

ORIGINAL

Saccharum spontaneum: for protection against complications associated with high fat diet induced obesity and hyperlipidemia in experimental rodents

Saccharum spontaneum: para la protección contra las complicaciones asociadas con la obesidad y la hiperlipidemia inducidas por una dieta rica en grasas en roedores experimentales

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ABSTRACT

Introduction: *Saccharum spontaneum* Linn. (Family: Poaceae) known as Kasa or wild sugar cane. It is considered as a precious remedial herb in traditional systems of medicine in India. The aim of this study was to determine the antiobesity and antihyperlipidemic activity of hydro-alcoholic root extract of *Saccharum spontaneum* (SPRE) in high fat diet (HFD) induced obese rats.

Method: the female Sprague-Dawley (SD) rats were divided into six groups ($n = 6$). SPRE at a dose of 100, 200, 400 mg/kg, p.o. and Sibutramine (5 mg/kg, p.o.) as reference drug was administered daily using animal feeding needles for 60 days. The experimental animals were allocated into normal control group (Group I) administered with regular lab. diet, control obese group (Group II) induced with HFD, reference drug sibutramine plus HFD group (Group III) and SPRE (100, 200 and 400 mg/kg) plus HFD group (Group IV, V and VI respectively). Its effect on body weight, organ fat pad, serum lipid profile (TC, HDL, TG, LDL, and VLDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN) and creatinine level were estimated in HFD induced obese rats.

Results: the administration of SPRE (200 and 400 mg/kg) supplemented with HFD showed controlled body weight and organ fat pad increment, and reduced the TC, LDL, VLDL, TG, and increased the HDL levels as well as reduced the AST, ALT, and BUN significantly ($P < 0.01$). The creatinine levels showed less significant effects when compared with HFD treated group. While SPRE at 100 mg/kg did not display any significant ($P > 0.01$) effect on these parameters.

Conclusion: the results of the present study scientifically proven the traditional use of *Saccharum spontaneum* as an antiobesity and antihyperlipidemic agent as it normalized the raised body weight and organ fat pad weight as well as antihyperlipidemic property by lowering the changed levels of lipid profile in female SD rats.

Keywords: Antihyperlipidemic; Antiobesity; Hydroalcoholic Extract; *Saccharum Spontaneum*.

RESUMEN

Introducción: *Saccharum spontaneum* Linn. (Familia: Poaceae), conocida como Kasa o caña de azúcar silvestre. Se considera una hierba medicinal muy valiosa en los sistemas de medicina tradicional de la India. El objetivo de este estudio fue determinar la actividad antiobesidad y antihiperlipidémica del extracto hidroalcohólico de la raíz de *Saccharum spontaneum* (SPRE) en ratas obesas inducidas por una dieta alta en grasas (HFD).

Método: las ratas Sprague-Dawley (SD) hembras se dividieron en seis grupos ($n = 6$). Se administró SPRE en dosis de 100, 200 y 400 mg/kg, p.o., y sibutramina (5 mg/kg, p.o.) como fármaco de referencia diariamente mediante agujas de alimentación animal durante 60 días. Los animales experimentales se asignaron a un grupo de control normal (grupo I) al que se le administró una dieta de laboratorio regular, un grupo de control obeso (grupo II) inducido con HFD, un grupo de sibutramina más HFD (grupo III) y un grupo de SPRE (100, 200 y 400 mg/kg) más HFD (grupos IV, V y VI, respectivamente). Se estimó su efecto sobre el peso corporal, la grasa orgánica, el perfil lipídico sérico (TC, HDL, TG, LDL y VLDL), el aspartato aminotransferasa (AST), la alanina aminotransferasa (ALT), el nitrógeno ureico en sangre (BUN) y el nivel de creatinina en ratas obesas inducidas por HFD.

Resultados: la administración de SPRE (200 y 400 mg/kg) complementada con HFD mostró un control del peso corporal y del incremento de la grasa orgánica, y redujo los niveles de TC, LDL, VLDL y TG, además de aumentar los niveles de HDL y reducir significativamente los niveles de AST, ALT y BUN ($P < 0,01$). Los niveles de creatinina mostraron efectos menos significativos en comparación con el grupo tratado con HFD. Mientras que el SPRE a 100 mg/kg no mostró ningún efecto significativo ($P > 0,01$) sobre estos parámetros.

Conclusión: los resultados del presente estudio demostraron científicamente el uso tradicional de *Saccharum spontaneum* como agente antiobesidad y antihiperlipidémico, ya que normalizó el aumento de peso corporal y el peso de la grasa orgánica, así como la propiedad antihiperlipidémica al reducir los niveles alterados del perfil lipídico en ratas SD hembras.

Palabras clave: Antihiperlipidémico; Antiobesidad; Extracto Hidroalcohólico; *Saccharum Spontaneum*.

INTRODUCTION

Current lifestyle, described by high consumptions of sugars, fats and calories as well as a reduced exercise and physical work out, leads to inflammatory and metabolic disorders, viz. obesity, hypertension, cancer, diabetes, and other chronic disorders. Nutrition could play a crucial role in order to avoid these lifestyle-related illnesses, and it is required to explore safe and effective useful ingredients in food.⁽¹⁾ Obesity is a grave problem worldwide and has been linked with rise in mortality, morbidity and reduced life expectancy.⁽²⁾ It happens as a consequence of energy imbalance between energy consumption and energy outgoes, leads to enhanced lipid levels in the blood and bigger fat mass.⁽³⁾ Though, fat is essential for good health, production of excess of fat is associated to a range of health hazards such as obesity, diabetes mellitus, dyslipidemia, asthma, hypertension, osteoarthritis, fatty liver disease, and cancers.^(4,5) Globally, the occurrence of obesity is increasing speedily. Currently 0,3 billion people are medically obese whereas more than 1 billion adults are overweight.⁽⁶⁾ World Health Organization also anticipated that this figure may increase to 3,3 billion by 2030. This disease has numerous factors which add to its etiology including sedentary lifestyle such as lack of physical activity, white collar jobs, excess calorie consumption, endocrine disorders, and psychiatric problems among others.^(7,8,9) Hyperlipidemia is a secondary metabolic disorder linked with augmented risk factors for progress of diabetes. Beside its causative relationship with diabetes, higher serum level of TG, TC and LDL are main risk factors for the premature advancement of cardiovascular disorders such as hypertension, atherosclerosis and coronary artery disease.⁽¹⁰⁾ Augmented level of plasma lipids mainly TC, TG, VLDL, and LDL along with reduced level of HDL are recognized to cause hyperlipidemia which is the cause for initiation and progression of atherosclerosis. Hyperlipidemia is triggered by excessive consumption of alcohol or foods.⁽¹⁰⁾ Enhanced lipid levels are the result from greater absorption through the gut or increased endogenous synthesis. So, consequently two ways are practicable to reduce hyperlipidemia either to block endogenous synthesis of cholesterol or to decrease cholesterol absorption from the diet.⁽¹¹⁾

Saccharum spontaneum Linn. recognized as Kasa or wild sugar cane(Family: Poaceae). It is considered as a precious remedial herb in traditional systems of medicine in India.⁽¹²⁾ Roots are used as diuretic and galactagogue. In Ayurveda system of medicine, roots are used as emollient, purgative, tonic, refrigerant, astringent, diuretic, aphrodisiac, and beneficial in the treatment of piles, burning sensation, dyspepsia, and sexual dysfunction.⁽¹³⁾ This herb is also used to treat gynecological problems and respiratory diseases. The stems are beneficial in impaired conditions of “pitta” and “vata” burning sensation, renal and vesical

calculi, haemorrhoids, dyspepsia, agalactia, phthisis, menorrhagia, dysentery, and general weakness.⁽¹⁴⁾ Aerial parts ownaphrodisiac and laxative properties, and are valuable in burning sensations, phthisis, vesical calculi, strangury, biliary disease, blood diseases, and haemorrhagic diathesis.⁽¹⁵⁾ The whole plant contains alkaloids, glycosides, carbohydrates, phenolic compounds, tannins, saponins, protein and amino acids, coumarins and flavonoids as active constituents.⁽¹⁶⁾

The current study was done to explore the antiobesity and antihyperlipidemic property of the hydro-alcoholic root extract of *Saccharum spontaneum* (SPRE) in HFD induced obesity in female Sprague-Dawley rats. Its effect on body weight, organ fat pad, serum lipid profile (TC, TG, LDL, VLDL and HDL) and on AST, ALT, BUN and creatinine level was estimated in HFD induced obese rats.

METHOD

Chemicals

Ketamine hydrochloride, trichloroacetic acid (TCA), and Disodium ethylenediaminetetraacetate were purchased from Himedia (Mumbai, India). TG, TC, LDL, VLDL, HDL, AST, ALT, BUN, and creatinine kits were procured from Span Diagnostics (Gujarat, India). High fat diet was procured from NIN, Hyderabad, India.

Plant material

Fresh *Saccharum spontaneum* L. roots were identified and collected from the field nearby Integral University and were authenticated by NBRI, Lucknow (Ref. No. NBRI/CIF/265/2011).

Preparation of SPRE

The plant roots were dried in shade at room temperature ($25 \pm 2^\circ\text{C}$) for 21 days to get steady weight. The dried roots were then ground to crude powder. The crude powder (200 g) was shaken separately in 50 % ethanol for 1 complete day on an orbital shaker at RT. The extract so prepared was filtered using a Buchner funnel and Whatman filter paper. The extract was filtered, dried and concentrated in rotary evaporator. The crude extract obtained was suspended in 0,3 % carboxyl-methyl cellulose (CMC) suspension.⁽¹⁵⁾

Preliminary phytochemical screening

An effort was carried out to detect the presence and absence of miscellaneous phytoconstituents in the SPRE viz., glycosides (Baljet test), flavonoids (Shinoda test), alkaloids (Wagner's test), phenolic compounds (Ferric chloride test), steroids and triterpenes (Lieberman-Burchard's test), tannins (Ferric chloride test), and saponins (Foam test) according to standard protocol.⁽¹⁷⁾

Animals

Female SD rats weighed $130-140 \pm 5$ g were procured from the CDRI, Lucknow, India. They were housed in polypropylene cages ($22,5 \times 37,5 \text{ cm}^2$) and maintained under standard environmental conditions of laboratory; room temperature $25 \pm 2^\circ\text{C}$, 12/12 h light/dark cycle and $60 \pm 5\%$ relative humidity with free approach to food and water, *ad libitum*. The experimental procedures were approved by the Institutional Animal Ethics Committee, which follow the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and conform to the international norms of the Indian National Science Academy. The study procedure was sanctioned by IAEC, Integral University, Lucknow (Registration number: IU/Pharm/Ph.D./CPCSEA/10/18), and observed for the first 4 h and then daily for 7 days for any signs of toxicity, including changes in skin, eyes, salivation, motor activity, mortality etc.

Acute toxicity study

Acute toxicity of the SPRE was assessed in Swiss albino mice weighed 25-30 g (either sex), and it was conducted as per OECD guidelines 425 for testing of substances for acute oral toxicity.⁽¹⁸⁾ Mice ($n = 6$) was administered with different doses of SPRE (50, 250, 500, 1000 and 2000 mg/kg, *p.o.*), while the control group received 0,3 % carboxymethyl cellulose suspension, *p.o.* Food or water was withdrawn for 1-2 h post drug administration. All the groups were observed for 14 days for any gross effect and then mortality rate was noticed after 4 h of treatment.

Experimental protocol

SPRE at a dose of 100, 200, 400 mg/kg, *p.o.* and Sibutramine, 5 mg/kg, *p.o.* as reference drug was administered daily using animal feeding needles for 60 days. There were six groups having six animals in each ($n = 6$). The animals were allocated into normal control (Group I) administered with regular standard diet, control obese group (Group II) induced with HFD, standard drug plus HFD group (Group III) and SPRE 100, 200 and 400 mg/kg treatment plus HFD group (Group IV, V and VI respectively).

Determination of body weight

Each group rat's body weight in gram was recorded on daily basis for 60 days.⁽¹⁹⁾

Determination of organ and fat pad weights

All the rats were sacrificed by cervical dislocation and then diverse organs viz. heart, liver, kidney, and uterus fat pads were removed and weighed.⁽²⁰⁾

Determination of SPRE on lipid profile

All the rats were anaesthetized with Ketamine HCl. The blood sample was collected through cardiac puncture at day 60. The serum level of TC, LDL, TG, VLDL, and HDL were estimated.⁽²¹⁾

Determination of SPRE on AST and ALT levels

AST and ALT levels were estimated by Maheshwari et al. method.⁽²²⁾

Determination of SPRE on BUN and serum creatinine

The BUN and serum creatinine were estimated.⁽²³⁾

Statistical analysis

The results were expressed as mean \pm S.E.M. (Standard Error Means) and they were analyzed by one-way ANOVA, followed by Dunett's multiple comparison tests using the software GraphPad Prism 5 (San Diego, CA, USA). The *P* value of less than 0,05 was considered significant.

RESULTS

Preliminary phytochemical screening

SPRE showed the presence of alkaloids, glycosides, flavonoids, tannins, saponins, phenolic compounds, steroids, and triterpenes.

Acute toxicity study

Saccharum spontaneum 50, 250, 500, 1000 and 2000 mg/kg, *p.o.* did not display any abnormal behaviour during first 4h post administration in the SD rats. No mortality was observed during 14 days post treatment with SPRE. One-tenth of maximum dose (200 mg/kg) was selected which served as middle dose for the antiobesity study. Further half of the middle dose (100 mg/kg) selected as low dose and double of the middle dose (400 mg/kg) selected as high dose for the study.

Determination of body weight and organ and fat pad weights

The final body weight of Group II (HFD treated) animals was significantly increased (*P*<0,01) when compared with normal Group I. The body weight of Group III (sibutramine plus HFD treated) was significantly reduced (*P*<0,01) when compared with Group II (HFD treated). The final body weight of Group V (SPRE 200 mg/kg plus HFD treated) and VI (SPRE 400 mg/kg plus HFD treated) was significantly reduced (*P*<0,01 and *P*<0,05) when compared with Group II while in Group IV, the effect of SPRE 100 mg/kg did not show significant effect when compared with Group II on the body weight. The drug treatment groups were able to restrict the elevation of body weight weekly when compared with HFD group animals. Group II showed highest weight gain when compared with Group I, while Group III, IV, V and VI showed reduction in weight gain when compared to Group II (table 1).

Table 1. Effects of SPRE on body weight in SD rats after 60 days of treatment

Group	Treatment	Initial body weight (g)	Final body weight (g)	% increase in body weight	% decrease in body weight
I	Normal Control (NC)	135,32 \pm 2,12	227,50 \pm 3,89	68,12	-
II	High Fat Diet (HFD)	135,51 \pm 2,30	319,58 \pm 6,42*	135,83	-
III	HFD + Sibutramine (5 mg/kg)	140,21 \pm 2,54	235,00 \pm 3,53##	67,60	68,23
IV	HFD + SPRE (100 mg/kg)	140,17 \pm 2,54	298,28 \pm 5,86 ^{n.s}	112,79	23,04
V	HFD + SPRE (200 mg/kg)	135,21 \pm 2,71	238,51 \pm 4,56##	76,39	59,44
VI	HFD + SPRE (400 mg/kg)	136,67 \pm 2,05	251,15 \pm 5,75#	83,76	52,07

Note: All values were expressed as Mean \pm SEM (*n* = 6); ^{n.s}: non-significant when compared with Group II; ^{*}*P*< 0,01= significant when compared with normal group I; [#]*P* < 0,05; ^{##}*P* < 0,01 = compared with Group II.

Group II (HFD treated) showed increased organ fat pad weight on heart, liver, kidney, and uterus significantly ($P<0,01$) when compared with Group I. While Group III (sibutramine plus HFD treated) significantly reduced organ fat pad weight ($P<0,01$), whereas Group V (SPRE 200 mg/kg plus HFD treated) and VI (SPRE 400 mg/kg plus HFD treated) significantly reduced organ fat pad weight ($P<0,01$ and $P<0,05$) when compared with Group II (HFD treated). However, Group IV (SPRE 100 mg/kg plus HFD treated) didn't show significant effect when compared with Group II (table 2).

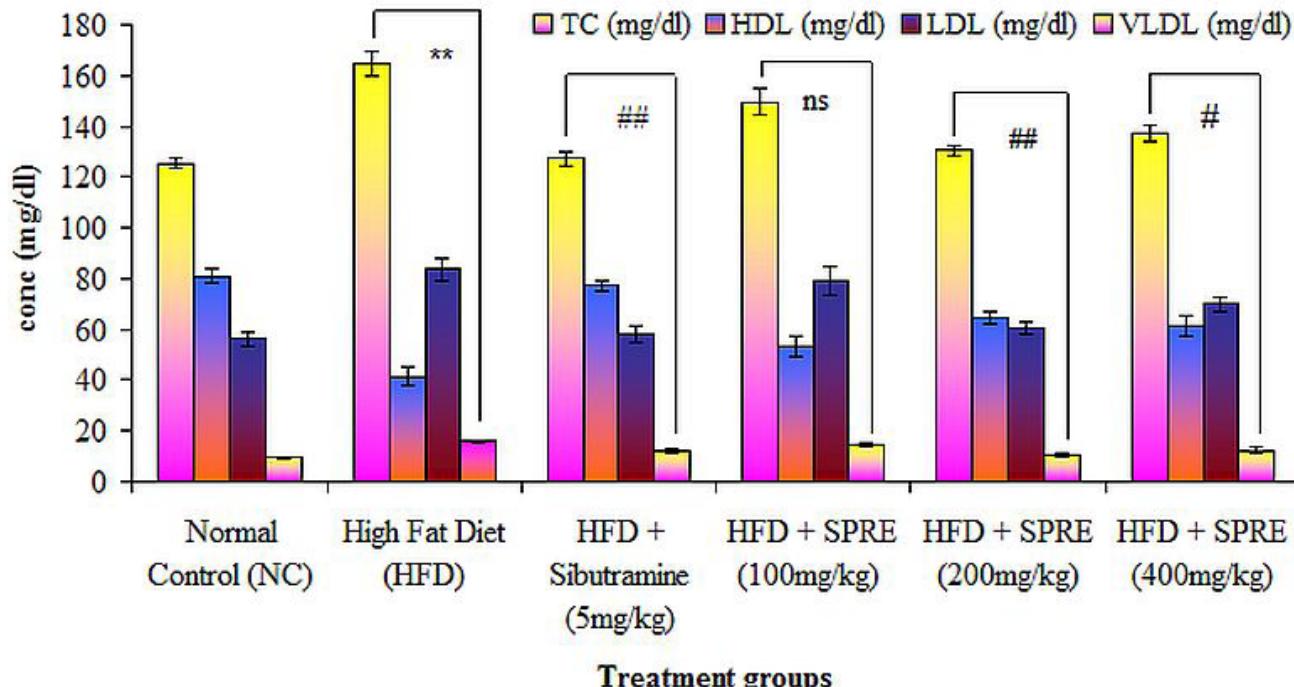
Table 2. Effects of SPRE on visceral fat pad in SD rats after 60 days of treatment

Group	Treatment	Heart (g)	Kidney (g)	Liver (g)	Uterus (g)
I	Normal Control (NC)	1,78 ± 0,07	1,21 ± 0,12	1,55 ± 0,12	1,45 ± 0,06
II	High Fat Diet (HFD)	2,32 ± 0,09*	1,77 ± 1,41*	2,65 ± 0,07*	2,71 ± 0,11*
III	HFD + Sibutramine (5 mg/kg)	1,89 ± 0,08##	1,23 ± 0,62##	1,57 ± 0,12##	1,47 ± 0,04##
IV	HFD + SPRE (100 mg/kg)	2,22 ± 0,6 ^{ns}	1,44 ± 1,21 ^{ns}	1,96 ± 0,03 ^{ns}	2,02 ± 0,10 ^{ns}
V	HFD + SPRE (200 mg/kg)	1,88 ± 0,09##	1,25 ± 0,94##	1,61 ± 0,13##	1,50 ± 0,04##
VI	HFD + SPRE (400 mg/kg)	1,98 ± 0,11*	1,29 ± 0,31*	1,83 ± 0,14*	1,85 ± 0,12*

Note: All values were expressed as Mean ± SEM ($n = 6$); ^{ns}: non-significant when compared with Group II; * $P<0,01$ = significant when compared with normal group I; # $P<0,05$; ## $P<0,01$ = compared with Group II.

Determination of SPRE on lipid profile

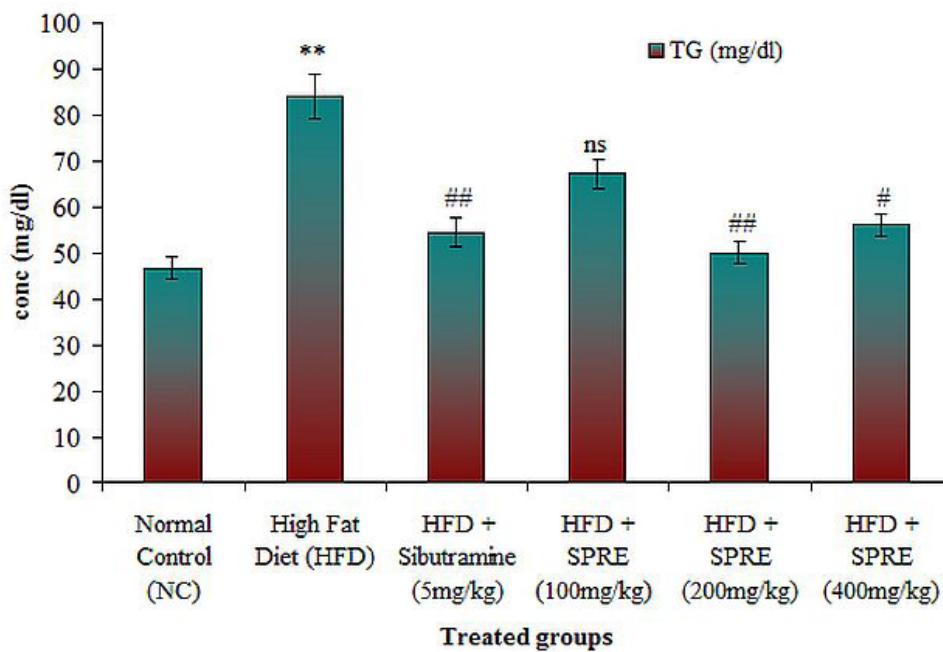
Group II (HFD treated) showed significantly increased ($P<0,01$) TC, LDL, VLDL and TG levels as well as significantly increased ($P<0,01$) HDL level when compared with normal Group I. While in Group III (sibutramine plus HFD treated), the TC, LDL, VLDL, and TG levels were significantly decreased ($P<0,01$) whereas HDL level significantly increased ($P<0,01$) when compared with Group II. Group V & VI significantly reduced ($P<0,01$ & $P<0,05$) the TC, LDL, VLDL, and TG levels when compared with Group II. While HDL levels significantly increased ($P<0,01$ & $P<0,05$) when compared with Group II whereas in Group IV, there was no significant effect on the TC, LDL, VLDL, TG, and HDL levels when compared with Group II (figures 1 and 2).



TC= Total cholesterol; HDL= High density lipoprotein; LDL= Low density lipoprotein; VLDL= Very low density lipoprotein;

Note: All values were expressed as Mean ± SEM ($n = 6$); ^{ns}: non-significant when compared with Group II; * $P<0,01$ = significant when compared with normal group I; # $P<0,05$; ## $P<0,01$ = compared with Group II.

Figure 1. Effects of SPRE on serum levels of TC, LDL, HDL and VLDL in SD rats



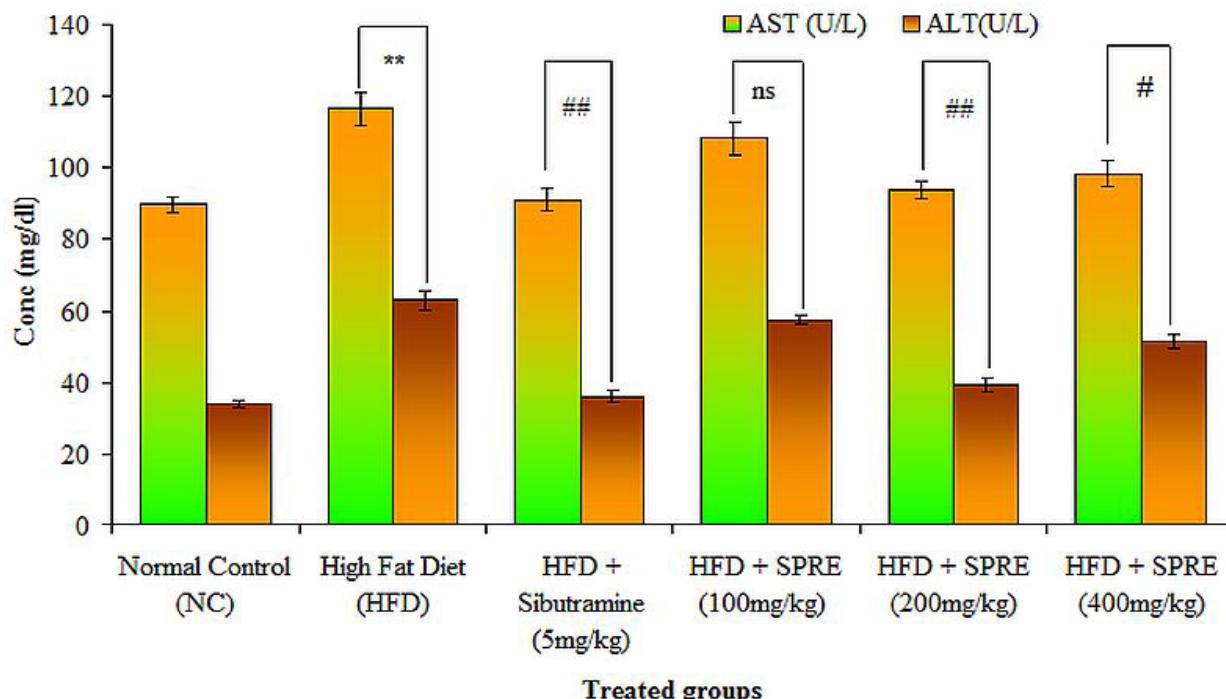
TG= Triglyceride.

Note: All values were expressed as Mean \pm SEM ($n = 6$); ns: non-significant when compared with Group II; ** $P < 0.01$ = significant when compared with normal group I; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ = compared with Group II.

Figure 2. Effects of SPRE on TG level in SD rats

Determination of SPRE on AST and ALT levels

In Group II, there was a significantly increase ($P < 0.01$) in the AST and ALT levels when compared with normal group (Group I) while in Group III, the AST and ALT levels significantly reduced ($P < 0.01$) when compared with Group II. In Group V and VI, the AST and ALT levels were significantly reduced ($P < 0.01$, $P < 0.05$) whereas in Group IV, there was no significant effect on the AST and ALT levels when compared with Group II (figure 3).



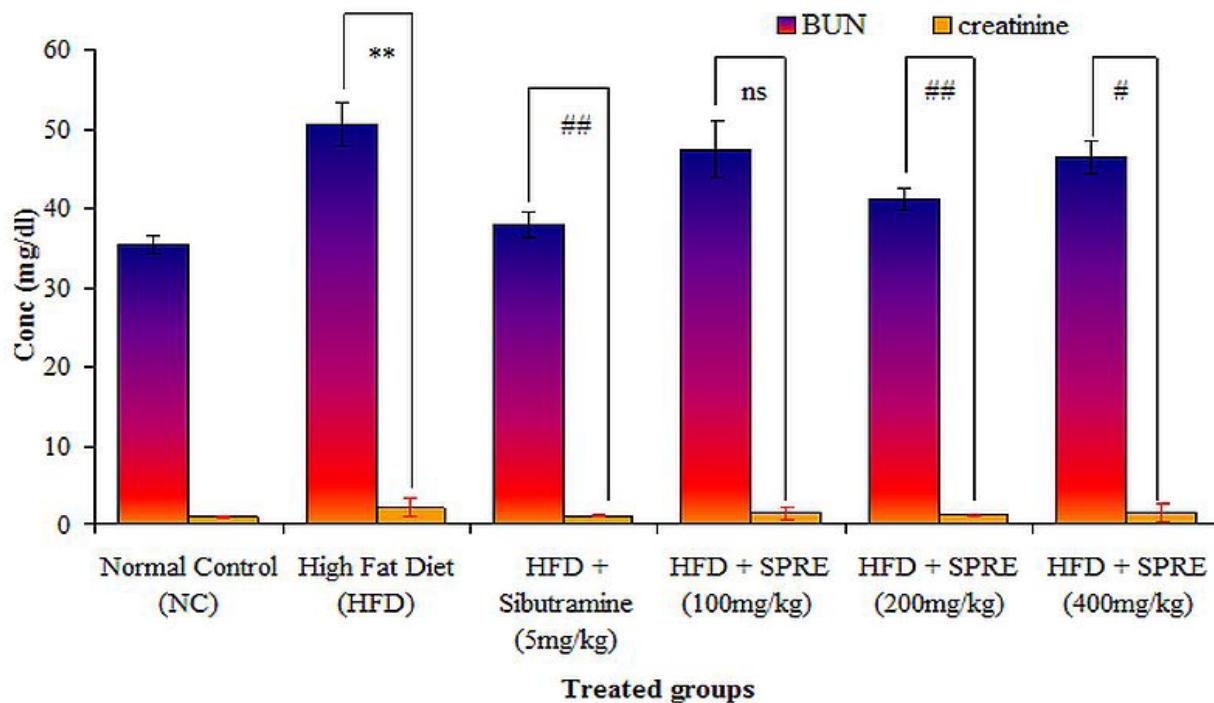
AST= Aspartate transaminase; ALT= Alanine transaminase.

Note: All values were expressed as Mean \pm SEM ($n = 6$); ns: non-significant when compared with Group II; ** $P < 0.01$ = significant when compared with normal group I; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ = compared with Group II.

Figure 3. Effects of SPRE on serum AST and ALT in SD rats

Determination of SPRE on blood urea nitrogen (BUN) and serum creatinine

BUN and creatinine levels were elevated in Group II when compared with normal group (Group I). Whereas in Group III, the BUN and creatinine levels significantly reduced ($P < 0,01$) when compared with Group II. Whereas in Group V & VI, the BUN and creatinine levels significantly reduced ($P < 0,01$, $P < 0,05$) when compared with Group II while in Group IV, there was no significant effect on the BUN and creatinine levels when compared with Group II (figure 4).



BUN= Blood Urea Nitrogen

Note: All values were expressed as Mean \pm SEM ($n = 6$); ns: non-significant when compared with Group II; ** $P < 0,01$ = significant when compared with normal group I; # $P < 0,05$; ## $P < 0,01$ = compared with Group II

Figure 4. Effects of SPRE on BUN and creatinine in SD rats

DISCUSSION

Obesity refers to the excessive amount of fat deposition in form of adipose tissues in various parts of the body including abdomen, heart, kidney, liver etc. it can be responsible for weight gain and number of metabolic and physiological changes.^(24,25) It was reported that the SPRE had the ability to reduce the body weight gain which could be due to its combined effects on the metabolic and serotonin pathways⁽²⁶⁾ and also reduced the food intake by inhibiting carbohydrate and fatty acid metabolism which may send a signal to the brain that result in a reduced appetite.⁽²⁷⁾ In present study the body weight and organ fat pad deposition of SPRE treated animals were reduced significantly when compared with HFD treated. Hyperlipidemia manifested by an elevation of serum cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG) concentration but diminished high density lipoprotein protein (HDL) concentration.⁽²⁸⁾ In the current study, the SPRE treatment along with HFD significantly decreased TC, LDL, VLDL, TG but increased HDL levels significantly when compared with HFD treated. The aspartate aminotransferase (AST) and alanine pyrophosphate (ALT) were present in high concentration in the liver under normal conditions whereas during hepatic necrosis these enzymes were released into the systemic circulation.^(29,30) In the present study, SPRE treatment significantly reduced both AST and ALT levels. In kidney diseases both plasma creatinine and blood urea nitrogen (BUN) levels rises.⁽³¹⁾ In this study BUN and plasma creatinine levels were significantly decreased in SPRE treated group when compared with HFD treated.

CONCLUSION

The present study demonstrated that SPRE possessed antiobesity and antihyperlipidemic activities in SD rats. Conclusively, observed reduction in body weight gain, lipids parameters, AST, ALT, blood urea nitrogen and creatinine suggests that hydroalcoholic root extract of *Saccharum spontaneum* possess significant anti-obesity and antihyperlipidemic potential.

Significance Statement

This study explored the antiobesity and antihyperlipidemic effect of SPRE that may be due to the presence of alkaloids, glycosides, flavonoids, tannins, saponins, phenolic compounds, steroids, and triterpenes. More studies, however, are needed to determine the exact mechanism of action of SPRE and the chemical constituents responsible for the observed effects.

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CONFLICT OF INTEREST

None.

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