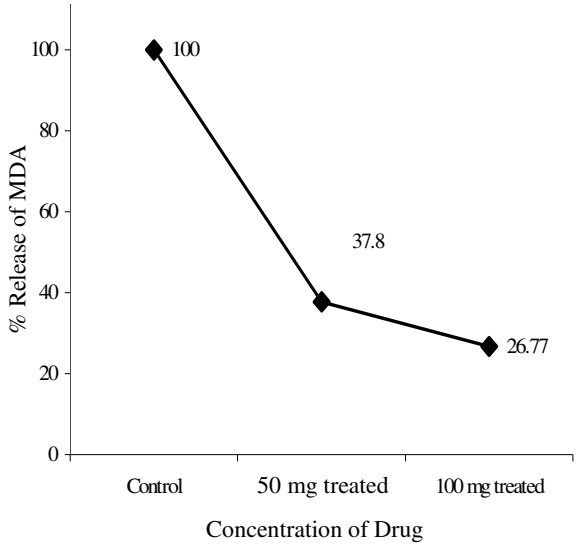

Figure 1. *In vitro* antioxidant effect of the drug.

The levels of urea, createnine and the activities of asparate and alanine transminases in the serum were also found to be elevated in diabetic condition indicating alloxan induced cell injuries in the liver and kidney as well as the negative nitrogen balance may be accounted for an enhanced catabolism of both liver and plasma proteins[23,24]. The rise in alanine transaminase activity is almost always due to hepatocellular damage and is usually accompanied by a rise in asparate transaminase[25]. The flavone glycoside treatment significantly lowered the levels of the urea, createnine and the activities of alanine transaminase and asparate transaminase. Administration of the drug has prevented the alloxan induced cellular damage in rats.

As the complication of diabetes involves oxidative stress, the antioxidant role of the flavone glycoside was determined *in vitro.* Strong antioxidant activity was found by inhibiting the release of the malondialdehyde product to 62.20% on 50 g and 73.23% on 100 g concentration of drug respectively.

The results of the present study confirm the antioxidant and hypolipidemic effort of flavone glycoside of *P.rubra* rather being an antidiabetic in alloxan induced rats.

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