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Short Communication

EFFECT OF PITHECELLOBIUM DULCE BENTH LEAVES IN DEXAMETHASONE INDUCED DIABETIC RATS

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ABSTRACT

Objective: The objective of the present study was to study the effect of *Pithecellobium dulce* Benth (*P. dulce*) leaves in dexamethasone-induced diabetic rats.

Methods: The authenticated *P. dulce* leaves were collected from a local area of Sangli, Maharashtra. The leaves of the plant were extracted with water and ethanol by maceration and soxhelation respectively. Acute toxicity studies of the both extracts were performed using rat and according to OECD 425 guidelines. The dose of 200 mg/kg and 400 mg/kg was selected for further studies. The albino rats were divided into seven groups with five animals in each group. The diabetes was induced by dexamethasone (10 mg/kg, s. c.) and treated with extract and standard drug for 10 d. Then blood glucose, triglyceride, total cholesterol and glycogen level in liver, muscle and kidney were estimated according to standard procedures.

Results: The study revealed that *P. dulce* at 200 mg/kg and 400 mg/kg showed significant ($p < 0.05$) antidiabetic activity. All the extract treated groups showed a significant reduction in blood glucose level on 11th day when compared to diabetic control group. The significant increase in blood glucose, triglyceride, and total cholesterol level was observed in the diabetic control group when compared to normal control group. The liver and muscle glycogen level was decreased significantly ($p < 0.05$) in the diabetic control group.

Conclusion: It can be concluded that *P. dulce* aqueous and ethanolic extract at two different doses (200 mg/kg and 400 mg/kg) possesses antidiabetic and hypolipidemic activity.

Keywords: *Pithecellobium dulce* Benth, Antidiabetic, Dexamethasone, Glucose, Lipid profile, Glycogen

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Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to either insufficiency of insulin or inability of cells to respond to insulin [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. The chronic complications of diabetes are microvascular damage, nephropathy, neuropathy and retinopathy which is mainly caused due to significantly increased the level of blood glucose level [2]. The World Health Organization (WHO) estimates that more than 220 million people worldwide have diabetes and this number is likely to more than double by 2030 [3]. The highest prevalence, as well as the long-term complications associated with diabetes, stimulates the search for the new antidiabetic agent.

In 1980 the WHO also suggested to study and find out the plants which are having potential hypoglycemic activity as the modern drugs have the less safety [4]. The present hypoglycemic drugs and insulin used for the treatment of diabetes is excessively costlier and having its own side effects which limits its use. Although many allopathic approaches are available for treatment of diabetes but none of these is ideal for treatment of diabetic patients. Insulin is associated with its stability when taken orally another hypoglycemic like sulphonyl urea and α glucosidase is associated with its own side effects [5]. Recently there is increasing trend for using plant preparations and its derivatives to treat diabetes and its complications [6].

Traditionally in ayurveda medicines consist of plant or plant product as a single plant or a combination of plants, which are considered less toxic and free from side effects when compared to synthetic drugs.

P. dulce (Leguminosae) is native to tropical Asia, America and cultivated throughout the India. It is evergreen; medium-sized tree grows up to 18 m in height [7]. It is commonly known as 'manila tamarind' and Indian jalebi as it resembles the sour taste of tamarind and Indian sweet jalebi. The leaves of *P. dulce* Contains cyclitol, dulcitol, octacosanol, α-spinasterol, kaempferol-3-rhamnoside, quercetin and afzelin [8]. The literature survey suggests that the leaves of the plant used traditionally for leprosy, intestinal disorders, peptic ulcer, toothache, ear ache, emollient, abortifacient and larvicidal in folk medicines [9]. The leaves of the plant have reported to contain the insulin-like content which may be useful for the treatment of diabetes [10]. The leaves also reported to show antifungal and antibacterial activity [11]. Estrogenic activity was observed by isolated isoflavonoids from the root of the plant [12]. The leaves of the plant reported to have free radical scavenging properties and antimycobacterial activity [8, 9]. The Neuro-pharmacological activities of this plant were also demonstrated [13].

Taking into consideration the traditional claims and reported activities, *P. dulce* has been studied for its antidiabetic activity in diabetic animals. Hence the present study was planned to investigate the effect of *P. dulce* leaves on dexamethasone-induced insulin resistance in the rat.

The fresh leaves of *P. dulce* were collected from Jaisingpur, Sangli District, Maharashtra, India. The plant material was taxonomically authenticated by acknowledged Botanist, Dr. Mrs. U. S. Yadav at Willingdon College, Sangli, Maharashtra, India (Voucher specimen No.: WILL/Bot/2009/03). The standard drug pioglitazone was obtained from Aarti Drugs Ltd, Mumbai. The inducer dexamethasone was obtained from Cipla Pharma R and D, Vikroli. All the diagnostic kits used are of Span Diagnostics, Surat.

The fresh leaves were separated and air dried under shade for seven days. The dried plant leaves were subjected to size reduction to a course by the dry grinder and passed through a sieve. The powder was subjected to aqueous and ethanolic extraction. The powder material was packed into soxhlet apparatus and extracted using ethyl alcohol as a solvent. This extract was oven dried at 40 °C giving a dried extract [14]. The aqueous extract was prepared with chloroform-water by maceration for six h at room temperature. During maceration, it was subjected to occasional shaking on an

orbital shaker and then stands for the next 18 h. The aqueous extract obtained was filtered with the help of Whatman filter paper and concentrated overheating on a water bath, and then the concentrated extract was air dried [15]. The solution of aqueous extract was prepared by distilled water as a solvent for the experiment. The aqueous extracts were administered orally through an orogastric tube. The suspension of ethanolic extracts was prepared by using 0.5% CMC in distilled water [16].

Wistar rats of either sex (150‑300 g) were acclimatized for seven days in the temperature (26±1 °C) and light controlled (12 hr light: 12 hr dark) room with the provision of food (Amrut Feed, Sangli) and water *ad libitum.* The study protocol was approved by the Institutional Animal Ethics Committee of Appasaheb Birnale college of Pharmacy, Sangli (Approval No: ABCP/IAEC/2009/08).

The acute toxicity of aqueous and ethanolic extracts of *P. dulce* was determined by using wistar albino rats (150‑300 g) which were maintained under the standard conditions. The animals (n= 5) were fasted 12 h prior to the experiment; OECD guideline 425 procedures were adopted for toxicity studies. A single dose of extract of *P. dulce* at 2000 mg/kg were administered to animals. The animals were observed for seven days as animals had not shown any sign of toxicity, behavioral changes and mortality the dose increased up to 5000 mg/kg. Then animals were observed up to 7 d for toxicity, behavioral changes, and mortality [17].

The experiment was designed for 11 d to evaluate antidiabetic activity of *P. dulce* leaves in dexamethasone-induced insulin resistance in albino rats. In the experiment total 35 overnight fasted rats were used. The 30 rats were rendered diabetic by the solution of dexamethasone (10 mg/kg, s. c.). Dexamethasone was dissolved in saline and administered intravenously into fasted rats at a dose of 10 mg/kg of body weight by subcutaneous route for 10 d and at the same time the test samples were administered orally. The animals were randomly divided into seven groups, five animals in each group. The animals were divided into seven groups as following.

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

Group-II: Dexamethasone 10 mg/kg (s. c)+vehicle

Group-III: Dexamethasone 10 mg/kg (s. c)+Pioglitazone 20 mg/kg

Group-IV: Dexamethasone 10 mg/kg (s. c)+Aqueous extract 200 mg/kg

Group-V: Dexamethasone 10 mg/kg (s. c)+Aqueous extract 400 mg/kg

Group-VI: Dexamethasone 10 mg/kg (s. c)+Ethanolic extract 200 mg/kg

Group-VII: Dexamethasone 10 mg/kg (s. c)+Ethanolic extract 400 mg/kg

At the last day of the treatment schedule, i.e. on day 11, the overnight fasted animals were anesthetized with diethyl ether and blood was collected by retro-orbital puncture method. Separation of serum from collected sample by centrifugation at 5000 rpm and stored at refrigeration temperature until use for estimation of serum glucose, triglyceride and total cholesterol levels. Animal were sacrificed by cervical dislocation method to collect the tissue samples of liver, skeletal muscle and kidney and evaluated for tissue glycogen levels.

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.

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 screening and dose of 200 mg/kg and 400 mg/kg p. o. of each extract (aqueous and ethanolic) was selected for the further study.

The effect of both aqueous and ethanolic extract on serum glucose, triglyceride and total cholesterol level is presented in table 1. The blood glucose level was found to be increased in dexamethasone-induced diabetic rats. The blood glucose level which was increased by dexamethasone significantly ($p < 0.05$) reduced by aqueous extract and ethanolic extract when compared with diabetic control group. The serum triglyceride level was found to be reduced significantly ($p < 0.05$) by both aqueous and ethanolic extract when compared with diabetic control group. The effect on total cholesterol level shows the significant ($p < 0.05$) reduction by both aqueous extract and ethanolic extract when compared with diabetic control group. The effect was found to be a dose-dependent increase in dose increases the effect for both aqueous extract and ethanolic extract.

Table 1: Effect of aqueous and ethanolic extracts of *P. dulce* on blood glucose, triglyceride and total cholesterol levels in dexamethasone-induced diabetic rats

Parameter	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Group-VII
Glucose	103.12± 2.34	176.35± 5.56	112.5± 4.23**	137.05± 2.34*	119.75± 3.12**	139.05± 3.12*	131.66± 5.23*
Triglyceride	125±4.01	168.74±3.30	145.83±4.02*	155.11±5.06*	144.9±4.9*	158.33±1.09*	148.66±3.50*
Total Cholesterol	103.12±2.34	176.35±1.56	112.50±4.23*	137.05±2.34*	119.75±3.12*	149.05±3.12*	141.66±5.23*

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

The effect of both aqueous and ethanolic extract on liver, skeletal muscle and kidney glycogen level is presented in table 2. The effect on liver glycogen level shows that there was a reduction in liver glycogen level. This reduced liver glycogen level was increased significantly ($p < 0.05$) by both aqueous extract and ethanolic extracts. The muscle glycogen level was reduced in dexamethasone-induced diabetic rats. The aqueous and ethanolic extracts show a significant increase in muscle glycogen level when treated for eleven days. The effect on kidney glycogen level was found to increase glycogen level in dexamethasone-induced diabetic rats. The standard drug, extract and ethanolic extracts reduce significantly increased glycogen level in kidney.

Table 2: Effect of aqueous and ethanolic extracts of *P. dulce* on liver, muscle and kidney glycogen levels in dexamethasone-induced diabetic rats

Parameter	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Group-VII
Liver	19.08± 2.9	7.73± 1.74	16.66± 1.82*	18.65± 2.06*	17.97± 2.86*	18.86± 2.75*	17.68± 1.95*
Muscle	3.30±0.20	0.76±0.21	2.55±0.29**	1.90±0.25*	2.42±0.41**	3.06±0.27**	2.32±0.40**
Kidney	0.70±0.08	2.14±0.28	1.48±0.17*	1.56±0.35*	1.52±0.39*	1.21±0.12*	1.28±0.35*

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

Effects of aqueous and ethanolic extracts of *P. dulce* leaves were tested for glucose, triglyceride, total cholesterol and glycogen level in dexamethasone-induced diabetic rats. The outcome of the present research demonstrates that *P. dulce* leaves produce significant pharmacological effects in the diabetic rats.

Glucocorticoid is extensively used as medicine for short-term acute steroid therapy can be seen in exacerbation of the chronic obstructive pulmonary disease, acute gout, chemotherapy protocols and bacterial meningitis. The chronic use of glucocorticoids suppresses the immune system and helps in organ transplantation [18]. During its use, it acts on the liver and shows increase in the level of blood glucose due to increasing in liver glycogen metabolism. Glucocorticoid also decreases the insulin sensitivity which act on cells and helps to increase glucose uptake through glucose transporters. The combined effect of this will result in an increase in blood glucose level and produces type-2 diabetes mellitus [29, 20]. It is observed that glucocorticoids and insulin have opposite effects on the metabolism of carbohydrate, protein, and fats. Insulin promotes synthesis of glycogen from glucose, so it is anabolic. Glucocorticoid increases metabolism of carbohydrate, protein and fat [21, 22]. The main mechanism by which the hyperglycemia and type-2 diabetes is produced by glucocorticoid is increased in glucose production and inhibition of insulin secretion [23, 24].

The present study reveals that significantly increased blood glucose, triglyceride, total cholesterol and decrease in muscle and liver glycogen level along with an increase in kidney glycogen level in diabetic rats compared to non-diabetic rats, which indicates diabetic was induced effectively.

In dexamethasone-induced type-2 diabetes due to insulin deficiency or due to insulin resistance results in elevation of blood glucose level [25]. However, the treatment with both aqueous and ethanolic extracts treatment significantly reduces blood glucose level. The effect of both extracts was dose dependent with an increase in dose there was a reduction in blood glucose level was observed. Reduction of elevated blood glucose level is an indicator of the antidiabetic activity of *P. dulce* leaves [26]. Furthermore, it is well known that these antidiabetic effects are mediated through the action of insulin on insulin receptors [27]. Therefore *P. dulce* induced enhancement of blood glucose level could be attributed to the increase in insulin secretion, decrease in insulin resistance or participation of insulin receptors. This effect may be due to the presence of flavonoids, saponins and tannins which may be responsible for the antidiabetic potential of *P. dulce* leaves [13].

Accumulation of triglyceride and total cholesterol is an indicator of abnormal lipid metabolism and is commonly associated with diabetes mellitus. In diabetes mellitus lipase enzyme required for hydrolysis of triglycerides was in the inactive state due to insulin deficiency which leads to increase in triglyceride level. The effect of both aqueous and ethanolic extract on lipid profile reveals that both extracts produced a significant decrease in triglyceride and total cholesterol level [28].

The result suggests that there is a significant improvement in liver and muscle glycogen level while kidney glycogen level was decreased significantly when compared with diabetic control group.

CONCLUSION

Our study shows that both aqueous and ethanolic extracts produce an antidiabetic effect in dexamethasone-induced diabetic rats. The effects on lipid profile indicate the hypolipidemic effect of both extracts in dexamethasone-induced diabetic rats. The extracts also increase liver and muscle glycogen level while kidney glycogen level was reduced significantly. Further studies are necessary to identify the active constituent present in the extract as well as to elucidate the mechanisms by which it produces its beneficial effects.

CONFLICTS OF INTERESTS

All authors have none to declare.

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