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## Research Article

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### Anti-Obesity Activity of Ethanol Extract of *Saccharum spontaneum*

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#### ABSTRACT

Obesity is of public health concern because of its association with medical complications that lead to increased morbidity and mortality. From time immemorial, traditional medicinal plants had gained importance in modulating obesity. The present study was designed to investigate the anti-obesity effect of ethanol extract of *Saccharum spontaneum*. Anti-obesity effect was investigated in male Wistar strain albino rats. Anti-obesity activity of ethanol extract of *Saccharum spontaneum* was evaluated at 200 and 400 mg/kg bd. wt. (p.o.). Animals were divided into five groups of six animals each. Group I animals received 1% gum acacia and served as normal control. Group II animals received only high fat diet for 40 days and served as negative control. Group III and IV animals received high fat diet for 40 days and treated with lower and higher doses of ethanol extract respectively from day 31 to day 40. Group V animals received high fat diet for 40 days and treated with Orlistat (standard) from day 31 to day 40. Anti-obesity activity was determined by assessing the body weight gain, food intake, lipid levels, glucose levels and organ weights in normal and obese rats. High fat diet induced obesity was characterized by increased food intake, body weight, organ weight increased lipid and glucose levels. Concurrent administration of ethanol extract reversed all the effects induced by high fat diet in dose dependent manner. The results of this study demonstrated that *Saccharum spontaneum* whole plant extract exerted significant anti- obese activity in rats fed with high fat diet.

**Keywords:** High Fat Diet, Obesity, *Saccharum spontaneum*

#### ARTICLE INFO

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## 1. Introduction

Obesity had been one of the leading public health concerns in industrialized and industrializing countries for past several years.[1] Obesity is a leading cause of death worldwide, it is one of the most serious public health problems of the 21st century.[2] It is a global epidemic resulting from sedentary life styles, improved socioeconomic conditions and availability of processed, high calorie foods and soft drinks.[3] The most common complications associated with obesity are Diabetes mellitus,[4] cardiovascular diseases,[5] dyslipidaemia [6] and fatty liver.[7] Many a number of synthetic anti-hyperlipidemic drugs are available for clinical use, but their

use is restricted because of their severe or life threatening side effects [24]. Tribal people of India and world use medicinal plants to treat obesity. *Saccharum spontaneum* (F: Poaceae) is one such plant which is used by tribal people of Andhra Pradesh and Tamilnadu to treat burning sensations, stranguiry, vesicles calculi, biliousness, galactagogue, diuretic and Obesity.[8-11] Till date no systematic study had been carried out on this plant for its anti-obesity activity. Hence present study was planned to evaluate anti-obesity activity of ethanol extract of *Saccharum spontaneum*.

## 2. Materials and Methods

### Plant material

*Saccharum spontaneum* L whole plant was collected from Tirumala hills, Chittor Dt., A.P. and authenticated by Botanist Dr. K. Madhava Chetti, Asst. Professor, Dept. of Botany, S.V. University, Tirupati and voucher specimen was deposited in S.V. University Botany Dept., Tirupati. The whole plant was allowed to dry under shade. The dried plant was powdered in a Wiley mill. 100gm of dry powder was macerated with petroleum ether (60-800c) for 12 hrs and refluxed for 3 hrs and then filtered and subjected to distillation under reduced pressure. The procedure was repeated for three times. Marc obtained was extracted with ethanol by hot extraction method. The procedure was repeated for three times to obtain residue which was used for pharmacological studies.

### Preliminary Phytochemical analysis

The ethanol extract was subjected to preliminary phytochemical screening for the presence of phyto constituents by following the methods mentioned in Harborne.[12]

### Pharmacological studies

#### Experimental animals

Colony inbred strains of male wistar rats weighing 150-180g were used for the pharmacological studies. The animals were kept under standard conditions (day/night rhythm) 8.00 am to 8.00 pm,  $22 \pm 10^\circ\text{C}$  room temperature, in polypropylene cages. The animals were fed on standard pellet diet and tap water *ad libitum*. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions. It was randomly distributed into five different groups with six animals in each group under identical conditions throughout the experiments. The animals were maintained in accordance with CPCSEA guidelines. All the procedures described were reviewed and approved by Institutional Animal Ethical Committee.

#### Acute toxicity studies

The acute oral toxicity studies were carried out following OECD 423 guidelines.[13]

#### Induction of obesity in experimental rats

Obesity was induced by high fat diet administration in rats. High cholesterol diet was prepared by mixing Normal diet, Cholic acid, Cholesterol, Olive oil in a ratio of 95: 0.5: 2: 2.5.[14] This diet was administered for 40 days.

#### Treatment Protocol

**Group I:** Animals received 1% gum acacia and served as normal control.

**Group II:** Animals received only high fat diet for 40 days and served as negative control.

**Group III:** Animals received high fat diet for 40 days and treated with lower dose of ethanol extract of *Saccharum spontaneum* (200mg/kg/body weight) suspended in 1% gum acacia from day 31 to day 40.

**Group IV:** Animals received high fat diet for 40 days and treated with higher dose of ethanol extract of *Saccharum spontaneum* (400mg/kg/body weight) suspended in 1% gum acacia from day 31 to day 40.

**Group V:** Animals received high fat diet for 40 days and treated with Orlistat (30mg/kg/body weight) suspended in 1% gum acacia from day 31 to day 40.

#### In-vivo Pharmacological Evaluation

Animals were observed for

**Body Weight:** Body weight (gm) was recorded every day for 40 days using digital weighing balance.

**Food Intake:** The food intake for group of 6 rats was measured daily for 40 days and expressed as mean daily food intake for each group.

**Biochemical studies :** On day 41 of experiment the animals were sacrificed by cervical decapitation and blood samples were collected by cardiac puncture into sterilized dry centrifugation tubes and allowed to stand for 30 minute at  $37^\circ\text{C}$ . The clear serum was separated at 2500 rpm for 10min using micro centrifuge and biochemical parameters like Blood glucose, Triglycerides, Total cholesterol, High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL), Very Low Density Lipoproteins (VLDL) were estimated.

**Organ weights:** At the end of the experiment organs (Kidney, liver, heart and spleen) of animals were removed and weighed on digital weighing balance.

**Statistical Analysis:** The statistical data was presented as mean  $\pm$  SEM. Parametric data which include all the biochemical parameters were analysed using a paired “t

“test for the paired data followed by one way analysis of variance (ANOVA). A probability value of  $P < 0.01$  was considered as significant.

### 3. Results and Discussion

**Preliminary Phytochemical Studies:** Ethanol extract gave positive tests for the alkaloids, carbohydrates, flavonoids,

amino-acids, tannins, phenols, glycosides and terpenoids (Table-1).

**Table 1:** Chemical tests carried out for ethanol extract of whole plant of *Saccharum spontaneum*”

Chemical constituent	Name of the test	Ethanol Extract
Alkaloids	Dragendorffs test	+Ve
Carbohydrates	Molischs Test	+Ve
Amino acids	Ninhydrin test	+Ve
Steroids	Libermann Burchard test	-Ve
Flavonoids	Shinoda test	+Ve
Tannins	Lead Acetate test	+Ve
Phenol	$\text{FeCl}_3$ Test	+Ve
Saponins	Froath test	-Ve
Glycosides	Borntragers test	+Ve
Terpenoids	Hirschosin reaction	+Ve

#### Pharmacological Studies

**Acute toxicity studies:** Ethanol extract of whole plant of *Saccharum spontaneum* was found to be safe since no

animal died even at the maximum dose of 2000 mg/kg body weight.

#### Effect of ethanol extract of *Saccharum spontaneum* on obesity:

**Table 2:** Effect of ethanol extract of *Saccharum spontaneum* on daily feed intake in rats

Group	Treatment	Feed intake (g)
I	Control	136.33 $\pm$ 1.56
II	HFD control	159.2 $\pm$ 2.38***
III	HFD+Ethanol extract (200 mg/kg bd wt)	144.33 $\pm$ 1.64 <sup>a</sup>
IV	HFD+Ethanol extract (400 mg/kg bd wt)	151.5 $\pm$ 2.75 <sup>cb</sup>
V	HFD+Orlistat (30 mg/kg bd wt )	126.2 $\pm$ 1.49 <sup>d</sup>

Each value represents the mean  $\pm$  SEM of 6 animals in each group

\*\*\* $p < 0.001$  when compared to Group-I

a: $p < 0.001$  when compared to Group-II

b: $p < 0.05$  when compared to Group-II

c: $p < 0.01$  when compared to Group-I

d: $p < 0.001$  when compared to Group-II

**Table 3:** Effect of ethanol extract of *Saccharum spontaneum* on weight gain (g) in rats

Groups	Treatment	Weight on day 1	Weight on day 40	Weight gain
I	Control	155.30 $\pm$ 1.82	167.7 $\pm$ 2.48	12.33 $\pm$ 1.92
II	HFD control	183.30 $\pm$ 3.49***	273.30 $\pm$ 6.66***	90.00 $\pm$ 7.98***
III	HFD+Ethanol extract (200 mg/kg bd wt)	168.3 $\pm$ 3.60*	206.40 $\pm$ 3.94***	38.17 $\pm$ 3.78 <sup>a</sup>
IV	HFD+Ethanol extract (400 mg/kg bd wt)	168.5 $\pm$ 3.21*	202.10 $\pm$ 5.70***	33.67 $\pm$ 3.35 <sup>bc</sup>
V	HFD+Orlistat (30 mg/kg bd wt )	170.00 $\pm$ 1.39**	203.5 $\pm$ 2.31***	33.50 $\pm$ 3.67 <sup>d</sup>

Each value represents the mean  $\pm$  SEM of 6 animals in each group

\*\*\* $p < 0.001$  when compared to Group-I

a: $p < 0.001$  when compared to Group-II

b: $p < 0.001$  when compared to Group-II

c: $p < 0.005$  when compared to Group-I

d:p<0.005 when compared to Group-II

**Table 4:** Effect of ethanol extract of *Saccharum spontaneum* on Serum total Cholesterol, Triglycerides in rats

Group	Treatment	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
I	Control	66.67±1.10	158.6±6.36
II	HFD control	163.2±4.86*	238.2±2.45*
III	HFD+Ethanol extract (200 mg/kg bd wt)	133.2±1.96 <sup>a</sup>	223.0±1.86 <sup>a</sup>
IV	HFD+Ethanol extract (400 mg/kg bd wt)	126.81±2.27 <sup>bc</sup>	200.14±4.01 <sup>bc</sup>
V	HFD+Orlistat (30 mg/kg bd wt)	90.83±1.08 <sup>d</sup>	173.7±2.19 <sup>d</sup>

Each value represents the mean ± SEM of 6 animals in each group

\*p<0.001 when compared to Group-I

a:p<0.001 when compared to Group-II

b:p<0.01 when compared to Group-II

c:p<0.001 when compared to Group-I

d:p<0.001 when compared to Group-II

**Table 5:** Effect of ethanol extract of *Saccharum spontaneum* on Serum HDL, LDL, VLDL Cholesterol in rats

Group	Treatment	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
I	Control	42.19±1.60	24.09±0.93	31.71±1.27
II	HFD control	32.49±1.61*	43.78±1.46*	47.63±0.49*
III	HFD+Ethanol extract (200 mg/kg bd wt)	31.31±0.50 <sup>a</sup>	36.47±0.40 <sup>a</sup>	44.59±0.37 <sup>a</sup>
IV	HFD+Ethanol extract (400 mg/kg bd wt)	36.72±0.98 <sup>bc</sup>	32.23±0.74 <sup>bc</sup>	40.02±0.65 <sup>bc</sup>
V	HFD+Orlistat (30 mg/kg bd wt)	46.76±1.09 <sup>d</sup>	28.36±1.28 <sup>d</sup>	35.19±0.82 <sup>d</sup>

Each value represents the mean ± SEM of 6 animals in each group

\*\*\*p<0.001 when compared to Group-I

a:p<0.001 when compared to Group-II

b:p<0.05 when compared to Group-II

c:p<0.05 when compared to Group-I

d:p<0.001 when compared to Group-II

**Table 6:** Effect of ethanol extract of *Saccharum spontaneum* on serum Blood glucose in rats

Group	Treatment	Blood glucose(mg/dl)
I	Control	60.24±1.79
II	HFD control	90.32±1.03***
III	HFD+Ethanol extract(200 mg/kg bd wt)	87.89±0.56 <sup>a</sup>
IV	HFD+Ethanol extract(400 mg/kg bd wt)	80.74±0.84 <sup>bc</sup>
V	HFD+Orlistat(30 mg/kg bd wt)	72.66±0.88 <sup>d</sup>

Each value represents the mean ± SEM of 6 animals in each group

\*\*\*p<0.001 when compared to Group-I

a:p<0.05 when compared to Group-II

b:p<0.001 when compared to Group-II

c:p<0.001 when compared to Group-I

d:p<0.001 when compared to Group-II

The parameters of group-II (HFD control) animals were compared with group-I animals (normal control) and group-III, IV, V animals were compared with group-II animals. Body weight was increased significantly (p<0.001) in rats

which were fed with high fat diet (HFD) when compared with control group animals, while animals which received ethanol extract exhibited significant reduction of weight gain. Food consumption increased significantly (p<0.001)

in HFD group compared with control. Treatment with ethanol extract of *Saccharum spontaneum* (200 and 400 mg/kg/bd.wt) showed significant ( $p<0.01$ ) decrease in daily food intake as compared with Group-II animals. Total cholesterol ( $p<0.01$ ), serum Triglycerides ( $p<0.001$ ), LDL ( $p<0.001$ ), VLDL ( $p<0.001$ ) were significantly elevated in animals which received only high fat diet, while HDL cholesterol was significantly ( $p<0.001$ ) decreased compared to the group which received normal diet.

Administration of ethanol extract along with high fat diet significantly reversed ( $p<0.001$ ) the effects induced by high fat diet in dose dependent manner. Animals which received high fat diet raised the levels of blood glucose significantly ( $p<0.001$ ) when compared with normal control. This alteration was ameliorated by administration of ethanol extract of *Saccharum spontaneum*. Weights of different organs were represented in Table-7.

**Table 7:** Effect of ethanol extract of *Saccharum spontaneum* on Organ weight (g) in rats

Group	Treatment	Kidney		Heart	Spleen	Liver
		Left	Right			
I	Control	0.68±0.01	0.71±0.04	0.75±0.01	0.76±0.01	7.28±0.05
II	HFD control	1.19±0.01**	1.20±0.03**	1.35±0.01**	1.84±0.02**	11.78±0.06**
III	HFD+Ethanol extract (200 mg/kg bd wt)	0.82±0.01 <sup>a</sup>	0.86±0.02 <sup>a</sup>	0.96±0.03 <sup>a</sup>	1.02±0.01 <sup>a</sup>	7.85±0.01 <sup>a</sup>
IV	HFD+Ethanol extract (400 mg/kg bd wt)	0.79±0.02 <sup>bc</sup>	0.81±0.03 <sup>bc</sup>	0.83±0.01 <sup>bc</sup>	0.87±0.03 <sup>bc</sup>	7.61±0.03 <sup>bc</sup>
V	HFD+Orlistat (30 mg/kg bd wt)	0.72±0.01 <sup>d</sup>	0.74±0.04 <sup>d</sup>	0.76±0.01 <sup>d</sup>	0.88±0.01 <sup>d</sup>	7.29±0.07 <sup>d</sup>

Each value represents the mean ± SEM of 6 animals in each group

\*\* $p<0.01$  when compared to Group-I

a: $p<0.01$  when compared to Group-II

b: $p<0.01$  when compared to Group-II

c: $p<0.01$  when compared to Group-I

d: $p<0.01$  when compared to Group-II

Weights of the internal organs like kidney, heart, spleen and liver were increased significantly ( $p<0.01$ ) in high fat diet animals when compared with normal animals. Treatment with ethanol extract of *Saccharum spontaneum* significantly ( $p<0.01$ ) reduced weight of organs when compared with animals fed with high fat diet. Animals which received Orlistat (Group V) along with the high fat diet significantly ( $p<0.001$ ) reversed all the effects induced by high fat diet.

### Discussion:

The present study was carried out to investigate the effects of ethanol extract of *Saccharum spontaneum* (200 and 400 mg/kg body weight) on body weight gain, food intake, lipid levels, glucose levels and organ weights in normal and obese rats. The current results showed that body weight increased significantly in high fat diet group compared with normal group as high fat diet may facilitate the development of a positive energy balance leading to an increase in visceral fat deposition and abdominal obesity in particular.[15] Decrease in body weights was observed in extract treated groups. This may be attributed to the presence of phytoconstituents in the extract which are useful in the treatment of obesity and hyperglycemia. The observed loss of body weight in obese rats is partly due to reduced food intake caused by *Saccharum spontaneum* as a result of its diuretic effect.[8]

In present study high fat diet resulted in dyslipidemic changes manifested by increased levels of triglycerides,

VLDL, total cholesterol, LDL and decreased levels of high density lipoprotein. Our results are in good agreement with Kamal et.al., 2009.[16] Supplementation of ethanol extract of *Saccharum spontaneum* produced significant decrease in serum triglycerides, VLDL, total cholesterol and low density lipoproteins while there was a significant increase in HDL cholesterol in obese rats.[17] The reduction of plasma LDL cholesterol, VLDL, triglycerides and increased levels of HDL is believed to be associated with the anti-oxidant principles such as tannins, terpenoids, flavonoids present in *Saccharum spontaneum*. [18,19] Our results showed that high fat diet exhibited significant increase in serum glucose, which parallels the results obtained from Uday Sasi Kiran et.al.[20] Diminished hepatic and muscular uptake of glucose produced hyperlipidemia due to increased fat mobilization from adipose tissue and resistance to the anti-lipolytic actions of insulin. Impaired insulin action is associated with an oversupply of lipids. Our findings indicated a significant decrease of serum blood glucose levels upon treatment with ethanol extract of *Saccharum spontaneum* at 200 and 400 mg/kg bd.wt. and these findings correlated with the results of González-Ortiz et al.,[21] Kamal A Amin et al.,[16] Tomoko Akase et al.,[22] and Tsutomu Shimada et al.[23]

### Organ weights

In present study animals which received HFD showed increase in weight of internal organs which may be because of accumulation of fat in different organs. Treatment with ethanol extract of *Saccharum spontaneum* significantly

decreased the weights of different internal organs

suggesting reduction in adipose tissue formation.

#### 4. Conclusion

In conclusion, the results of this study demonstrated that *Saccharum spontaneum* whole plant extract exerted significant anti-obese activity in rats fed with high fat diet.

Our results clearly suggest that anti-obesity activity of *Saccharum spontaneum* may be due to its hypophagic, hypoglycaemic, and hypolipidemic properties.

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