



Hypolipidemic activity of ethanolic extract of *Saccharum spontaneum* in atherogenic diet induced rats

Geetha Kodali* and Ahmadi Banu

Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad (T.S), India.

Received on:17-10-2015; Revised on: 21-11-2015; Accepted on: 29-12-2015

ABSTRACT

Background: *Saccharum spontaneum* (Poaceae) commonly known as Wild sugarcane is a popular folk medicine in traditional systems of medicine in India. The whole plant is used to treat mental diseases, abdominal disorders, dyspnoea, anaemia, obesity, diuretic, lithotriptic, purgative, tonic, aphrodisiac, gynecological troubles, respiratory troubles etc. The present investigation was aimed to study the effect of ethanolic extract of *Saccharum spontaneum* whole plant (ESSW) in attenuating hyperlipidemia which is associated with many cardiovascular diseases like atherosclerosis. **Methods:** Hyperlipidemia was induced to rats by feeding atherogenic diet (AD) for a period of 21 days. The lipid profile was taken as the major marker of Hyperlipidemia and accordingly the changes in serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL-C), and high density lipoprotein (HDL-C) were measured (using enzymatic kits) after treatment with the ethanolic extract of *Saccharum spontaneum* whole plant (ESSW) and standard (atorvastatin). **Results and discussion:** The oral administration of ESSW (100 and 200 mg/kg body weight) for 21 days significantly lowered the TC, TG and LDL-C levels while increase in HDL levels were observed in a dose dependent manner. **Conclusion:** Based on the results obtained, it can be concluded that ESSW has significant antihyperlipidemic properties and can be used in the treatment of vascular disorders including atherosclerosis. The potential activity of the extracts can be attributed to the presence of antioxidant principles such as phenolic compounds which were reported from the plant.

KEYWORDS: *Saccharum spontaneum*, Antihyperlipidemic, Atorvastatin, Atherogenic diet.

INTRODUCTION

Cardiovascular diseases are the most common causes of mortality worldwide¹ and high plasma levels of cholesterol along with generation of reactive species (ROS) play's key role in the development of coronary artery diseases (CAD) and atherosclerosis^{2,3}. The general suggested measure for the treatment of hyperlipidemia associated diseases is through restriction of caloric intake or increased caloric expenditure. Plant derived polyphenols may provide a similar effect without restricting caloric intake and change in life style⁴. These polyphenols are secondary metabolites synthesized in the plants as a result of major stress event and hence the amount of polyphenols present in our commonly consumed food is very low. Therefore dietary supplements rich in polyphenols are recommended for achieving beneficial results. A number of plants have been found to be useful in treatment of hyperlipidemia such as *Allium sativum*, *Commiphora mukul*, *Boswellia serrata*, *Embllica officinalis*⁵, *Garcinia cambogia*⁶, *Terminalia arjuna*, *Trigonella foenum-graecum*, *Ocimum sanctum*, *Withania somnifera*⁷ and *Zingiber officinale*⁸.

Many allopathic anti-hyperlipidemic drugs like statins are available in the market, but they were found to cause many side effects like hyperuricemia, diarrhoea, myositis, hepatotoxicity, etc⁹. As they are basically enzyme inhibitors, so it is likely that they may be inhibiting other critical enzymes in the body. Moreover, statins are ingested on a long term basis so there may be a risk of chronic toxic effects over a life time of use¹⁰. Therefore attention is now paid much to search natural hypolipidemic agents from plant sources. A vast number of plants listed in the traditional medicine are yet to receive scientific documentation.

Saccharum spontaneum (Poaceae) commonly known as Wild sugarcane is widely found throughout India along the streams and moist places. Scientific information on their pharmacognosy, phytochemistry and pharmacology are very scant. It is a popular folk medicine and considered as valuable medicinal herb in traditional systems of medicine in India. The rural people in Vellore district of Tamilnadu and Andhra Pradesh use fresh juice of the stem of *Saccharum spontaneum* plant for the treatment of mental illness and mental disturbances. The whole plant is used to treat mental diseases, abdominal disorders, dyspnoea, anaemia, obesity, diuretic, lithotriptic, purgative, tonic, aphrodisiac, gynecological troubles, respiratory troubles etc¹¹.

*Corresponding author.

Dr. Geetha Kodali,
Shadan Women's College of Pharmacy,
Khairatabad, Hyderabad, (T.S), India.

An exhaustive literature survey on the *Saccharum* species revealed that the genus is a rich source of carbohydrates, flavonoids, terpenoids, alkaloids, phenolics, etc.¹². Many of these compounds have been found to possess significant therapeutic uses such as diuretic, antioxidant, anti-diarrhoeal, antiestrogenic and hypocholesterolemic etc. The effect of *Saccharum spontaneum* on hyperlipidemia was not reported. Hence, the present study was aimed to assess the possible effect of the selected plant extract in the lowering of cholesterol levels in atherogenic diet fed rats.

MATERIALS AND METHODS

Plant material

Fresh plant material was collected from the Hills of Tirumala region, Chittoor (dist).A.P, India and authenticated by Dr. Madhava chetty, Assistant Professor, Department of Botany, S.V. University, Tirupathi. A voucher specimen was kept in our herbarium, Dept of Pharmacognosy and Phytochemistry, Shadan Women's College of Pharmacy, Hyderabad, State of Telangana.

Preparation of ethanolic extract

Freshly collected plant material was dried under shade and coarsely powdered in a wiley mill. The coarsely powdered material (1 kg) was extracted with petroleum ether to remove the fatty material and further extracted in a Soxhlet apparatus and subjected to continuous extraction with ethanol (95%). The liquid extract was collected and concentrated under reduced pressure until a waxy mass was obtained. The concentrate obtained was weighed in each case¹³ [Harborne JB., 1998]. The concentrate was thoroughly air dried to remove all traces of the solvent and the percentage yield was calculated. The ethanolic extract was suspended in Tween – 80 for oral administration.

Preliminary Phytochemical Screening

The ethanolic extract of *Saccharum spontaneum* (ESSW) were tested for the presence or absence of phytoconstituents like steroids, alkaloids, saponins, tannins, carbohydrates, proteins, flavonoids and other phenolic compounds according to the standard procedures available in the literature¹⁴. The total phenolic content of the extract was determined by the Folin-ciocalteu assay¹⁵ and expressed as milligrams of gallic acid equivalents (GAE) per 100 grams dry mass (mg GAE/100 g dw).

Gallic Acid Equivalents = Absorbance (at 765 nm)/0.0508 (GAE)

Experimental animals

In-house laboratory bred healthy male Sprague Dawley (SD) rats

weighing 180-200 gm were used for the experiment. Rats were chosen as it accumulates cholesterol in both the liver and blood more readily than the mouse¹⁶. Also studies have been reported where SD rats have shown higher levels of TC and TG levels in serum in comparison to Wistar rats when fed with AD diet which show that SD rats are better model than Wistar rats for hyperlipidemic studies^{17,18}. The animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by institutional Animal Ethics Committee (IAEC). They were housed, three per polypropylene cage under standard laboratory conditions at room temperature $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with 12 h light/dark cycle. They had free access to standard pellet diet and water ad libitum except during experimentation.

Acute toxicity studies

The acute toxicity of ethanolic extract of *Saccharum spontaneum* was carried out using albino rats as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423, received from Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Experimental induction of Atherosclerosis

In rats, hyperlipidemia was induced by daily administration of atherogenic diet (AD) consisting of 2% Cholesterol, 1% Choline Chloride and 2% Lard in normal pellet diet over a period of 21 days.

Experimental design

The male SD rats were randomly divided into five groups with 6 animals per group.

Group 1: Control group treated with saline solution

Group 2: AD Control

Group 3: AD + Atorvastatin at (10 mg/kg)

Group 4: AD+ ESSW (100 mg/kg bw)

Group 5: AD+ ESSW (200 mg/kg bw)

Body weight

The body weight of animals was measured on day 1 before dosing and on day 22. The percentage change in body weight was calculated.

Assay for lipid profiles

On day 22nd, animals were anaesthetized with diethyl ether and blood

was collected by retro orbital puncture. The blood was allowed to clot for 30 min at room temperature and then was subjected to centrifugation at 2000 rpm for 15 minutes to obtain serum¹⁹. The resulting upper serum layer was collected in clean, dry and labeled micro-centrifuge tubes. This serum was analyzed for serum triglycerides (TG), total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) using commercially available kits (Swemed diagnostics). Serum Low density and very low density lipoprotein cholesterol (LDL-C, VLDL-C) and atherogenic index were determined by calculation.

Calculation:

$$AI = \frac{LDL + VLDL}{HDL}$$

$$LDL-C (mg/dl) = TC - (HDL-C + \frac{Triglycerides}{5})$$

$$VLDL-C (mg/dl) = \frac{Triglycerides}{5}$$

Estimation of lipid peroxidation (LPO)

The lipid peroxidation was measured with thiobarbituric acid reacting substance (TBARS) using malondialdehyde (MDA) as the standard (Lepage G et al., 1991). The content of MDA expressed as n moles formed per milligram of protein in tissue was calculated using the formula:

$$Conc. = A \times (V/E) \times P$$

Where, A = Absorbance

V = Volume of solution

E = Extinction Coefficient (1.56 x 10⁻⁶ x m⁻¹ x cm⁻¹)

P = Protein content of tissue calculated as mg of protein per gram of tissue.

Statistical analysis

The results were expressed as mean ± S.D (n=6). The statistical analysis involving five groups was performed by one way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test. P value < 0.05 was considered as statistically significant. Data were processed with graph pad prism version 5.00 software.

RESULTS

The preliminary phytochemical investigations of the ethanolic extract of *Saccharum spontaneum* showed the presence of phytoconstituents like steroids, terpenoids, flavonoids, glycosides and tannins. The percentage yield of the ethanolic extract was found to be 4.2% w/w. The total phenolic content of the extracts was determined spectrophotometrically by Folin-ciocalteu method and calculated as Gallic acid equivalents (GAE). The amount

of phenols in ethanolic extract of *Saccharum spontaneum* was found to be 9.82 mg/g of the crude drug. The acute toxicity studies of ESSW showed no symptoms of toxicity or behavioral changes at the maximum dose (2000 mg/kg). Hence 1/10th of the maximum dose viz. 200 mg/kg b.w was set as high dose for further studies. The results of in-vivo antihyperlipidemic studies of the extracts like the changes in body weight, serum lipid parameters and lipid peroxidation were presented in tables 1-3. A significant decrease (p<0.01) in the TC, TG and LDL-C levels were observed in the treatment groups with an increase in HDL-C in a dose dependent manner.

Table 1: Effect of the alcoholic extracts on % body weight

Parameter	Normal Control	AD diet Control	
% Body weight gain	21.99 ± 4.60 ^a	73.78 ± 9.22 ^b	
Parameter	Atorvastatin 10 mg/kg	ESSW 100 mg/kg	ESSW 200 mg/kg
% Body weight gain	34.55 ± 7.44 ^a	61.41 ± 8.40 ^b	56.52 ± 8.94 ^{bq}

Values are mean ± S.D (n = 6)

Table 2: Changes of serum lipid parameters in control and treated rats

Parameter	Normal Control	AD Control	
TC (mg/dL)	77.76±4.19 ^a	165.87±3.64 ^b	
TG (mg/dL)	75.07±4.24 ^a	127.62±7.21 ^b	
HDL (mg/dL)	27.08±1.23 ^a	21.92±0.89 ^b	
LDL (mg/dL)	35.66±4.14 ^a	118.43±4.08 ^b	
AI	1.32±0.19 ^a	5.41±0.35 ^b	
Parameter	AD + Atv 10 mg/kg	AD + ESSW 100 mg/kg	AD + ESSW 200 mg/kg
TC (mg/dL)	98.91±4.59 ^{bq}	159.67±10.17 ^b	144.27±9.18 ^{bq}
TG (mg/dL)	78.63±3.46 ^a	121.15±8.35 ^b	108.94±11.20 ^{bq}
HDL (mg/dL)	25.62±1.03 ^a	23.22±0.86 ^{bp}	22.09±0.71 ^b
LDL (mg/dL)	57.58±4.59 ^{bq}	112.22±9.17 ^b	100.39±7.82 ^{bq}
AI	2.25±0.18 ^{bq}	4.85±0.53 ^{bp}	4.55±0.43 ^{bq}

ESSW: Ethanolic extract of *S.spontaneum* whole plant, AD: Atherogenic diet, Atv: Atorvastatin, TC: total cholesterol, TG: triglycerides, HDL: high density lipoprotein cholesterol, LDL: low density lipoprotein cholesterol, AI: Atherogenic index.

Values are mean ± S.D (n = 6), p values: a<0.05, b<0.01, c<0.001, as compared with normal control; p values: p<0.05, q<0.01, r<0.001, as compared with AD control. (by one-way ANOVA followed by Dunnett multiple comparison test)

Table 3: Effect of alcoholic extracts on lipid peroxidation

Parameter	Normal Control	AD diet Control	
LPO	3.93± 0.35 ^a	7.53± 0.47 ^b	
Parameter	Atorvastatin 10 mg/kg	ESSW 100 mg/kg	ESSW 200 mg/kg
LPO	3.84 ± 0.14 ^a	5.53± 0.48 ^{bq}	5.58± 0.41 ^{bq}

DISCUSSION

Though lipids are essential in the formation of cell membrane and hormone synthesis, excess of cholesterol leads to development of atherosclerosis. LDL-C is considered as the major bad cholesterol as it transports cholesterol not only to peripheral tissues but also to the arterial wall²⁰. The increase in concentration of blood cholesterol through diet results in accumulation of LDL-C whose oxidative modification is implicated in atherosclerosis.

In the present study the lipid levels of atherogenic diet control group were significantly higher than the control group which indicates that AD is a potential source of dietary cholesterol. Treatment with ESSW (100 and 200 mg/kg) showed significant decrease in body weight and lipid levels in a dose dependent manner. Atorvastatin (10 mg/kg) showed a marked decrease in the levels of serum TC, TG, LDL and atherogenic index. Besides, the levels of HDL were elevated in ESSW treated groups. HDL is considered as good cholesterol as it takes up the excess cholesterol from the cells and delivers to the liver for metabolism²¹. Therefore an increase in HDL levels indicates lower risk of atherosclerosis. Atherogenic index (AI) which is defined as LDL-C to HDL-C is an important factor for the diagnosis of atherosclerosis. The results clearly indicate that ESSW significantly decreased the AI.

Oxidative stress caused by atherogenic diet is associated with peroxidation of cellular lipids, which is determined by measurement of thiobarbituric acid reacting substance (TBARS). The concentration of LPO products may reflect the degree of oxidative stress. The increased level of TBARS results in increase of oxygen free radicals, which attacks the polyunsaturated fatty acids in cell membranes and cause LPO. The malondialdehyde (MDA) content, a measure of LPO was assayed in the form of TBARS. Treatment with ESSW and Atv significantly ($p < 0.01$) prevented the peroxidation by reducing the concentration of TBARS (Table 3).

CONCLUSION

The present study is the first to demonstrate the hypolipidemic activity of *Saccharum spontaneum* and thereby reducing the risk of cardiovascular complications like atherosclerosis. The results found were encouraging for further studies on the plant. Though the exact mechanism of hypolipidemic action was not established, the possible beneficial effect of polyphenols can be taken into account as they are potent antioxidants and can prevent the oxidation of Low density lipoprotein cholesterol. Further studies are necessary to establish the exact mechanism of action.

REFERENCES

1. Epstein FM. Age and the cardiovascular system. New

England Journal of Medicine. 1992; 327: 173.

2. Stokes J, Kannel WB, Wolf PA, Cupples LA, D'agostino RB. Circulation. 1987; 75: V65–V73.
3. Visavadiya NP, Narasimhacharya AVRL. Hypolipidemic and antioxidant activities of *Asparagus Racemosus* in hypercholesteremic rats. Indian J Pharmacol. 2005; 37: 376-80.
4. Lamming DW, Wood JG, Sinclair DA. Small molecules that regulate life span: evidence for xenohormesis. Mol. Microbiol. 2004; 53: 1003–1009.
5. Mathur R, Sharma A, Dixit VP, Varma M. Hypolipideamic effect of fruit juice of *Embllica officinalis* in cholesterol.-fed rabbits. Journal of Ethnopharmacology. 1996; 50: 61–686.
6. Koshy AS, Vijayalakshmi NR. Impact of certain flavonoids on lipid profiles—potential action of *Garcinia cambogia flavonoids*. Phytotherapy Research. 2001; 15: 395–400.
7. Cox PA. Ethnopharmacology and the search for new drugs Bioactive Compounds from Plants Ciba Foundation Symposium 154, Chichester, John Wiley & Sons, 1990: pp.40.
8. Bhandari U, Sharma JN, Zafar R. The protective action of ethanolic ginger (*Zingiber officinale*) extract in cholesterol fed rabbits. Journal of Ethnopharmacology. 1998; 61: 167–171.
9. Kumar AS, Mazumder A, and Saravanan VS. Antihyperlipidemic activity of *Camellia sinensis* leaves in triton wr-1339 induced albino rats. Phcog Mag. 2008; 4: 60-64.
10. Dhar ML, Dhar MM, Dhawan BN, Mehora BN, and Ray C. Indian J. Exp. Bio. 1968; 6: 232.
11. Sharma P C , Yelne M B , Dennis T J . Database on Medicinal Plants used in Ayurveda . New Delhi: Central Council for Research in Ayurveda and Siddha ; 2002.
12. M. Khalid, H.H. Siddiqui, Pharmacognostical Evaluation and Qualitative Analysis of *Saccharum spontaneum* (L.) Root, International Journal of Pharmaceutical Sciences and Drug Research, 3 (2011) 338-341.
13. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London. 1998: pp. 1-32.
14. Khandelwal KR. Practical Pharmacognosy, 9th Edn., Nirali Prakashan, 2002: 149-56.
15. Marinova D, Ribarova F, Atanassova M. J. Univ. Chem. Tech. Met. (Sofia). 2005; 40(3): 255-260.
16. Beher WT, Baker GD, Penney DG. A comparative study of the effects of bile acids and cholesterol on cholesterol metabolism in the mouse, rat, hamster and guinea pig. J Nutr. 1963; 79: 523-30.
17. Kühn ER, Bellon K, Huybrechts L, Heyns W. Endocrine

- differences between the Wistar and Sprague-Dawley laboratory rat: influence of cold adaptation. *Horm Metab Res.* 1983; 15: 491-8.
18. Gao Y, Li K, Tang S, Xiao Y. Study on animal models for hyperlipidemia. *J Hyg res.* 2002; 31: 97-9.
 19. Xu L, Liu Y, Wang T, Qi Y, Han X, Xu Y et al. Development and validation of a sensitive and rapid non-aqueous LC-ESI-MS/MS method for measurement of diosgenin in the plasma of normal and hyperlipidemic rats: A comparative study. *J Chrom B.* 2009; 877: 1530-6.
 20. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med.* 1989; 320: 915-24.
 21. L. Martinez, S. Jacquet, F. Terce, X. Collet, B. Perret, R. Barbaras, New insight on the molecular mechanisms of high-density lipoprotein cellular interactions, *Cellular and Molecular Life Sciences CMLS*, 61 (2004) 2343-2360

Source of support: Nil, Conflict of interest: None Declared