Pharmacodynamic studies on the isolated active fraction of *Acacia farnesiana* (L.) willd

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ABSTRACT

Background: *Acacia farnesiana* is a medicinal plant that grows throughout tropical parts of Indian subcontinent, particularly in sandy soils of river beds in Northern India. The objective of the present study was to evaluate the anti-hyperglycemic activity of the extracts using glucose tolerance test. Isolation of an active fraction (AF) from the active extract (water extract) using alcohol precipitation and to get insight to the mechanism of action of the AF of *A. farnesiana.* **Materials and Methods:** Glucose uptake by isolated rat diaphragm of the AF was performed. Further the effect of release of Insulin from isolated and cultured pancreatic β-cell was determined. Besides, effect of oral administration of the AF was compared with that of intraperitonial administration. The effect of AF on serum glucose levels in orally glucose loaded rats was compared with that of intraperitoneal glucose loaded rats. **Results:** The water extract significantly lowered the blood glucose level. When precipitated with alcohol, the activity was found in the soluble fraction. Glucose uptake in the isolated rat hemidiaphragm, was increased by the AF at 40 µg/ml concentration, the AF did not significantly influence insulin release from cultured islets. The AF was found to be effective in orally glucose loaded in contrast to intraperitonial route. **Conclusion:** Our findings suggest that this plant is promising for further studies leading to the development of valuable medicine for diabetes.

Key words: *Acacia farnesiana,* active fraction, glucose tolerance test, diabetes mellitus

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Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia arising as a consequence of a relative or absolute deficiency of insulin secretion, resistance to insulin action or both.[1] This is a major health problem throughout the world, with a world-wide prevalence of 171 million people in 2000 and is expected to increase 366 million people by 2030. In particular, number of people with diabetes in India, currently around 40.9 million, is expected to rise to 69.9 million by 2030.[2] India leads the world with the largest number of diabetic subjects thus earning the dubious distinction of being termed the "Diabetes Capital of the World."[3] The basis of abnormalities in carbohydrate, fat and protein metabolism in diabetes is deficient action of insulin on its target tissues, resulting from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action.[4] Although the prevalence of diabetes is consistently increasing, an effective treatment is still lacking. Current pharmacotherapeutics insufficiently reverse hyperglycemia, have limited tolerability, and induce side-effects.[5] Hence, the identification of new pharmacological approaches to effectively prevent, treat and cure this metabolic disorder is of crucial importance. In experimental diabetes, enzymes of glucose metabolism are markedly altered and produce hyperglycemia, which leads to the pathogenesis of diabetic complications. There is an urgent need to identify indigenous natural resources in order to procure them. In recent times, many traditionally used medicinally important plants have been tested for their antidiabetic potential by various investigations in experimental animals.[6-8]

Acacia farnesiana is a medicinal plant that grows throughout tropical parts of Indian subcontinent, particularly in sandy soils of river beds in Northern India and other parts of Tamil Nadu. It is used in folk medical practices to treat DM in certain remote villages of Thirunelveli district of Tamil Nadu. In ethno-medical practices, the plant is also used as diuretic, treat antiulcer, anti-pyritic etc., Recent

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preliminary studies have showed promising anti-diabetic activity of the plant.[9] Phytochemical analysis showed the presence of tannins, phenol, glycosides, terpinoids, in the active fraction (AF).[10] Hence, the present study was carried out to determine the mechanism of action with an aim to scientifically prove the traditional claim of this plant.

MATERIALS AND METHODS

Chemicals and reagents

Insulin was purchased from Knoll Pharmaceuticals Ltd., India. All other chemicals and reagents used were of analytical grade and purchased from E. Merck Ltd., Mumbai.

Plant materials

Aerial parts *A. farnesiana* were collected from Tirunelveli district of Tamil Nadu and identified by Dr. Mathew Don, Taxonomist of Tropical Botanic Garden and Research Institute (TBGRI) and a voucher specimen, TBGRI 8283, was deposited in the herbarium of TBGRI.

Animals

Inbred Wistar rats (150-200 g weight) reared in TBGRI animal house were used for *in vivo* experimentation. Animals were caged in uniform hygienic conditions and fed with standard pellet diet (Lipton Indian Ltd, Bangalore) and water *ad libitum* as per the guidelines of Institute Animal Ethics Committee (IAEC). IAEC is approved by National Committee for the Purpose of Control and Supervision of Experiments on Animals. (Approval No: B-31/03/2010/ ppd-7 dated 31-03-2010).

Plant extraction

The aerial parts of the plant were collected, cleaned, dried and powdered. To prepare water extract, the powder was extracted with distilled water (5 g/100 ml) by stirring for 4 h and then filtering through filter paper (Watman No. 1). This process was repeated thrice with the residue. The combined filtrate was freeze dried in a lyophilizer. The alcoholic and n-hexane extract of the aerial part of the plant powder was prepared similarly using ethyl alcohol and n-hexane respectively. The process was repeated three times. The alcohol and hexane extracts were dried using a rotary evaporator under reduced pressure at 40°C.[11]

Isolation of an AF

The water extract of the plant was precipitated with ethyl alcohol (1:1) and separated into precipitate fraction and alcohol soluble fraction; both of the fractions were tested for anti-hyperglycemic activity using glucose tolerance test (GTT). The AF was subjected to mechanism action studies.

GTT

This was done as described elsewhere.[12] Rats were divided into indicated number of groups. Control group received the vehicle (water, 1 ml, p.o). The experimental groups received indicated doses of the AF in an identical manner. The rats of all groups were loaded with 60% glucose (3 g/kg, p.o) 30 min after herbal drug (AF) administration. Blood samples were collected by retro-orbital puncture just 1 min prior to drug administration and at 30 and 90 after glucose loading. Serum glucose levels were measured immediately. Six normal fed animals were used in each group.

Effect of AF on glucose uptake by isolated rat hemidiaphragm

Glucose uptake by isolated rat hemidiaphragm was estimated as described.[13] Briefly, 15 overnight fasted male Wistar rats weighing 150-175 g were sacrificed by cervical dislocation. The diaphragms were dissected out, washed in PBS and divided into two equal halves. Weight of each hemidiaphragm was taken and grouped in to 5 of 6 hemidiaphragms each. Sterile, graduated test tubes (20 ml) were grouped in to six sets of six each. The first set (control group) of test tubes was taken with 2 ml of Tyrode's solution with 2% (w/v) glucose. Second group of test tubes was taken with 2 ml of Tyrode's solution with 2% glucose and 10 μg/ml of the AF of *A. farnesiana*. The third set of test tubes was taken with 2 ml of Tyrode's solution with 2% glucose and 20 μg/ml of AF. The fourth set of the test tube was taken with 2 ml of Tyrode's solution with 2% glucose and 40 μg/ml of AF. The fifth set of the test tube was taken with 2 ml of Tyrode's solution with 2% glucose and 20 μg/ml of AF and 0.4 IU of insulin. The sixth set of the test tube was the insulin (0.4 IU) control group. The volumes in all test tubes were made up to 4 ml with Tyrode's solution without glucose. The isolated rat hemidiaphragms were immediately immersed in the respective solutions in each test tube of all six groups. Two hemidiaphragms from the same animal were not used for the same set of experiments. All the test tubes were incubated for 30 min at 37°C in an atmosphere of 100% oxygen with shaking at 140 cycles/min. Glucose uptake per gram of hemidiaphragm was calculated as the difference between the initial and final glucose content in the incubated medium.

Determination of the effect of AF on the release of insulin from rat pancreatic β-cells

Isolation of rat pancreatic β-cell islets and insulin release assay with or without the AF were carried out as per the method.[14] The islets cultured for 48 h in RPMI-1640 medium supplemented with 10% fetal calf serum were checked for viability by Trypan blue exclusion method.

Insulin release assay

Ten cultured viable β-cell islets were placed in each of the well in a 24 well plate in such a manner to get triplicate of four groups. All these wells contained 1 mL of KRBH (pH-7.4), supplemented with 2.75 mmol/L of glucose. The islets were pre-incubated for 1 h at 37°C in a CO_2 incubator, gassed with 5% CO_2 in the air and supernatants were separated. After pre-incubation, the four groups were incubated again for 1 h with 1 mL of KRBH with or without glucose and AF. The first group (control) of β-cell islets was incubated in 1 mL of KRBH. The second group (glucose control group) was incubated in 1 mL of KRBH with 16.5 mmol/L glucose. Third and fourth groups were incubated with AF and 16.5 mmol/L glucose. After incubation, the islets were spun down and supernatants were collected. The insulin released in the supernatant was measured by radio-immuno-assay method.[15]

Glucose tolerance in normal rats: Effect of intra-peritoneal versus oral administration of the herbal drug

To compare the efficacy of oral administration of the drug with that of i. p. administration, male rats (200-240 g) were divided into 4 groups of 6 rats in each group. Control groups (Groups 1 and 2) received 1 ml of water i.p. and oral respectively; and experimental groups(Groups 2 and 3) received i. p. and oral administration respectively of 1 ml AF (25 mg/kg). After 30 min, the rats were orally loaded with glucose and glucose tolerance was determined as above.

GTT in intra peritoneal glucose loaded rats

To investigate tolerance to intraperitoneal glucose loading, rats were divided into 2 groups of 6 each. Control group received 1 ml of water and the experimental group received orally 25 mg/kg AF. After 30 min oral AF administration, rats of both groups were loaded with 60% glucose (3 g/kg, p.o.) by intra peritoneal injection. Blood samples were collected at 0, 60 and 90 min after AF administration and glucose levels were measured.

Statistical analysis

Statistical comparisons were performed using one-way analysis of variance followed by Dunnetts' test. $P < 0.05$ were considered to be significant.

RESULTS

When different extracts of the plant were tested for their glucose lowering effects, the water extract at a dose of 50 mg/kg showed significant glucose lowering activity at 30 and 90 min after glucose loading in normal fed rats. The alcohol and hexane extracts of these plants were almost inactive [Table 1]. The yield of precipitate and soluble fractions of the water extract obtained by alcohol precipitation was approximately 48 and 52%, respectively. The glucose lowering effects of the fractions are given in [Table 2]. The soluble fraction showed significant glucose lowering activity in normal glucose loaded rats at a dose of 25 mg/kg. The precipitate fraction was devoid of the activity. As shown in [Figure 1] under tissue culture conditions, in the isolated rat hemidiaphragm, glucose uptake was increased by the AF treatment at 40 μg/ml concentration. Under this *in vitro* condition, insulin (0.1 IU/ml) also stimulated glucose uptake by the hemidiaphragm. When the herbal drug (20 μg/ml) and insulin (0.1 IU/ml) were added together, to a large extent, the effect of insulin was observed on glucose uptake. As shown in [Figure 2], glucose markedly stimulated insulin release into the medium from cultured pancreatic β-cell islets. The AF did not influence the insulin release as studied both in the presence or absence of high level of glucose level. As shown in [Table 3], in GTT, intraperitoneal as well as oral route of AF administration showed almost the same level of anti-hyperglycemic activity in orally glucose loaded rats. As shown in [Table 4], AF did not influence significantly the levels of serum glucose in intra-peritoneal glucose loaded rats, in contrast to oral administration.

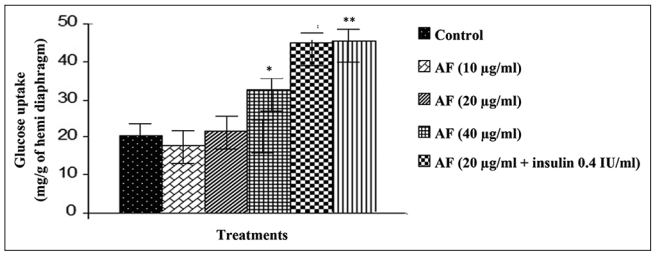

Figure 1: Effect of active fraction of Acacia farnesiana on glucose uptake in isolated rat hemidiaphragm. Values are mean±standard deviation, Values marked with ostrich are significantly different from control values, $^*P \geq 0.05$, $^{**}P \geq 0.001$

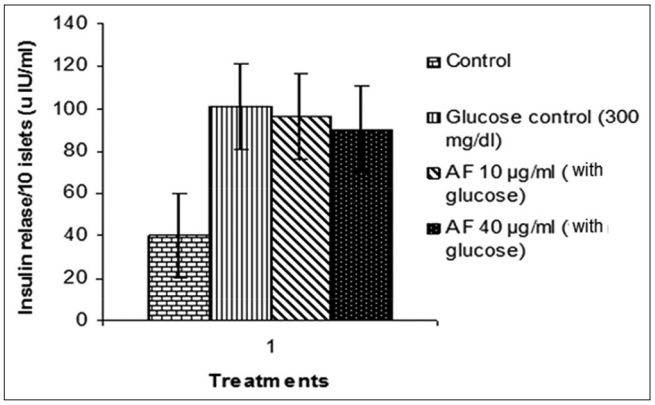

Figure 2: Effect of active fraction of Acacia farnesiana on insulin release from isolated and cultured pancreatic β-cell islets. Values are mean±standard deviation; $n = 3$; insulin values in glucose added groups are significantly different compared to control, $P < 0.001$

Table 1: Effect of different extracts of *Acacia farnesiana* on glucose tolerance in fed and glucose loaded normal rats

Treatment	Serum glucose levels (mmol/l)		
	0 min	30 min	90 min
Control	6.13±0.30	9.85±0.43	6.68±0.23
Water extract (50 mg/kg)	6.19±0.27	6.55±0.29**	6.94±0.19*
Alcohol extract (50 mg/kg)	6.23±0.26	9.41±0.48	7.76±0.44
Hexane extract (50 mg/kg)	6.39±0.33	9.35±0.60	7.80±0.36

Values are mean±SD; *n*=6; ***P*<0.001, ***P*<0.05 (compared to respective control values, Student's *t* test); SD: Standard deviation

Table 2: Effect of the two fractions of the aqueous extract of *Acacia farnesiana* on glucose tolerance in fed and glucose loaded normal rats

Treatment	Serum glucose levels (mmol/l)		
	0 min	30 min	90 min
Control	5.94±0.30	9.57±0.50	7.09±0.29
Water extract (50 mg/kg)	5.07±0.26	6.16±0.05**	5.40±0.24*
Precipitatefraction (25 mg/kg)	5.95±0.34	9.31±0.40	7.17±0.29
Soluble fraction (25 mg/kg)	5.12±0.24	6.03±0.37**	5.45±0.26*

Values are mean±SD; *n*=6; ***P*<0.001, ***P*<0.05 (compared to respective control values, Student's *t* test); SD: Standard deviation

Table 3: Effect of different routes of administration of active fraction of *Acacia farnesiana* on glucose tolerance in glucose loaded normal rats

Treatment	Serum glucose levels (mmol/l)		
	0 (initial)	30	90
Control (oral)	5.31±0.30	9.29±0.50	7.17±0.22
Control (i.p.)	5.36±0.20	9.26±0.46	7.04±0.26
Active fraction (25 mg/kg) (Oral)	5.49±0.26	6.36±0.8**	6.05±0.5*
Active fraction (25 mg/kg) (i.p)	5.43±0.25	6.29±0.47**	5.86±0.26**

Values are mean±SD; *n*=6; ***P*<0.001, **P*<0.05 (compared to respective control values, Student's *t* test); SD: Standard deviation

Table 4: Effect of active fraction of *Acacia farnesiana* on serum glucose levels in intra-peritonially glucose loaded normal, rats

Treatment	Serum glucose levels (mmol/l)		
	0 (initial)	30	90
Control	5.30±0.31	9.22±0.52	6.97±0.29
Active fraction (25 mg/kg) (Oral)	5.28±0.28	8.42±0.59	6.55±0.39

Values are mean±SD; *n*=6; Values are not significantly different from respective control values (Student's *t* test); SD: Standard deviation

DISCUSSION

The AF increased glucose uptake by isolated hemidiaphragm at a relatively higher concentration (40 μg/ml). These results suggest a direct stimulatory effect of the fraction on glucose uptake without the involvement of insulin.[16] This may be the major mechanism of action of this drug.

Unlike sulfonyl urea drugs the AF did not influence the insulin release from cultured pancreatic islet cells. In contrast to oral administration of glucose, AF did not substantially influence serum glucose levels, when glucose was loaded through intraperitoneal route to normal rats. Thus, it appears that glucose absorption from the intestinal tract is inhibited by the AF. It can be assumed that the herbal drug directly or indirectly inhibits to a large extent glucose absorption/transport from the intestine.[17] In the fasted rats, the AF did not significantly influence serum glucose levels even in orally glucose loaded rats. Thus, interestingly, the herbal drug showed anti-hyperglycemic effect, not hypoglycemic activity. Since the AF was found to be effective in orally glucose loaded, fed rats, not fasted rats, in GTT, in fed rats the drug may be stimulating the release of one or more known or unknown factors from the gastrointestinal tract, which may block absorption/transport of glucose directly or indirectly from the intestinal tract.[18]

CONCLUSION

The AF of *A. farnesiana* is an attractive material for further studies leading to the likely development of safe phytomedicine or conventional medicine for diabetes.

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