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α-Glucosidase inhibitory and antidiabetic activities of ethanolic extract of *Pisonia alba* Span. leaves

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Abstract
In this study, the ethanolic extract of *Pisonia alba* has been studied for its α-glucosidase inhibitory and antidiabetic properties. The extract shows α-glucosidase inhibitory activity in a concentration dependent manner (IC_{50} of 416.7 µg/ml). On 15 days administration of the extract (250 and 500 mg/kg) on alloxan induced diabetic rats resulted in significant ($P<0.01$) decrease in blood glucose, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (SALP), cholesterol, triglycerides levels and increase in HDL levels as compared to pathogenic diabetic rats. These results indicate that the extract is effective in hyperglycemia and also protects significantly from other metabolic aberrations caused by alloxan.

Keywords: *Pisonia alba*, alloxan, α-Glucosidase, antihyperglycemic, glibenclamide.

INTRODUCTION

Diabetes mellitus (DM) is a common metabolic disease characterized by elevated blood glucose levels, resulting from absent or inadequate pancreatic insulin secretion, with or without current impairment of insulin action. Currently, diabetes has been estimated to affect 177 million people world wide and this figure is projected to increase to 300 million by 2025 (Porter *et al.*, 2005). Epidemiological studies and clinical trials strongly support that hyperglycemia is the main cause of complications related with coronary artery disease, cerebrovascular disease, renal failure, blindness, limb amputation, neurological complications and pre-mature death (Lopez 2001). Recent studies suggest that postprandial hyperglycemia could induce the non-enzymatic glycosylation of various proteins, resulting in the development of chronic complications. Therefore, control of postprandial plasma glucose levels is critical in the early treatment of DM and in reducing chronic vascular complications (Shim *et al.*, 2003).

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One of the therapeutic approaches for reducing postprandial hyperglycemia in patients with DM is to prevent absorption of carbohydrates after food uptake. Only monosaccharides, such as glucose and fructose, can be transported out of the intestinal lumen into the blood stream. Complex starches, oligosaccharides and disaccharides must be broken down into individual monosaccharides before being absorbed in the duodenum and upper jejunum. This digestion is facilitated by enteric enzymes, including pancreatic α-amylase and α-glucosidases that are attached to the brush border of the intestinal cells. Acarbose and miglitol are competitive inhibitors of intestinal α-glucosidases and reduce the postprandial digestion and absorption of starch and disaccharides (Davis *et al.*, 1996). Screening of α-glucosidases inhibitors from plants and synthetic sources is increasing. Inhibitors of these enzymes have been recently developed from natural sources (Shim *et al.*, 2003).

In herbal medicinal practice many plants are used to treat diabetes mellitus in south India. Most of these medicinal plants are not scientifically validated for their

therapeutic efficacy and safety (Babu *et al.*, 2002). Though there are numerous traditional medicinal plants reported to have hypoglycemic property, many of them have proven to be not effective in lowering glucose levels in severe diabetes (Nagarajan *et al.*, 1987). Furthermore, most of the hypoglycemic agents used in allopathic medicine are reported to have side effects including hematological, coma and disturbances of liver and kidney. Therefore, there is a need to search for more effective and safer drugs for diabetes (Pari *et al.*, 1999).

Pisonia alba (Nyctaginaceae), commonly known as Lettuce Tree, is an evergreen tree 9-12 m high found sparsely wild in the beach forests of Andaman Islands, cultivated to a small extent in India and Ceylon. The fresh leaves moistened with Eau-de-Cologne are used to subdue inflammation of a filariosis nature in the legs and other parts (Kiritikar *et al.*,1935). They are used as diuretic. The root is purgative. A survey of literature revealed that *Pisonia alba* is an untapped candidate for antidiabetic activity though it is extensively used in traditional healing of diabetes in Kerala (Anonymous, 1969).

MATERIALS AND METHODS

Plant material and extraction procedure

The aerial parts of *Pisonia alba* were collected from Trivandrum district of Kerala. The plant was authenticated by Dr. Mathew Dan, Plant taxonomist of Tropical Botanical Garden and Research Institute. A voucher specimen has been deposited at the herbarium of TBGRI (TBGT 57019 dated 19/01/07) for future reference. The aerial parts of *Pisonia alba* were washed thoroughly in tap water, dried under shade and powdered to coarse particles. The powder (22 gm) was extracted with 3000 ml of ethanol with constant stirring. The residue was removed by filtration and the filtrate was evaporated to dryness at 40ºC under reduced pressure in a rotary evaporator. The yield of the ethanol extract was found to be 0.165 gm. The extract was dried in a desiccator and it was referred to as PAEE. It was diluted with 2% Tweeen-80 to desired concentrations and used for the experiments.

Animals

Adult male albino rats (250-350 gm), reared in Tropical Botanical Garden and Research Institute's animal house were used for the experiments. They were grouped and housed in Poly-acrylic cages with six animals per cage and maintained under standard laboratory conditions. (Temperature 24-28ºC, relative humidity 60-70% and 12 h dark-light cycles). They were fed with commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water, *ad libitum*. All animal experiments were carried out according to NIH guidelines, after getting the approval of the Institute's Animal Ethics Committee (Reg. No. 176/99/CPCSEA).

Acute toxicity studies

Healthy adult wistar albino rats of either sex, starved overnight were divided into five groups (n=6) and were orally fed with the PAEE in increasing dose levels of 100, 500, 1000, 3000 and 5000 mg/kg body weight (Ghosh, 1984). The animals were observed continuously for 2 h under behavioral, neurologial, autonomic profiles (Turner, 1965).

Inhibition assay for the α-glucosidase activity

In order to investigate the inhibitory of PAEE, an *in vitro* α-glucosidase inhibition test was performed. Normally α-Glucosidase from yeast is extensively used as a screening material for α-glucosidase inhibitors, but the results do not always agree with those obtained in mammals. Therefore, we used the mouse small intestine homogenate as an α-glucosidase solution because we speculated that it would better reflect the *in vivo* state. The inhibitory effect was measured using the method slightly modified from Dahlqvist (Dahlqvist, 1964). After fasting for 20 h, the small intestine between the part immediately below duodenum and the part immediately above the cecum was cut, rinsed with ice cold saline and homogenized with 12 ml of maleate buffer (100 mM, pH 6.0). The homogenate was used as the α-glucosidase solution. The assay mixture consisted of 100 mM maleate buffer (pH 6.0), 2% (w/v) each sugar substrate solution (100 µl), and the PAEE (50-1000µg/ml). It was pre-incubated for 5 min at 37ºC, and the reaction was initiated by adding the crude α-glucosidase solution (50 µl) to it, followed by incubation for 10 min at 37ºC. The glucose released in the reaction mixture was determined with the kit described above. The rate of carbohydrate decomposition was calculated as percentage ratio to the amount of glucose obtained when the carbohydrate was completely digested. The rate of prevention was calculated by the following formula:

$$\text{Inhibition rat (\%)} = \frac{(A_0 - A_1) - B}{A_0} \times 100$$

A_0 - Amount of glucose produced by the positive control.
A_1 - Amount of glucose produced by the addition of PAEE
B - Glucose production value in blank.

Effect of PAEE on Oral Glucose Tolerance Test (OGTT)

The oral glucose tolerance test (Bonner-Weir, 1988) was performed in overnight fasted (18 h) normal rats. Rats divided into three groups (*n*=6) were administered

Table 1: *In vitro* α-glucosidase inhibition using PAEE.

Concentration (µg/ml)	% of inhibition of α-glucosidase		
	PAEE	Acarbose	IC_{50} (µg/ml)
50	5.75 ± 0.05	74.53 ± 0.01	
100	16.84 ± 0.06	81.23 ± 0.02	
200	34.76 ± 0.02	87.98 ± 0.01	416.7
400	45.00 ± 0.00	91.78 ± 0.02	
500	55.54 ± 0.02	94.08 ± 0.04	
1000	83.29 ± 0.02	96.71 ± 0.03	

All the values represent (mean ± SE) taken from two experiments each with 3 replicates per dose was tabulated.

Table 2: Effect of PAEE Oral Glucose Tolerance Test.

Groups	Blood glucose (mg/dl)			
	0 min	30 min	90 min	150 min
Nomal control	61 ± 1.6	150 ± 3.8	160 ± 1.5	140 ± 8.3
PAEE (250 mg/kg)	62.5 ± 0.6	100 ± 4.1**	106 ± 2.2**	77 ± 4.4**
PAEE (500 mg/kg)	62 ± 2.1	104 ± 1.1**	110 ± 1.6**	80 ± 4.8**

Values are the mean ± SD, n=6 for all groups significance **P<0.01, compared with vehicle treated control group (One way ANOVA using Dunnett's test).

drinking water, PAEE 250 and 500 mg/kg, respectively. Glucose (2 gm/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the retro orbital sinus under ether inhalation at 30, 90 and 150 min of glucose administration and glucose levels were estimated using the standard glucose reagent kit (Accurex Biomedical Pvt. Ltd. Thane).

Effect of PAEE on Alloxan-induced diabetic rats

Rats were injected with alloxan (60 mg/kg) through tail vein (Babu *et al.*, 2002). Five days later, blood samples were drawn and glucose levels were determined to confirm the development of diabetes. The diabetic rats exhibiting glucose levels in the range of 400-450 mg/dl were selected to determine the efficacy of PAEE.

Experimental setup

The animals were divided into five groups with six animals in each group.

- Group I normal control rats administered 2% Tween-80 (1 ml), p.o.
- Group II diabetic control rats administered a single daily dose of 2% Tween-80 (1 ml), p.o.
- Group III diabetic rats administered PAEE (250 mg/kg b.w/d)
- Group IV diabetic rats administered PAEE (500 mg/kg b.w/d)
- Group V diabetic rats administered reference drug glibenclamide (600 µg/kg b.w/rat/d) in aqueous solution orally for 15 days.

Blood samples were collected in the morning, 1hr after drug administration on days 1, 4, 7, 10 and 15 for glucose estimation. After 15 days of treatment, the fasted rats were sacrificed by cervical decapitation. Blood was collected into heparinized tubes. The serum was analysed for glucose content, serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, serum alkaline phosphatase, cholesterol, triglycerides and HDL in an autoanalyzer Micro lab 2000 using Ecolin kits.

Statistical analysis

The experimental data were expressed as mean±SEM. The significance of difference among the various treated groups and control group was analyzed by means of one-way ANOVA followed by Dunnett's multiple comparison test using Graphat Instat Software (San Diego, CA, USA).

RESULTS AND DISCUSSION

The acute toxicity studies revealed the nontoxic nature of the PAEE. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period. This is not surprising as PAEE is extensively used as vegetable and salad.

The PAEE showed a significant inhibitory action of α-glucosidase enzyme (Table 1). The results revealed that PAEE showed 5.75% of inhibition at 50 µg/ml and for 1000 µg/ml inhibition was found to be 83.29%. There was a proportionate increase in the percentage of α-glucosidase inhibition in a concentration dependent manner (IC_{50} of 416.7 µg/ml). Acarbose was used as a reference standard for the evaluation of α-glucosidase inhibitory action. α-glucosidase is one of a number of glucosidases located in the brush-border surface membrane of intestinal cells, and is a key enzyme of carbohydrate digestion (Caspary, 1978). α-glucosidase inhibitors block the actions of α-glucosidase enzymes in the small intestine, which limits the conversion of oligosaccharides and disaccharides to monosaccharides, necessary for gastrointestinal absorption. Postprandial glucose peaks may be attenuated by delayed glucose absorption. The main benefits attributable to α-glucosidase inhibitors are reductions in both postprandial glycemic levels and in the total range of postprandial glucose levels (Lebovitz, 1997). The antidiabetic action of PAEE is attributed to the intestinal α-glucosidase inhibitory activity.

Oral administration of PAEE at 250 and 500 mg/kg doses resulted in a significant fall in blood glucose level, 2½ h after a single dose of treatment in glucose loaded rats (Table 2). PAEE was effective at both the doses used in depressing the peak value of blood sugar at 30 min after glucose loading. At both the dose levels, PAEE completely prevented the elevation of blood

Table 3: Effect of 15 days treatment with PAEE on glucose levels of diabetic rats

Treatments	Blood glucose levels (mg/dl)				
	Day 1	Day 4	Day 7	Day 10	Day 15
Normal control	88 ± 4.2	89.3 ± 3.9	85.3±4	88 ± 5.1	86 ± 4
Diabetic control	565.2 ± 2.7***	567.4 ± 3***	568.7 ± 2.8***	570.2 ± 1.4***	572.5 ± 3***
PAEE (250 mg/kg)	271.3 ± 2.7	266.7 ± 2.4	245.2 ± 2.5**	200.7 ± 2.4**	200 ± 2.5**
PAEE (500 mg/kg)	268.7 ± 1.6	260.8 ± 4.1	241.8 ± 4.9**	204.2 ± 3.7**	205.95 ± 3.4**
Glibenclamide (600 µg/kg)	274 ± 5.2	264.5 ± 3.6	247.4 ± 3.8**	200.1 ± 4.2**	203.35 ± 4.7**

Values are the mean ± SD, n=6 for all , significance **P<0.01, ***P<0.001 compared with vehicle treated control group (One way ANOVA using Dunnett's test).

Table 4: Effect of PAEE on rat serum biochemical parameters after alloxan administration

Groups	SGPT (IU/L)	SGOT (IU /L)	ALP (U/l)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)
Normal control	83 ± 7.5	123 ± 2	207 ± 19.3	50 ± 4.7	75.32 ± 1.81	76.42± 2.76
Diabetic control	185 ± 9.02***	272 ± 44***	293 ± 3.5***	91.75 ± 6.4***	183.45 ± 13.22***	28.73 ± 1.71***
Diabetic +PAEE (250 mg/kg)	90 ± 2.7	205 ± 34**	222 ± 27	57.25 ± 10.06	87.51 ± 4.71**	72.52 ± 2.54**
Diabetic + PAEE (500 mg/kg)	85 ± 26.4**	204 ± 14**	206 ± 18**	61.25 ± 8.7	80.72 ± 3.23**	75.25 ± 1.63**
Diabetic + Glibenclamide (600 µg/kg)	84 ± 3.5**	203 ± 74**	160 ± 36**	59 ± 26.2**	82.32 ± 2.44	75.63 ± 1.84**

Values are the mean ± SD, n=6 for all groups significance **P<0.01, compared with vehicle treated control group (One way ANOVA using Dunnett's test).

glucose level caused by oral glucose feeding. This finding indicates that the PAEE extract might be producing its hypoglycemic activity by a mechanism independent from the insulin secretion, *e.g.* by the inhibition of endogenous glucose production (Eddouks *et al.*, 2003) or by the inhibition of intestinal glucose absorption (Platel *et al.*, 1997).

In alloxan induced diabetic rats, the blood glucose levels were in the range of 400-450 mg/dl which can be considered as severe diabetes (Table 3). Administration of PAEE upto 15 days tends to bring the blood glucose levels towards normal. The PAEE treated groups showed blood glucose levels of 200 mg/dl and 205.95 mg/dl, when compared with that of diabetic control groups (203.35 mg/dl). A similar reduction in blood glucose level was noticed in glibenclamide treated rats (203.35 mg/dl). A significant ($p<0.01$) reduction in blood glucose level was noticed among PAEE and standard drug treated groups when compared with diabetic control. Alloxan, a β-cytotoxin, induces chemical diabetes in a wide variety of animal species including rats by damaging the insulin-secreting β-cells and almost complete destruction of the pancreas. In the present study, PAEE significantly reduced the blood glucose levels of alloxan diabetic rats indicating the mechanism possibly by potentiation of pancreatic secretion of insulin from existing residual β-cells of islets or enhanced transport of blood glucose to peripheral (Tulay *et al.*, 2008). Inhibition of proximal tubular reabsorption mechanism for glucose in the kidney may also contribute to the blood glucose lowering effect (Jafri *et al.*, 2000).

An increase in the SGPT, SGOT and SALP activities was recorded in diabetic rats in comparison with non diabetic rats, indicating an altered liver function in diabetic condition. PAEE significantly controlled SGPT, SGOT and SALP values in the alloxan diabetic rats (Table 4). In diabetic animals a change in the serum enzymes is directly related to changes in the metabolism in which these enzymes are involved. The increased levels of transaminases which are active in the absence of insulin because of increased availability of amino acids in diabetes (Bondy *et al.*, 1949; Felig *et al.*, 1970) are responsible for the increased gluconeogensis and ketogenesis observed in diabetes. In the present study, the PAEE significantly decreased Aspartate aminotransferase (ASAT) and Alanine aminotransferase (ALAT) enzyme activities. Hence, the improvements noticed in the levels of the enzymes studied, namely Glutamate oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminases (GPT) are as a consequence of an improvement in the carbohydrate, fat and protein metabolism. The restoration of GOT and GPT after treatment also

indicates a revival of insulin secretion. Elevation of Alkaline phosphatase (ALP) has been reported in diabetic rats (Mishima, 1967) and rabbits (Begum *et al.*, 1978). This increase in ALP was significantly reversed by the PAEE.

A decrease in the serum triglycerides and total cholesterol levels and an increase in the HDL cholesterol levels were observed (Table 4). A marked increase in total cholesterol and decrease in HDL cholesterol have been observed in untreated diabetic rats. Under normal circumstances insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzymes thereby causing hypertriglyceridemia (Annie *et al.*, 2006) The significant control of the levels of serum lipids in the aqueous extract treated diabetic rats may be directly attributed to improvement in insulin levels upon PAEE therapy.

Further pharmacological and biochemical investigations at the cellular and molecular level are underway to elucidate its exact mechanism of action.

CONCLUSION

The results obtained from the present study show that the PAEE had beneficial effects on blood glucose levels in glucose-fed hyperglycemic and diabetic rats and it also protects significantly from other metabolic aberrations caused by alloxan, thus scientifically verifying the traditional claim. PAEE appears to be an attractive material for further studies leading to possible drug development for diabetes. Development of phytomedicines is relatively inexpensive and less time consuming; it is more suited to our economic conditions than allopathic drug development which is more expensive and spread over several years.

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