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Anti-Hyperglycemic Screening of Ethno-Medicinal Plants

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Abstract: Extracts of plant parts of *Stereospermum tetragonum, Acacia farnesiana, Acacia chundra, Symplocos spicata* was investigated for anti-hyperglycemic activity. Evaluation of anti-hyperglycemic property of *Stereospermum tetragonum,Acacia farnesiana, Acacia chundra, Symplocos spicata.* The plant parts of *Stereospermum tetragonum, Acacia farnesiana, Symplocos spicata* and *Acacia chundra* were collected, extracted and evaluated for their anti –hyperglycemic activity. Statistical comparisons were done using one-way analysis of variance followed by Dunnetts' test. P values < 0.05 were considered significant. Wister albino rats in fed and fasting state after administration of plant extracts were tested by Glucose tolerance test (GTT) for anti-hyperglycemic activity. *Stereospermum tetragonum* showed promising anti-hyperglycemic activity in fed rats. *Acacia farnesiana* and *Acacia chundra* showed the activity in fed as well as fasted rats. *Symplocos spicata* was devoid of any such activity. The results of the present study suggest that *Stereospermum tetragonum* showed promising anti hyperglycemic activity and this appears to be of interest and is likely to be safe in the treatment of diabetes mellitus.
Keywords: *Stereospermum tetragonum Acacia farnesiana, Acacia chundra, Symplocos spicata,* Anti-hyperglycemic activity.

Introduction

Type 2 diabetes mellitus is the most common type of diabetes mellitus which is managed by a combination of diet, exercise, oral hypoglycemic drugs and sometimes insulin injections. Synthetic oral hypoglycemic drugs which are currently the main form of treatment for type 2 diabetes mellitus has undesirable side effects and high secondary failure rates. [1], [2] In addition, these drugs cannot be afforded by people living in rural communities because of their high cost. [1] These limitations in currently available antidiabetic pharmacological agents have prompted researchers all over the world to investigate alternative antidiabetic remedies. Consideration is given to plants and herbs used by traditional healers and herbalists as antidiabetic remedies with the hope of discovering new natural products that can be used or developed into safe, inexpensive and effective antidiabetic remedies. In this context, a number of medicinal plants and herbs have been studied and validated for their anti-hyperglycemic potential using experimental animal models of diabetes.

[3] [4] as well clinical studies involving diabetic patients. [5], [6] In addition, bioactive compounds of most of these plants have been isolated and identified. [7], [8] However, mechanism of action of these plants and their products in their blood glucose lowering effects on tissue or organs remain unknown. In remote villages and tribal areas of India numerous medical plants are used for various ailments. These are not known to the mainstream population. Further, they are not scientifically verified. Ethno-medical exploration and follow up scientific studies could bring to light many valuable medicinal plants for drug development. In this context, an exploration was carried out in selected villages of Tamil Nadu to understand plants used in folklore medicine to treat diabetes mellitus (DM). The following 4 plants *Stereospermum tetragonum* (root), *Aacacia farnesiana* (aerial part), *Acacia chundra* (heart wood) and *Symplocos spicata* (stem bark) used by local traditional healers to treat diabetes mellitus were identified in the exploration. These plants were screened for their anti-diabetic activity using glucose tolerance test (GTT) in rats.

Materials and Methods

Plant material

The plant material was collected from the remote villages of Thirunelveli, Dindigul, Virudunagar and Thirupur districts in Tamilnadu, India.The plant identification was confirmed by Tropical Botanic Garden and Research Institute (TBGRI) and a voucher specimen TBGRI 8282, 8283, 8284 and 8285 is deposited in the herbarium of TBGRI.

Extraction:

Root of *Stereospermum tetragonum*, aerial part of *Acacia farnesiana*, heart wood of *Acacia chundra* and stem bark of *Symplocos spicata* were cut into small pieces and air dried. The dried parts were powdered and 10 % w/v water suspension was prepared by using 2% gum acacia. Water suspension of these plants was used for initial screening. Solvent extract was extracted with distilled water (5 g/100 ml) by stirring for 4 hours and then filtered through filter paper (Watman No.1) The alcoholic and n-hexane extract of the plant powder was prepared using ethyl alcohol and n-hexane and the alcohol and hexane extracts were dried using a rotary evaporator under reduced pressure at 40°C. [9] This process was repeated thrice with the residue. The combined filtrate was freeze dried in a lyophilizer.

Animals

Wister albino rats reared in the animal house of TBGRI were used for the present study. The animals were fed with standard rodent pellets (Lipton India Ltd. Bangalore) and water *ad libitum.* The animals were maintained under the standard laboratory conditions (temperature 24-28°C, relative humidity 60-80 and 12 hours dark-light cycle). TBGRI animal house and breeding facilities are registered with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Government of India. CPCSEA guidelines were followed and approval was obtained from Institute Animal Ethics Committee (IAEC) for this study. IAEC is approved by CPCSEA (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). Animals described as fasted were deprived of food for 12 hours but had free access to water.

Glucose tolerance test (GTT)

GTT was used to investigate the glucose lowering effect of the suspension and extracts. GTT was done in fed as well as fasted rats. Normal rats were divided into 2 groups. Control group received the vehicle (2% gum acacia, 1 ml/ rat, p.o.). The experimental group received the extract. In the initial phase, a relative high dose (500 mg/kg) of the water suspension of plant powder in 2% gum acacia was used. The rats of all the groups were loaded with 60% glucose (3 g/kg, p.o.), 30 min after herbal drug or vehicle (control) administration. Blood samples were collected by retro-orbital puncture under local anesthesia just 1minute prior to herbal drug administration and at 30 and 90 min after glucose loading. Serum glucose levels were measured immediately. [10]

Statistical analysis

Statistical comparisons were done using one-way analysis of variance (ANOVA) followed by Dunnetts' test. p value < 0.05 was considered significant.

Results

When two relatively high doses (250 and 500 mg/kg) of the water suspension of the four plants were tested in overnight fasted normal rats by GTT, two of the plants (*A. farnesiana* aerial part and *A. chundra* heart wood) showed slightly varying levels of blood glucose lowering activity. The other 2 plants (*S. tetragonum and S. spicata*) did not exhibit this property [Table 1]. Interestingly, when GTT was performed in fed rats instead of fasted rats, *S. tetragonum* root showed remarkable anti-hyperglycemic activity. *S. spicata* was not active in fed rats also [Table 2]. When different extracts of *S. tetragonum* root was tested in fed rats, water extract (50 mg/kg) was found to be the active extract [Table 3]. As given in [Table 4]. *A. farnesiana* aerial part, the water extract (50 mg/kg) was found to be active. However, in the case of *A. chundra* heart wood, the n-hexane extract (20 mg/kg) exhibited glucose lowering activity [Table 5]. Since the water extract of *S. tetragonum* root showed promising anti-hyperglycemic activity in fed rats, the water extract of root and aerial parts of this plant was compared for their glucose lowering effect. As shown in [Table 6], the root part was found to be active than aerial part in lowering the blood glucose level.

Table 1: Effect of water suspensions of selected plants on glucose tolerance in fasted and glucose loaded normal rats

Serum glucose levels (mmol/dl)

Treatment	Time (min) after glucose administration.		
	0 (initial)	30	90
Control (2% gum acacia)	3.07± 0.18	6.38 ± 0.33	3.95 ± 0.23
A. farnesiana (250 mg/kg)	3.20 ± 0.19	4.96± 0.24* *	4.17± 0.26
A. farnesiana (500 mg/kg)	3.00 ± 0.17	4.74± 0.23* *	3.86± 0.21
A. chundra (250 mg/kg)	3.11 ± 0.16	4.64± 0.22* *	3.85± 0.27
A. chundra (500 mg/kg)	3.01 ± 0.16	4.09± 0.26* *	3.40± 0.19*
S. tetragonum (250 mg/kg)	3.05± 0.20	6.17 ± 0.34	4.18 ± 0.22
S. tetragonum (500 mg/kg)	3.00± 0.21	6.14 ± 0.31	4.10 ± 0.24
S. spicata (250 mg/kg)	3.17± 0.19	6.09 ± 0.31	4.19 ± 0.26
S. spicata (500 mg/kg)	3.18 ± 0.16	6.26 ± 0.22	3.90 ± 0.23

Values are mean ± S.D.; n=6; **, P<0.001, *, P<0.05 (compared to control values, Student's *t* test)

Table 2: Effect of water suspension of *S. tetragonum* (root) and *S. spicata* (stem bark) on glucose tolerance in fed and glucose loaded normal rats

	Serum glucose levels (mmol/dl)		
Treatment	Time (min) after glucose administration.		
	0 (initial)	30	90
Control (2% gum acacia)	4.76 ± 0.24	9.67± 0.40	7.05± 0.31
S. tetragonum (250 mg/kg)	4.87± 0.25	7.46 ± 0.27*	5.90± 0.27*
S. tetragonum (500 mg/kg)	4.86± 0.29	5.96 ± 0.26*	5.35± 0.25*
S. spicata (250 mg/kg)	4.72± 0.25	8.97± 0.40	6.85± 0.31
S. spicata (500 mg/kg)	4.61± 0.25	9.00± 0.40	7.10± 0.31

Values are mean ± S.D.; n=6; *, P<0.001 (compared to control values, Student's *t* test)

Table 3: Effect of different extracts of *S. tetragonam* root on glucose tolerance in fed and glucose loaded normal rats

	Serum glucose levels (mmol/l)		
Treatment	Time (min) after glucose administration.		
	0 (initial)	30	90
Control	5.28± 0.23	9.56±0. 41	7.00 ± 0.24
Water extract (50 mg/kg)	5.20 ± 0.27	6.04± 0.27**	6.46± 0.19*
Alcohol extract (50 mg/kg)	5.02 ± 0.24	8.73± 0.54	6.79 ± 0.26
Hexane extract (25 mg/kg)	5.15 ± 0.30	8.79± 0.66	7.11± 0.27

Values are mean ± S.D.; n=6; **, P<0.001, *, P<0.05 (compared to control values, Student's *t* test)

[The yields of water, alcohol and h-hexane extract, for 500 mg root powder, were 45, 25, 10 mg respectively. Therefore a lower dose was taken in the case of hexane extract]

Table 4: Effect of different extract of *A. farnesiana* (aerial part) on glucose tolerance in fed and glucose loaded normal rats

Serum glucose levels (mmol/l)

Treatment	Time (min) after glucose administration.		
	0 (initial)	30	90
Control	5.13± 0.30	9.85± 0.43	7.68± 0.23
Water extract (50 mg/kg)	5.19± 0.27	6.55± 0.29* *	6.94± 0.19*
Alcohol extract (50 mg/kg)	5.23± 0.26	9.41± 0.48	7.76± 0.44
Hexane extract (25 mg/kg)	5.39 ± 0.33	9.35± 0.60	7.80± 0.36

Values are mean ± S.D.; n=6; **, P<0.001, *, P<0.05 (compared to control values, Student's t test)

Table 5: Effect of different extracts of *A. chundra* (heart wood) on glucose tolerance in fed and glucose loaded normal rats

Serum glucose levels (mmol/l)

Treatment	Time (min) after glucose administration.		
	0 (initial)	30	90
Control	4.79 ± 0.27	8.99 ± 0.42	7.04 ± 0.25
Water extract 40 mg/kg	4.66 ± 0.30	8.35 ± 5.9	6.68 ± 0.29
Alcohol extract 40 mg/kg	4.69 ± 4.4	8.18 ± 0.55	6.80 ± 0.32
Hexane extract 20 mg/kg	4.59 ± 0.30	4.91 ± 0.49* *	5.67 ± 0.27*

Values are mean ± S.D.; n=6; **, P<0.001, *, P<0.05 (compared to respective control values, Student's t test)

Table 6: Effect of water extract of root and aerial part of *S. tetragonum* on glucose tolerance in fed and glucose loaded normal rats

Serum glucose levels (mmol/l)

Treatment	Time (min) after glucose administration.		
	0 (initial)	30	90
Control	5.13 ± 0.28	9.78± 0.50	7.06± 3.64
Root extract (50 mg/kg)	5.24 ± 0.27	6.49± 0.27* *	6.73±0.20*
Aerial part extract (50 mg/kg)	5.04 ± 0.32	7.57 ± 0.33* *	6.90 ± 0.39

Values are mean ± S.D.; n=6; **, P<0.001, *, P<0.05 (compared to respective control Values, Student's *t* test)

Discussion

Although all the four plants selected were used in folklore medicine to control DM, the anti-hyperglycemic property was found in three plants only. *S. spicata* stem bark was devoid of anti-hyperglycemic property in both fed and fasted rats. However, the possibility of having beneficial effects to diabetic patients after repeated dose administration or short term treatment remains to be studied. The water extract of *S. tetragonum* and *A. farnesiana* exhibited almost the same level of anti-hyperglycemic activity. However, *S. tetragonum* appeared to be slightly better than *A. farnesiana* because, *S. tetragonum* extract showed anti-hyperglycemic activity in fed rats and not in fasted rats. This appears to be of interest and it is likely to be safe even in accidental over dose. In the case of *A. chundra* the activity was found in n-hexane extract. Thus the active principle of this plant was not soluble in water and alcohol. This could be a hindrance in the fixation of correct doses for oral administration, bioavailability and is likely to produce hypo-glycemic activity at high doses. In view of the above points *S. tetragonum* is a potential antidiabetic plant for the treatment of diabetes.

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