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Antidiabetic activity of *Smilax china* roots in alloxan-induced diabetic rats

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Abstract: A study of ancient literature indicates that diabetes was fairly well known and well conceived as an entity in India. Plant-based drugs have been used against various diseases since a long time. The nature has provided abundant plant wealth for all the living creatures, which possess medicinal virtues. The essential values of some plants have long been published, but a large number of them have remained unexplored to date. Therefore, there is a necessity to explore their uses and to conduct pharmacological studies to ascertain their therapeutic properties. In fact, nowadays, diabetes is a global problem. Hence, the present study aims to open new avenues for the improvement of medicinal uses of *Smilax china* (Liliaceae) for the selected area for diabetes. Another important objective of such a study is to bring the anti-diabetic medicinal plants sector on a firm scientific footing, raise awareness and add value to the resource. Dried aqueous, alcoholic, and petroleum ether (60-80°C) extracts of roots of *Smilax china* were subjected for hypoglycaemic activity in Wistar rats (150-200 g). Blood sugar level was determined using digital glucometer. The oral administration of root extracts at doses of 200 mg kg^{-1} lead to a significant blood glucose reduction. This laid the foundation to study the active compounds of such anti-diabetic plants that are responsible for the hypoglycemic activities.

Keywords: Alloxan-induced diabetes, hypoglycaemic activity, *Smilax china.*

Introduction:

A study of ancient literature indicates that diabetes was fairly well known and well conceived as an entity in India. The knowledge of the system of diabetes mellitus, as the history reveals, existed with the Indians since prehistoric age. 'Madhumeha' is a disease in which a patient passes sweet urine and exhibits sweetness all over the body, i.e., in sweat, mucus, breathe, blood, etc. The practical usage of juices of various plants achieved the lowering of blood glucose by 10-20%. [1] Diabetes mellitus occurs throughout the world; however, it is more common in the more developed countries. Diabetes is in the top 10, perhaps in the top 5, of the most significant diseases in the developed world and is still gaining significance.[2] Therefore, it is advised to allow such remedial measures as supplements to other modes of therapy. Plant-based drugs have been used against various diseases since long time. The primitive man used herbs as therapeutic agents and medicament, which they were able to procure easily. The nature has provided abundant plant wealth for all living creatures, which possess medicinal virtues. The essential values of some plants have long been published; however, a large number of them remain unexplored as yet. Therefore, there is a necessity to explore their uses and pharmacological studies to ascertain their therapeutic properties. In fact, nowadays, diabetes is a global problem. The present study aims to open new avenues for the improvement of medicinal uses of *Smilax china* (Liliaceae) for diabetes. Another important objective is to bring the anti-diabetic medicinal plants sector on a firm scientific footing, raise awareness, add value to the resource and contribute to the socio-economic well being of our country particularly on the national and international levels.

Materials and Methods:

Smilax China (Liliaceae) is distributed throughout the tropic and sub tropic parts of the world. Some pharmacological activities of *Smilax* spp. rhizome have been studied. Oral administration of the extract from *S. sarsaparilla* at the dose of 500 mg/kg reduced the paw edema induced by carrageenan in rats[3]. The methanol extract of rhizomes of *S. glabra* (100mg/kg, i.p.) reduced the blood glucose of normalmice and KK-Ay mice[4] .The aqueous extract (400, 800 mg/kg, p.o.) from rhizome of *S. glabra* inhibited the swelling of the adjuvant arthritis in rats[5].The ethyl acetate, butanol and aqueous extracted fractions from *S. china* root showed high levels of DPPH free radical scavenging activity[6] . The decoction of *S. china* (90 and 180 mg/kg, p.o.) could significantly inhibit inflammatory swelling on adjunctive arthritis mouse[7].

Plant:

The roots of *Smilax China* for the proposed study was purchased from a commercial source, at Visakhapatnam, and was authenticated by Professor K. Venkiah, Department of Botany , Andhra university, Visakhapatnam. A voucher specimen has been deposited at the museum of our college. After collection the roots were washed thoroughly under running tap water, cut into pieces, shade dried at room temperature (24-26^{0}C) and ground into a coarse powder. The powdered roots was extracted by using ethanol in soxhlet apparatus (Yield 14.52%). The preliminary phytochemical screening was carried out and revealed the presence of mainly glycosides, flavanoids, tannins and triterpenoids

Drugs:

Alloxan monohydrate was purchased from Sigma chemicals (St Louis, USA). All other chemicals used for this study were of analytical grade

Animals:

Wistar rats (190-220 g) of either sex were employed in this study. The rats were maintained under standard laboratory conditions at 25 ± 2°C, relative humidity 50 ± 15% and normal photo period [12 h dark/12 h light] were used for the experiment. Commercial pellet diet [Hindustan liver, Kolkotta, India] and water were provided *ad libitum* . The experimental protocol has been approved by the Institutional Animal Ethics committee and by the Regulatory body of the government

Toxicity Studies:

Acute and chronic toxicity studies were carried out.[8] Animals of either sex were fasted for 18 h Then, they were observed for motor reflexes for 48 h. Since no mortality was observed and the behavioural pattern was unaffected, further studies were carried out Table-1 and Table-2. In chronic toxicity studies, 22 animals were used. They were divided into two groups: 6 as control and 16 as test animals. In the test group, a dose of dried extract of *Smilax china* roots of 250 mg/kg was administered daily for a period of 15 days. The body weights were recorded for both the groups at an interval of 5 days. Finally, the haematological parameters were studied in both the groups Table-3 and Table-4.

Experimental Design:

Diabetes was induced using alloxan monohydrate (100 mg/kg). Only alloxanised hyperglycaemic animals were used for further studies. Animals were fasted for 18 h before the experiment and divided into 5 groups (6 animals in each group). The first group (control) received normal saline and the second group received alloxan monohydrate alone. The three test groups received 200 mg/kg of different extracts before the dose of alloxan. All the animals were regularly observed for their general behaviour.

Effects on Blood Glucose Levels:

Dried aqueous, alcoholic, and petroleum ether (60-80°C) extracts of leaves of *Smilax china* (200 mg/kg) were suspended in 1% bentonite and subjected for hypoglycemic activity in Wistar rats (190-220 g). Diabetes was induced by the intravenous administration of alloxan (100 mg/kg) [9-10] after anaesthesia with ethyl ether. Forty-eight hours later, the blood (1 mL) was collected from the orbital sinus into tubes and immediately used for the determination of glucose. Only animals that presented with glycaemic levels equal to or above 200 mg/dL were submitted to treatments, which consisted of a daily administration of extracts of roots of *Smilax china* for 7 days. The oral treatments (by gavage) of all groups were carried out at the same time (in the morning) and and used. In acute toxicity studies, a dose of 250 mg/kg of dried extract were orally administered to 12 mice; additionally, 3 mice were kept as control. under the same conditions. One hour after the last administration, the blood was collected again for blood glucose measurements using a glucometer.

Statistical Analysis:

All the values were expressed as mean ± Standard error mean (SEM). The differences were compared using one-way analysis of variance (ANOVA) followed by Dunnet's t test. *P* values < 0.01 were considered as significant.

Result:

The effect of oral administration of aqueous, alcoholic and petroleum ether extracts of *Smilax china* roots are shown in Table-5. Experimental studies reveals that the aqueous and alcoholic extracts from *Smilax china* roots (200 mg/kg) orally administered for 7 days produced a significant decrease in the blood glucose level in the model of alloxan-induced diabetes in rats. Petroleum extract exhibits a very weak anti-diabetic activity.

Discussion:

Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas. [11-12] *In vitro* studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis. [13-14] The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells.[15-16] Experimental studies revels that the aqueous and alcoholic extracts from *Smilax china* leaves (200 mg/kg) orally administered for 7 days produced a significant decrease in the blood glucose level in the model of alloxan-induced diabetes in rats. Petroleum extract exhibits very weak anti-diabetic activity. It also proves the traditional claim with regard to *Smilax china* for its anti-diabetic activity.

Table-1 acute toxicity studies of extract for 48hrs

Group	Dose(mg)	Extract toxicity(48hrs)					
		motor reflexes			behavioral patterns		
		Aqueous	Alcohol	P.E	Aqueous	Alcohol	P.E
Test	200	Normal	Normal	Normal	Normal	Normal	Normal
Control		Normal	Normal				

P.E=Petroleum ether

Table-2 acute toxicity studies of extract for 72hrs

Group	Dose(mg)	Extract toxicity(72hrs)					
		motor reflexes			behavioral patterns		
		Aqueous	Alcohol	P.E	Aqueous	Alcohol	P.E
Test	200	Normal	Normal	Normal	Normal	Normal	Normal
Control		Normal	Normal				

P.E= Petroleum ether

Table-3 chronic toxicity studies of extract

Time in days	average body weight(Gms)	
	Test	Control
5	23.24	23.25
10	24.13	24.46
15	25.56	25.45

Table-4 Hematological parameters

Parameter	test	control
Bleeding time (min)	3.96±0.08	4.17±0.08
Clotting time(s)	38.78±0.02	39.12±0.06
Total WBC /CC^3	6045±0.01	6077±0.02
Total RBC/CC^3 (Mil)	8.49±0.75	8.74±0.04
Hemoglobin mg/dl	14.50±0.04	15.00±0.01

Mil-million

Table-5 Effect of aqueous, alcohol and petroleum ether extract of *Smilax china* roots on glucose levels

Group	Blood Glucose Level (mg %)	
	Before treatment	After treatment
Control	91.36±0.015	91.36±0.014
Alloxan control	90.89±0.015*	356.85+0.028*
Aqueous extract	91.42±0.014*	98.54±0.018
Alcohol extracts	90.45±0.016*	91.54±0.017
Petroleum ether	92.42±0.011*	284.74±0.135*

n=6 all values are expressed as mean± SEM, $p < 0.01$ was considered statistically significant as compared to 0 hour of their respective group.

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