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Antidiabetic activity of Ethyl acetate and Ethanolic extract of *Scindapsus officinalis* fruit in Alloxan Induced Diabetic rats.

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Abstract: The climber *Scindapsus officinalis*[1] *(Roxb.)* belongs to family Araceae which is known as Anaittippilli in Tamil[2]. In the present study ethyl acetate (EAESOF) and ethanolic (EESOF) extract of *Scindapsus officinalis* fruit were subjected to the phytochemical investigation and evaluated for antidiabetic activity on blood glucose level, lipid profiles and on the body weight in alloxan induced diabetic rats. EAESOF & EESOF (200 mg/kg) and Glibenclamide (10mg/kg) were administered orally in alloxan (120 mg/kg, i.p.) induced diabetic rats. In this antidiabetic study, maximum reduction in blood glucose was observed in EAESOF & EESOF(160.8, 96.7 mg/dl) at the dose of 200 mg/kg on 21st day respectively. The EAESOF & EESOF showed the significant effect ($p<0.005$) in the various biochemical parameters like protein, triglycerides, cholesterol and total lipid levels. EAESOF & EESOF prevented further loss of body weight. EAESOF & EESOF (200 mg/kg) was found to have significant ($p<0.001$) blood glucose lowering effect. Preliminary Phytochemical investigation revealed the presence of alkaloids, flavonoids, saponins and tannins as the major constituents in the ethyl acetate & ethanol extract. These results suggest that EAESOF & EESOF (200 mg/kg) showed antidiabetic activity in alloxan induced diabetic rats.

Key words: *Scindapsus officinalis,* Alloxan, Glibenclamide, Lipidprofiles, Bloodglucose, Antidiabetic activity.

Introduction:

Diabetes mellitus[3] is a group of metabolic diseases characterized by hyperglycemia arising as a result of a relative or absolute deficiency of insulin secretion, resistance to insulin action, or both. Diabetes is an ailment in which the body does not produce or properly use insulin. The cause of diabetes continues to be anonymity, although both genetics and environmental factors such as obesity and lack of exercise ppear to play roles. It is predicted that by 2030, the numbers of people with DM will be more than double (about 366 million) among which India, China and the United States will have the largest figure[4]. The pathogenesis of diabetes mellitus is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides[5]. Several medicinal plants have been used as dietary adjunct[6] and in the treatment of numerous diseases without proper knowledge of their function. The essential value of some plants has long been published and the large numbers of them remain unexplored as yet. One such plant is *Scindapsus officinalis* which consists of secondary metabolites like flavonoids, tannins, glycosides, alkaloids, terpenes, etc. The folk lore claim of *Scindapsus officinalis*[7] fruits are antidiabetic, anthelmintic, aphrodisiac, galactagogue, stimulant, diaphoretic, antidiarrhoeal,

carminative, expectorant, tonic, antiprotozoal, anticancer, sharpening hearing, aphrodisiac, cardiotonic and regulating the bowel and appetite. It is also used in dysentery,asthma, troubles of the throat, rheumatism, asthma, worm infestations, pharyngopathy, helminthiasis and bronchitis. There is scientific report for the evaluation of antidiabetic activity in *Scindapsus officinalis* fruit. Hence, the objective of the present study was designed to investigate the antidiabetic activity of ethyl acetate & ethanol extract of *Scindapsus officinalis* fruit in alloxan induced diabetic rats.

Material and Method

Collection of plant material:

The *Scindapsus officinalis* fruit were collected from the local area collected from the local market of Chennai, Tamil Nadu state, India. They were idnentified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Tambaram, Chennai, Tamil Nadu, the voucher specimen no:Parc/2009/363 has been deposited at the herbarium unit of the Department of Pharmacognosy, velsuniversity, Pallavaram, Chennai.

Preparation of ethylacetate and ethanol extract of *scindapsu sofficinalis*:

The fruit were shade dried and coarsely powdered. About 300 gm powdered drug was extracted successively by cold maceration method with different solvents of increasing polarity i.e. hexane, chloroform, ethyl acetate and 50% ethanol. After 72 hrs of maceration it was filtered. The marc was dried each time before extraction with next solvent. After complete extraction, the extracts were concentrated by distilling off the solvent and then evaporated to dryness on water-bath. Colour of the extracts was observed and percentage yield was calculated on the air-dried basis. The results were expressed: (tab:1)

Table 1. Percentage yield of successive extracts of *Scindapsus officinalis* fruit

Type of Successive Extract	Colour	Percentage Yield
Hexane extract	Brown	1.323%
Chloroform extract	Brown	2.26%
Ethyl acetate extract	Brown	0.38%
50% Ethanolic extract	Brown	2.256%

Chemicals:

Glucometer (Acucheck-Sensor) was purchased from Roche Diagnostics, Mumbai, India Glibenclamide was obtained as gift sample from IPCA Laboratories, Mumbai, India. Alloxan monohydrate was purchased from Sigma, USA. Ethanol was purchased from Ranbaxy Fine Chemicals Ltd., New Delhi, India.

Qualitative chemical tests[10]:

Ethyl acetate and ethanolic extract was tested to know the different constituents present in it by the standard procedures. The extract showed the presence of alkaloids, saponins, flavonoids, and tannins.

Animals:

All the experiments on animal were conducted according to protocols that were approved by the Institutional Animal Ethics Committee (IAEC, Reg. No.290 / CPCSEA / dated 6-10-09) of Vels University. Wister albino rats (150–200 g) of either sex were used for this study. Animals were maintained under standard environmental conditions and had free access to feed and water *ad libitum*. Acute toxicity study was carried out using albino mice.

ACUTE TOXICITY STUDY[11]:

The acute toxicity study was carried out by using Swiss albino mice of either sex, weighing about 25–30g. This study was performed as per OECD (Organization for economic co-operation and development)-423 guidelines. Animals were kept in a temp controlled environment (23 ± 2ºC) at 12 hours light/dark cycle. All the protocols were performed in accordance with Institutional Animal Ethics Committee (IAEC, Reg. No.290 / CPCSEA / dated 6-10-09) of Vels University. It was found that the tolerated dose level is 2000 mg/kg body weight.

Alloxan-induced diabetes[12,13,14]:

The albino rats weighing 150-200 g of either sex were allowed to fast for 24 hours prior to experimentation and rendered diabetic by a single dose of intraperitoneal injection of alloxan 120 mg/kg body weight.15 After 18 hours of injection of alloxan, diabetes was confirmed by testing blood sugar level more than 250 mg/dl were selected for the further study. Animals were maintained for four days in diabetic condition for well establishment of diabetes.

Animal Grouping and drug administration:

They were divided into five groups.

Group 1: Animals as served as a non-diabetic control, received food and distilled water *ad libitum* (10ml/kg body weight/day) orally.

Group 2: Untreated but diabetes induced animals (Alloxan) served as a disease control group.

Group 3: Diabetes induced animals treated with glibenclamide suspended in 2% w/v CMC at the dose 10 mg/kg body weight/day orally.

Group 4: Diabetes induced animals treated with EAESOF suspended in 2% CMC at the dose 200 mg/kg body weight/day orally.

Group 5: Diabetes induced animals treated with EESOF suspended in 2% w/v CMC at the dose 200mg/kg body weight/day orally.

Table 2. Effect of Extracts (EAESOF & EESOF) on Blood glucose level against Alloxan induced Diabetic rats.

Group	Treatment and Dose	Blood-glucose level (mg/dl)			
		Day 1	Day 7	Day 14	Day 21
I	Vehicle control (food and distilled water *ad libitum*, 10ml/kg/day orally)	97.64±0.39	95.66±0.23	93.62±0.46	94.46±0.33
II	(Alloxan suspended in saline, 120mg/kg i.p.)	276.5±0.51a*	303.6±0.3a*	342.2±0.54a*	411.8±0.4a*
III	Diabetic+Standard (Glibenclamide 10mg/kg/day orally)	264.6±0.37b**	201.7±0.28b**	163.8±0.55b**	90.02±0.8b**
IV	Diabetic + EAESOF 200 mg/kg/day orally	259.6±0.54b*	246.9±0.21b*	215.5±0.44b*	160.8±0.5b*
V	Diabetic + EESOF 200 mg/kg/day orally	260.7±0.66b**	204.2±0.32b**	156.3±0.35b**	96.7±0.59b**

Values are expressed as mean ± SEM (n=6). *P<0.05, ** P<0.01
a is used to indicate the significance between Group II VS Group I
b is used to indicate the significance between Group II VS Group III, IV, & V
Data were analyzed by One-way ANOVA followed by Dunnett's t-test

Table 3. Effect of EAESOF and EESOF on Body weight of Alloxan-induced Diabetic rats.

Group	Treatment & Dose	Body weight (g)			
		Day 1	Day 7	Day 14	Day 21
I	Vehicle control (food and distilled water *ad libitum*, 10ml/kg/day orally)	201.50±3.31	202.2±2.31	204.7±2.33	206.8±1.94
II	Diabetic control (Alloxan suspended in saline,120mg/kg i.p.)	206.30±4.88	175.21±7.16a*	162.2±3.54a*	149.79±2.31a*
III	Diabetic+Standard (Glibenclamide 10mg/kg/day orally)	205.66±2.48	196.2±1.48	192.2±1.23	191.7±1.49
IV	Diabetic + EAESOF 200 mg/kg/day orally	206.81±2.31	185.56±0.21	181.18±2.14	179.8±0.31**b***
V	Diabetic + EESOF 200 mg/kg/day orally	205.72±2.33	193.02±2.36	191.28±2.41	189.21±1.48**b***

Values are expressed as mean ± SEM (n=6); *P<0.05
* is used to indicate the significance , a is used to indicate the significance between Group II VS Group I
b is used to indicate the significance between Group II VS Group IV & V
Data were analyzed by One-way ANOVA followed by Dunnett's t-test

Table 4. Effect of *Scindapsus officinalis* fruit extracts on biochemical parameters

Group	Treatment & Dose	Parameters at Day 21st			
		Protein (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Total Lipids
I	Vehicle control (food and distilled water *ad libitum*, 10ml/kg/day orally)	2.56±0.07	151.51±1.11	86.85±5.6	143.88±0.59
II	Diabetic control (Alloxan suspended in saline, 120mg/kg i.p.)	0.55±0.02a*	269.32±12.5a*	201.82±9.2a*	285.13±0.34a*
III	Diabetic+Standard (Glibenclamide 10mg/kg/day orally)	1.87±0.02b*	147.81±7.01b*	98.15±4.78b*	146.75±0.42b*
IV	Diabetic + EAESOF 200 mg/kg/day orally	1.52±0.02b*	173.82±4.7b*	127.46±0.48b*	176.93±0.66b*
V	Diabetic + EESOF 200 mg/kg/day orally	1.76±0.05b*	156.51±6.7b*	108.33±0.41b*	153.11±0.45b*

Values are expressed as mean ± SEM (n=6); *P<0.05
* is used to indicate the significance
a is used to indicate the significance between Group II VS Group I
b is used to indicate the significance between Group II VS Group IV & V
Data were analyzed by One-way ANOVA followed by Dunnett's t-test

Assessment of Antidiabetic Activity:

Effects of consumed extracts on blood-glucose level of rats

The blood samples were collected from the tail vein of the rats and blood glucose levels was estimated at 1st, 7th, 14th and 21st days after extract administration by using one touch basic glucose strips (Johnson & Johnson Ltd., Mumbai). The results were mentioned (table 2).

Effects of consumed extracts on body weight of rats

The body weight of each group was estimated after the 1st, 7th, 14th and 21st day intervals and the findings were mentioned (table 3).

Serum analysis

On the twenty first day of experiment the animals were sacrificed and blood was collected from various groups by puncturing the retro-orbital plexus, kept aside for half an hour for clotting. Serum was separated by centrifuging the blood samples at 6000 rpm for 20 mins and stored in the refrigerator until analyzed. The serum was analyzed for various biochemical parameters such as protein, cholesterol, triglycerides and total lipids. The findings were mentioned (table 4).

***Effects of consumed extracts on histopathology of pancreas* (Histomorphologic Changes of Pancreas)**

The pancreas was removed for identifying histopathological changes. Pancreatic sections stained with hematoxylin and eosin (H & E x40). The sections revealed that alloxan caused severe necrotic changes of pancreatic islets, especially in the centre of islets. Nuclear changes, karyolysis, disappearance of nucleus and in some places residue of destroyed cells were visible. The cellular integrity and architecture were intact in the non-diabetic control group (Figure 1). Relative reduction of size and number of islets especially around the central vessel and severe reduction of beta cells were clearly seen in diabetic control group (Figure 2). Pancreas of the diabetic group III which consumed 10mg/kg body wt Glibenclamide (Fig. 3), showed similarity to group I (Fig. 1). Study of pancreas of treated diabetic groups IV and V showed increased size of islets and hyperchromic nucleus. There was also a relative increase of granulated and normal beta cells in the group V (Fig. 4) which consumed 200mg/kg body wt EESOF, when compared with the diabetic group IV (Fig. 5) which consumed 200mg/kg EAESOF.

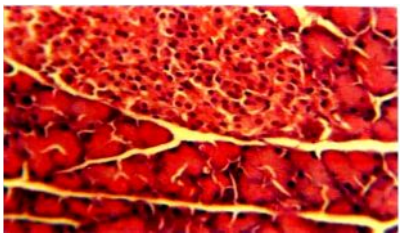

Figure 1.
Normal Pancreas, H&E Staining (40X)
Section shows degeneration of β-cells granules in β-cells.

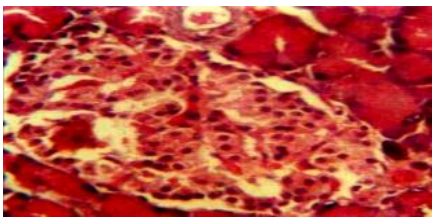

Figure 2.
Diabeteic pancreas H&E Staining (40X)
Section shows normal pancreas with insulin in pancreas

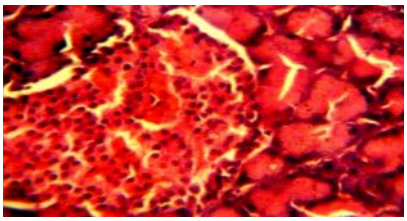

Figure 3.
Pancreas treated with standard (Glibenclamide10mg/kg) H&E Staining (40X)
Section shows pancreas with mild damage

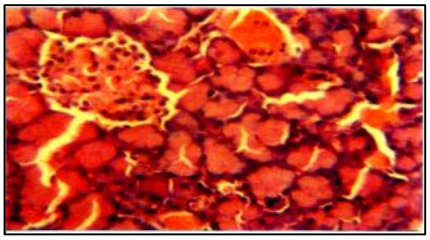

Figure 4.
Pancreas treated with test drug (EAES OF 200mg/kg) H&E Staining (40X)
Section shows increased size of islets.

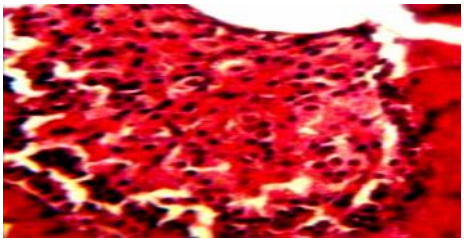

Figure 5.
Pancreas treated with test drug 1 (EESOF 200mg/kg) H&E Staining (40X)
Section shows increase in granulated and normal beta cells

Statistical Analysis:

For *in-vivo* experiments values are represented by mean ± SEM. The mean values are analyzed by one way ANOVA followed by Dunnets test. The $p<0.05$ & $p<0.01$ was considered as statistically significant.

Results:

Anti- diabetic effect of EAESOF&EESOF in Alloxan induced diabetic rats:

In the Anti- diabetic study, repeated administration (once a day for 21 days) of the EAESOF & EESOF as well as Glibenclamide causes significantly ($p<0.001$) reduction in the blood glucose level as compared with diabetic control group. Maximum reduction in blood glucose level was observed (160.8, 96.7 mg/dl respectively) on 21st day in the diabetic rats treated with EAESOF & EESOF at 200mg/kg. Glibenclamide treated animals showed maximum reduction in blood glucose level (90.02 mg/dl) on 21st day (Table 2). Subacute treatment for 14 days with the EAESOF & EESOF in the treated doses brought about improvement in bodyweights, indicating its beneficial effect in preventing loss of body weight in diabetic rats. The ability of EAESOF & EESOF to prevent body weight loss seems to be due to its ability to reduced hyperglycaemia (Table 3). The EAESOF & EESOF showed short onset and short duration of antihyperglycaemic action. Subacute treatment for 21 days with the in the EAESOF & EESOF treated doses brought about improvement in body weights indicating its beneficial effect in preventing loss of body weight in diabetic rat. The EAESOF & EESOF showed the significant effect ($p<0.005$) in the various biochemical parameters like protein, triglycerides, cholesterol and total lipid levels. Flavonoids, alkaloids, tannins and phenolics are known to modulate the activities of various enzymes due to their interaction with various

biomolecules. The fruit of the plant *Scindapsus officinalis* have been reported to contain alkaloids, flavonoids, saponin and tannins. Preliminary phytochemical analysis indicated that, the ethyl acetate and ethanol extract of *Scindapsus officinalis* fruit contain flavonoids, alkaloids, phenolic compound and tannins. The antihyperglycaemic activity of EAESOF & EESOF may probably be due to the presence of several bioactive antidiabetic principles. It is thus apparent that EAESOF & EESOF possesses antihyperglycaemic activity.

Histopatholgy:

The effect of EAESOF & EESOF at 200mg/kg dose on histopathlogical findings on the pancreas shown in plate 1-5. It is observed that diabetogenic agent alloxan produced lesion in the pancreatic islets as viewed by very scanty islets with acinar tissue. Treatment with Glibenclamide has decreased the degree of lesions as indicated by partial intact pancreatic cells with acini. However attenuation of pancreatic degeneration was observed in diabetic animals treated with EAESOF&EESOF 200mg/kg.

Conclusion:

The climber *Scindapsus officinalis* (Roxb.) belongs to family Araceae is known as Anaittippilli in Tamil. The findings of antidiabetic study support the traditional use of *Scindapsus officinalis* fruit for controlling hyperglycemia in diabetics. Further characterization of active principles flavonoids, alkaloids, tannins in Scindapsus and studies are in progress to isolate, identify and characterize such active components.

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