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Antidiabetic Activity of Extracts of *Pithecellobium dulce* Benth Leaves in Alloxan Induced Diabetic Rats

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ABSTRACT

The objective of the present study was to study effect of *Pithecellobium dulce* Benth (*P. dulce*) leaves in alloxan induced diabetic rats. The *P. dulce* leaves were extracted by maceration and soxhelation method by using water and ethanol as solvent. Acute toxicity study was performed according to OECD 425 guidelines for both aqueous and ethanolic extracts of *P. dulce* leaves. The dose of 200 mg/kg and 400 mg/kg was selected for further studies. Animals were rendered diabetic by administration of alloxan (130 mg/kg, *i.p.*). The albino rats were divided in to seven groups with five animals in each group. Diabetic animals were treated with aqueous and ethanolic extract for 20 days. Then blood glucose, triglyceride, total cholesterol, urea, uric acid, creatinine, aspartate aminotransferase (AST), alanine transaminase (ALT) and glycogen level in liver, muscle and kidney were estimated according to standard procedures. The result shows significant decrease in blood glucose, triglyceride, total cholesterol, urea, uric acid, creatinine, AST and ALT level when compared to diabetic group. The liver and muscle glycogen level was increased significantly in extract treated groups when compared to diabetic control group. Both extract of *P. dulce* posses antidiabetic and hypolipidemic potential.

Keywords: *Pithecellobium dulce* Benth, antidiabetic, alloxan, lipid profile, glycogen, OGTT.

INTRODUCTION

Diabetes mellitus (DM) is commonest form of diabetes caused by a deficiency of the hormone insulin or increase in insulin resistance, which results in hyperglycemia. [1] Diabetes is the most serious and common metabolic disease all over the world and it is caused mainly due to pancreatic β cell dysfunction and insulin resistance. [2] DM is the condition which depends on the level of hyperglycemia causing microvascular and macrovascular complications. The microvascular damage increases the risk of retinopathy, nephropathy and neuropathy. Macrovascular complications like ischemic heart disease, stroke and peripheral vascular disease can results in reduced life expectancy and significant morbidity. [3] According to World Health Organization (WHO) 1.5 million peoples were died because of diabetes in year 2012. Globally, in 2014 total 422 million adult were living with diabetes; the number was 108 million in 1980. [4] In 1980 the WHO also suggested to study and find out the plants which are having potential hypoglycemic activity as the modern drugs has the less safety. [5] DM is difficult to control with currently available drugs which are associated with many side effects. [6] Thus today there is need to search for the novel drug for treatment of DM.

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Historically, many natural plant products were proved to be effective for treatment of DM. The natural plants products have received considerable attention now days as these products are the templates for the development of new molecule for DM.

P. dulce is medium sized plant which grows up to 18 m height. It is commonly known as Indian jalebi, mania tamarind or Indian sweet jalebi. [7] The common constituents present in leaves of the plant are cyclitol, dulcitol, octacosanol, α- spinasterol, kaempferol-3-rhamnoside, quercetin and afzelin. [8] Traditionally the leaves of the plant are used for leprosy, intestinal disorders, peptic ulcer, tooth ache, ear ache, emollient, abortifacient and larvicidic activity. [9] The leaves also reported to show antifungal and antibacterial activity. Estrogenic activity was observed by isolated isoflavonoids from root of the plant. Literature survey also demonstrates the neuropharmacological profile of the plant in mice. [10-11] The leaves of the plant reported to have free radical-scavenging properties and antimycobacterial activity. The leaves of the plant have reported to contain the insulin like content which may be useful for treatment of diabetes. [9] Taking into consideration the traditional claims, presence of insulin like principle in P. *dulce* plant and free radical scavenging activity it may be useful in diabetes so present study was planned to investigate the effect of *P. dulce* leaves on alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant Material Collection

The plant material was collected from Jaisingpur, Sangli District, Maharashtra, India. The voucher specimen of the plant was identified by acknowledged botanist, Dr. (Mrs.) U. S. Yadav at Willingdon College, Sangli, Maharashtra, India (Voucher specimen No.: WILL/Bot/2009/03). The voucher specimen was deposited at same college.

The standard drug pioglitazone was obtained from Aarti Drugs Ltd, Mumbai. The inducer alloxan was obtained from Research lab Fine Chem Industries, Mumbai. All the diagnostic kits used are of Span Diagnostics, Surat.

Preparation of extracts

The fresh leaves were subjected for observation for removal of damaged leaves and any other mixed materials. Then the separated leaves were dried in fresh circulating air under shade for seven days. The dried leaves were subjected to size reduction by dry grinder. [12] The leave powder after passing through the sieve subjected to aqueous and ethanolic extraction. In ethanolic extraction leaves powder was subjected for soxhlation extraction using ethyl alcohol as solvent. [13-14] The extract was dried at 40°C in oven to give dried extract. In aqueous extraction leaves powder was subjected for maceration using chloroform water as solvent. In maceration process leaves powder was subjected to occasional shaking on orbital shaker and then stands for the next 18 hours. Then the extract was filtered by whatman filter paper and concentrated by heating on water bath. Concentrated aqueous extract was air dried and used for further study. [13-14] The aqueous extracts were administered orally through orogastric tube. The suspension of ethanolic extracts was prepared by using 0.5% CMC in distilled water. [15]

Animals

Wistar albino rats of either sex (150-300 g) were obtained from animal house of Appasaheb Birnale college of Pharmacy, Sangli, Maharashtra, India. The rats were kept at appropriate temperature of (26 ± 1°C) and light controlled (12 h light: 12 h dark) room with provision of food (Amrut Feed, Sangli). The study protocol was approved by the Institutional Animal Ethics Committee of Appasaheb Birnale College of Pharmacy, Sangli (Approval No: ABCP/IAEC/2009/08).

Acute toxicity study

Acute toxicity study was performed according to the Organization of Economic Cooperation and Development (OECD) guidelines 425. [16] Fifteen healthy male albino rats which were fasted 12 h prior to experiment were divided into three groups with five animals in each group. The first group serves as control group where as second and third group serves as aqueous and ethanolic treatment groups. Extract of *P. dulce* at dose of 2000 mg/kg were administered to animals. The animals were observed for seven days as animals had not shown any sign of toxicity, behavioural changes and mortality the dose increased up to 5000 mg/kg. Then animals were observed up to 7 days for toxicity, behavioural changes and mortality.

Induction of Experimental Diabetes

Diabetes was induced using alloxan from Research lab Fine Chem Industries, Mumbai. The animals were fasted overnight and diabetes was induced by a single intra peritoneal injection of a freshly prepared solution of Alloxan (130 mg/kg b.w.) in a saline solution. [17] 10% dextrose was there after administered orally to combat the immediate hypoglycemia that could occur. [18] Then on 4th day fasting blood sugar level was checked in each animal by using glucose kit and animals with fasting blood sugar above 200 mg/dl were included in study.

Experimental Design

The experiment was designed for 21 days to evaluate antidiabetic activity of *P. dulce* leaves in alloxan induced diabetes in albino rats. [19] The animals were randomly divided into seven groups as follow:

Group- I: Normal control 2 ml/kg normal saline (vehicle)

Group- II: Alloxan 130 mg/kg + vehicle

Group- III: Alloxan 130 mg/kg + Pioglitazone 20 mg/kg

Group- IV: Alloxan 130 mg/kg + Aqueous extract 200 mg/kg

Group- V: Alloxan 130 mg/kg + Aqueous extract 400 mg/kg

Group- VI: Alloxan 130 mg/kg + Ethanolic extract 200 mg/kg
Group- VII: Alloxan 130 mg/kg + Ethanolic extract 400 mg/kg

Oral Glucose Tolerance Test [20]

The acclimatized animals were fasted for 24 hours with water *ad libitum*, fasted animals were divided into seven groups of five rats. First Groups served as control which received distilled water. Second group serves as diabetic control. Third Group received Pioglitazone at an oral dose 20 mg/kg and groups 4-7 received aqueous and ethanolic extract at the dose of (200 and 400 mg/kg) respectively, after withdrawing the initial (0 hours) blood samples and after 30 min of extracts administration, the rats of all groups were orally treated with 2 g/kg glucose. Blood glucose level was checked at the interval of 30, 60, 90, 120 min, after glucose loading, from tail vein by glucose strips.

Biochemical Parameters

At the end of the treatment schedule, i.e. on day 21, the overnight fasted animals were anaesthetized with diethyl ether and blood was collected by retro orbital puncture method. [21] The serum was separated from serum sample by centrifugation at 5000 rpm and stored at refrigeration temperature until use. This Serum sample was used to estimate glucose, triglyceride, total cholesterol, high density lipoprotein (HDL), creatinine, urea, uric acid, AST and ALT level. Further the animals were sacrificed by cervical dislocation and glycogen level was estimated in collected samples of liver, skeletal muscle and kidney by anthrone reagent. [33]

Statistical analysis

Results are expressed as mean±SEM. The statistical analysis of data was made by analysis of variance (ANOVA) followed by Dunnett's test. A value of $P < 0.05$ was considered significant.

RESULTS

Acute toxicity

Under the presence of the experimental conditions the absence of toxic symptoms and mortality in animals indicates that the extract might be having the LD_{50} value above 2000 mg/kg *p.o.* body weight. Thus the extracts were considered to be safe for further pharmacological study and dose of 200 mg/kg *p.o.* and 400 mg/kg *p.o.* of each extract (aqueous and ethanolic) was selected for the further study.

Effect of extract in Oral Glucose Tolerance Test

Fig. 1.1 shows the blood glucose levels in different groups after oral administration of glucose at the dose of 2 g/kg b.w. Aqueous and ethanolic extract of P. *dulce* showed significant decrease in glucose level from 30 min after oral glucose administration when compared with diabetic control group.

Effect of extract on mean blood glucose level in alloxan induced diabetic rats

Table 1 shows the effect on fasting blood glucose in different group during 21 days study in different experimental groups. Induction of diabetes in the experimental rats was confirmed by the presences of a high fasting glucose level when compared with normal group. Treatment of diabetic rats with AE and EE for 21 days significantly ($p < 0.05$) reduced their blood glucose levels when compared with those of diabetic control group.

Effect of extract on different biochemical parameters in alloxan induced diabetic rats

The effect of AE and EE on triglyceride, total cholesterol, HDL, creatinine, urea, uric acid, AST and ALT in different experimental groups are presented in Table 2. Alloxan induced diabetic rats showed significant increase in triglyceride as compared to control group. The increased triglyceride level in diabetic rats was significantly ($p < 0.05$) prevented by the treatment with AE and EE. The alloxan induced diabetic rats showed significant increase in total cholesterol level as compared to normal control group. The administration of AE and EE to diabetic rats significantly restored the total cholesterol level. The diabetic control group showed significant ($p < 0.05$) decrease in HDL level when compared with normal control group. Treatment with AE and EE at different doses significantly increased HDL levels relative to diabetic control group. Diabetic rats treated with AE and EE at the graded dose showed statistically significant ($p < 0.05$) reduction in serum urea, creatinine, uric acid, AST and ALT levels when compared to diabetic control group.

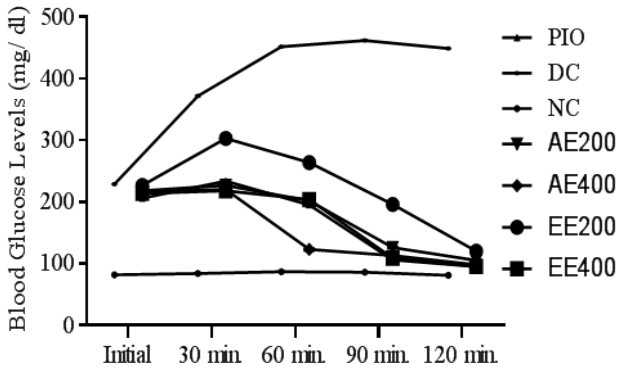

Fig. 1.1: Effect of aqueous and ethanolic extract of *P. dulce* leaves on glucose tolerance test in alloxan induced diabetic rats

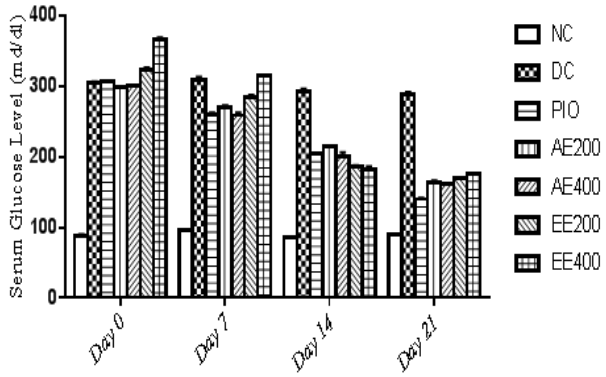

Fig. 1.2: Effect of aqueous and ethanolic extract of *P. dulce* leaves on mean fasting blood glucose level in alloxan induced diabetic rats.

Table 1: Effect of aqueous and ethanolic extract of *P. dulce* leaves on mean fasting blood glucose level in alloxan induced diabetic rats.

	Mean Fasting Blood Glucose (mg/dl)						
	Group-I	**Group-II**	**Group-III**	**Group-IV**	**Group-V**	**Group-VI**	**Group-VII**
Day-0	87.87 ± 2.56	304.63 ± 2.12	306.81 ± 1.20	297.96 ± 1.56	299.99 ± 2.23	323.33 ± 3.53	366.66 ± 2.23
Day-7	95.83 ± 1.45	309.37 ± 4.23	260.34 ± 2.30*	270.00 ± 2.67*	258.75 ± 4.23*	284.84 ± 3.12*	315.14 ± 1.33
Day-14	85.53 ± 0.45	292.98 ± 3.55	204.13 ± 1.30*	215.00 ± 1.24*	201.00 ± 5.23*	185.71 ± 2.46*	182.24 ± 4.21*
Day-21	89.00 ± 2.55	287.49 ± 4.30	140.00 ± 2.12*	163.80 ± 2.78*	161.80 ± 0.24*	170.00 ± 1.23*	176.66 ± 0.98*

Values are expressed as mean ± SEM (n=5), $^{**}p < 0.01$, $^{*}p < 0.05$ when compared with control group

Table 2: Effect of aqueous and ethanolic extracts of *P. dulce* on biochemical parameters in alloxan induced diabetic rats

Parameter	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Group-VII
Triglyceride (mg/dl)	118.62 ± 8.05	257.94 ± 6.94	131.00 ± 2.03*	136.00 ± 3.33*	135.60 ± 4.45*	139.99 ± 1.92*	129.88 ± 5.88*
Total Cholesterol (mg/dl)	139.75 ± 5.23	216.51 ± 6.66	121.66 ± 8.33*	137.77 ± 5.88*	127.66 ± 8.77*	129.34 ± 1.13*	127.68 ± 2.99*
HDL (mg/dl)	52.44 ± 2.56	26.62 ± 1.40	48.54 ± 4.56*	38.26 ± 6.45*	44.60 ± 5.20*	33.20 ± 2.68*	39.42 ± 6.12*
Serum Urea (mg/dl)	38.40 ± 0.46	53.70 ± 1.23	36.66 ± 1.04*	37.92 ± 3.38*	34.60 ± 0.44*	42.38 ± 0.34*	42.73 ± 1.05*
Serum Creatinine (mg/dl)	1.04 ± 0.08	1.43 ± 0.02	0.79 ± 0.09*	1.10 ± 0.66*	0.96 ± 0.08*	1.36 ± 0.39*	1.12 ± 0.02*
Serum Uric acid (mg/dl)	1.12 ± 0.03	3.28 ± 0.08	1.27 ± 0.05*	1.79 ± 0.23*	1.30 ± 0.04*	1.82 ± 0.07*	1.46 ± 0.09*
AST (U/l)	140.00 ± 3.44	456.00 ± 6.30	144.00 ± 5.34 *	234.00 ± 7.34 *	184.00 ± 8.20 *	248.00 ± 3.43 *	204.00 ± 6.62*
ALT (U/l)	63.00 ± 4.32	380.00 ± 6.48	82.00 ± 4.20*	196.00 ± 6.24*	146.00 ± 3.08*	208.00 ± 5.08*	182.00 ± 4.30*

Values are expressed as mean ± SEM (n=5), $^{**}p < 0.01$, $^{*}p < 0.05$ when compared with control group

Table 3: Effect of aqueous and ethanolic extracts of *P. dulce* on liver, muscle and kidney glycogen levels in alloxan induced diabetic rats

Organ	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Group-VII
Liver	16.59 ± 2.54	8.07 ± 1.20	20.22 ± 2.03*	23.11 ± 0.32*	22.87 ± 0.15*	17.8 ± 0.18*	18.12 ± 0.04*
Skeletal Muscle	3.17 ± 0.27	1.53 ± 0.24	2.48 ± 0.31*	2.54 ± 0.31*	2.17 ± 0.03*	3.10 ± 0.45*	2.16 ± 0.09*
Kidney	0.72 ± 0.08	1.40 ± 0.32	1.18 ± 0.21*	1.41 ± 0.24*	1.59 ± 0.15*	1.11 ± 0.18*	1.57 ± 0.04*

Values are expressed as mean ± SEM (n=5), $^{**}p < 0.01$, $^{*}p < 0.05$ when compared with control group

Effect of extract on liver, skeletal muscle and kidney glycogen diabetic rats

The effect of extract on liver glycogen level shows that there was significant increase in liver glycogen level. The reduced Liver glycogen level in diabetic rats was increased significantly by both AE and EE. The muscle glycogen level was reduced in alloxan induced diabetic rats. The AE and EE showed significant ($p < 0.05$) increase in muscle glycogen level when treated for 21 days. The effect on kidney glycogen level was found to increase glycogen level in alloxan induced diabetic rats. Standard drug, AE and EE reduce significantly glycogen level in kidney.

DISCUSSION

Considering the presence of insulin like principle in the leaves of *P. dulce*, the present study was conducted to study the antidiabetic activity of *P. dulce* leaves extracts. The present era is gaining the more focus on plant drugs as it is having less side effects and lower price as compared to present allopathic drugs. [22] The present study reveals that the treatment with aqueous and ethanolic extract of *P. dulce* leaves for 21 days shows significant antidiabetic effect on alloxan induced diabetic rats.

The acute toxicity study indicates that aqueous and ethanolic extract of *P. dulce* leaves when administered orally at the dose of 5000 mg/kg did not produced any sign of toxicity or death in treated animals. This suggests that LD_{50} of plant extracts is above 5000 mg/kg indicating the extract is non toxic for further use in study. Alloxan is commonly used chemical agent to induce diabetes in rats. It acts on pancreatic β cells of langerhan's causing partial destruction and induces diabetes mellitus through reduced insulin secretion. [23] The intraperitoneal administration of alloxan at the dose of 130 mg/kg destroys pancreatic β cells giving rise to diabetic rats. [24] The induction of diabetes was confirmed by fasting blood glucose level above 200 mg/kg on fourth day of administration of alloxan intraperitoneally using glucose strips.

The significant reduction in blood glucose level was observed in extracts treated rats when compared with diabetic control group. The rats treated with alloxan shows continuous rise in blood glucose level for 21 days. Traditionally many plants are used for the treatment of diabetes mellitus and which were found to be very effective alternative for present drugs which are having many side effects. So considering this we made an attempt to evaluate the antidiabetic potential of *P. dulce* leaves extracts in alloxan induced diabetic rats. Both aqueous and ethanolic extracts on single dose administration were not able to reduce the blood glucose level. The repeated administration of *P. dulce* leaves extracts for 21 days significantly reduces the blood glucose level when compared with diabetic control group. In present study we found that both extract of *P. dulce* when administered to diabetic rats reduces blood glucose level when compared with diabetic control group. The extracts might be produced their antidiabetic activity by increasing production or release of insulin from pancreas or reducing insulin resistance. [25] It is well known that the pioglitazone produces hypoglycemia by decreasing the peripheral resistance to insulin and increasing glucose transporter activity in cells. In our study both extracts of *P. dulce*

reduces blood glucose level which may be possibly due to increase in insulin release or due to increase in peripheral glucose uptake. The extracts may be acting on some of the surviving pancreatic β cells and increases the release of insulin responsible for antidiabetic potential. [26-27]

In oral glucose tolerance test blood glucose level was estimated at the intervals of 0, 30, 60, 90 and 120 minutes. This test shows the glucose utilization by body and which is used to diagnose the diabetes. The blood glucose level by administration of two different doses of *P. dulce* aqueous and ethanolic extract were found to be dose dependent when compared to diabetic control group.

In lipid metabolism alloxan produces hyperlipidemia in diabetic rats which may be due to increase in release of free fatty acids from stored fats. [28-29] Our present study also reveals that the lipid profile i.e. triglyceride and total cholesterol were significantly decreased by aqueous and ethanolic extracts of *P. dulce* when compared to diabetic control group. In contrast, the mean HDL level of *P. dulce* treated rats was significantly higher as compared to diabetic control group. Thus the treatment with aqueous and ethanolic extract of *P. dulce* may acts on stored fats which inhibits the release of free fatty acids and decreases the triglyceride and total cholesterol level. The extracts also increase HDL level in diabetic rats after treatment for 21 days.

Diabetic rats treated with *P. dulce* shows significant decrease in uric acid level when compared with diabetic control group. Uric acid is the product of purine metabolism which is excreted by kidney. In diabetic rats the increase in uric acid level may be produced due to increased purine metabolism or decreased uric acid excretion. [30] The extract may act by decreasing the purine metabolism of increasing the excretion of uric acid through kidney. Diabetic rats treated with *P. dulce* shows significant decrease in urea level when compared with diabetic control group. Urea is the metabolic product of proteins in target cell. This urea is filtered and removed by kidney as waste product. In alloxan induced diabetic rats due to kidney dysfunction there was accumulation of urea level and increase in blood urea level. The treatment with P. *dulce* significantly decreases increased blood urea level in diabetic rats revealing its protective effect on kidney in diabetic rats. Diabetic rats treated with P. *dulce* shows significant decrease in creatinine level when compared with diabetic control group. This effect of P. *dulce* on uric acid, urea and creatinine indicates the protective effect on kidney in alloxan induced diabetic rats. [29-31]

During alloxan treatment the level of AST and ALT is increased which are considered to be important markers of liver damage. AST and ALT level is increased due to reduced insulin level responsible for liver dysfunction. [32-34] This finding supports the toxic effect of alloxan on liver increasing the levels of AST and ALT. The extract of *P. dulce* decreases significantly AST and ALT level when compared with diabetic control group supporting its protective effect on the liver.

The result suggests that in treated groups there is significant improvement in liver and muscle glycogen level while kidney glycogen level was decreased significantly when compared with diabetic control group. This improvement in liver and muscle glycogen level may be due to increase in uptake of glucose from blood. This effect on glycogen level support the probable increase in glucose uptake due to treatment with *P. dulce* extracts.

Our study reveals that both aqueous and ethanolic extract produces its antidiabetic effect in alloxan induced diabetic rats. The effect on lipid profile indicates the hypolipidemic activity of both extract which is dose dependent. Oral glucose tolerance test concludes the aqueous and ethanolic extract of *P. dulce* promotes glucose tolerance. The effect of extracts on blood urea, uric acid and creatinine indicates protective effect on kidney. As the extracts of *P. dulce* decreases significantly AST and ALT level it may have protective effect on liver. *P. dulce* leaves extracts also increases significantly liver and muscle glycogen which may be due to increase in glucose uptake. Further detail study is required to identify the mechanism of action of extracts which have antidiabetic potential.

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