Hypolipidemic effect of *Sesbania grandiflora* on cigarette smoke exposed rats

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Summary

Sesbania grandiflora (*S. grandiflora*) is widely used in Indian traditional medicine for the treatment of a large spectrum of diseases. In the present study, we assessed the possible effects of aqueous suspension of *S. grandiflora* (ASSG) leaves against cigarette smoke induced hyperlipidemia in rats. Adult WKY rats exposed to cigarette smoke for a period of 90 days were assigned randomly to a control group and a treatment (ASSG, 1000mg kg^{-1}) group and were treated orally for 3 weeks. The efficacy of ASSG on total lipids, total cholesterol (TC), triglycerides (TG), phospholipids, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) were estimated in cigarette smoke exposed (CSE) rats. Total lipids, total cholesterol, triglycerides, LDL-C, and VLDL-C were significantly increased while phospholipids and HDL-C was significantly decreased in cigarette smoke exposed rats. These results were reversed to near normal in ASSG treated with cigarette smoke exposed rats. These observations clearly demonstrated the hypolipidemic effect of *S. grandiflora* in cigarette smoke exposed rats. These results provide further support to the traditional use of *S. grandiflora* for the treatment of smoking induced hyperlipidemia associated diseases.

Key words: Cigarette smoke; Hypolipidemia; Hyperlipidemia; Sesbania grandiflora

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Introduction

Cigarette smoking has been implicated as an independent and strong risk factor for cardiovascular diseases [1]; the underlying pathophysiological role of cigarette smoke is partially understood. Common feature of cigarette smoke associated cardiovascular pathology, including atherosclerogenesis and myocardial infarction is associated with impaired lipid and lipoprotein metabolism. Several smoking effects have been described as being atherogenic, such as direct vascular actions [2], oxidative stress [3], thrombogenic factors [4] and secondary dyslipidemia [5,6] whereas a large number of research groups depicted increased plasma levels of atherogenic lipoproteins (LDL-C and VLDL-C) and decreased levels of antiatherogenic lipoprotein (HDL-C) in smokers [7]. Besides, total cholesterol and triglycerides were increased and phospholipids was decreased in cigarette smoke exposed rats [8]. To modulate the lipid and lipoprotein alterations several antioxidant and hypolipidemic herbs has been used that may be useful adjuncts in reducing the risk of cardio vascular diseases.

Sesbania grandiflora (L.) pers (Febaceae), commonly known as 'sebania' and 'agathi', has been used as an important dietary nutrition source in Southeast Asian countries [9]. *S. grandiflora* leaves are a richest source of amino acids and contain several essential minerals and vitamins. In addition, it contains saponin, an aliphatic alcohol and grandiflorol (α-5-methyl-5-pentacosanol) [10,11] which could reduce the blood cholesterol level.

Different parts of this plant are used in Siddha system of Indian traditional medicine for the treatment of a wide spectrum of ailments including anemia, bronchitis, fever, headache, ophthalmia, nasal catarrh, inflammation, leprosy, gout and rheumatism [12]. It also possesses anxiolytic and anticonvulsive [13] and hepatoprotective [14,15] properties. In addition, *S. grandiflora* is mentioned as a potent antidote for tobacco and smoking-related diseases [16] However, the mechanisms underlying its beneficial effects against chronic smoking associated diseases are yet to be determined.

In the present study, we depicted the effects of *S. grandiflora* against cigarette smoke-induced hyperlipidemia in rats. For that purpose, adult Wistar-Kyoto (WKY) rats were exposed to cigarette smoke for a period of 90 days and subsequently treated for 3 weeks with aqueous suspension of leaves of *S. grandiflora* (ASSG).

Materials and Methods

Drugs and chemicals

Phosphotungstic acid, Magnesium chloride, Adenosine tri phosphate, Cholesterol and phospholipid standard were obtained from the Sigma chemicals company (MO, St.Louis, USA). All other chemicals and solvents utilized in this study were purchased from Glaxo Laboratories (P) Ltd (Mumbai, India).

Plant material

Fresh *S. grandiflora* leaves were collected from a local plantation (Poovathur, Thanjavur, India). The leaves were washed for any contaminants, dried thoroughly under shade and then was crushed in a motor and pestle.

The powder form of the leaves of *S. grandiflora* was reconstituted in distilled water to form a suspension. The aqueous suspension of *S. grandiflora* (ASSG) leaves was prepared freshly every day prior to administration.

Animals

All the experimental procedures utilized were performed in accordance with guidelines issued by the Institutional Animal Care and Ethics Committee. Male Wistar-Kyoto (WKY) rats weighing 125-150 gm were obtained from Venkateshwara Animal Breeding Centre, Bangalore, India. Animals were housed in polypropylene cages with filter tops under controlled conditions of a 12 hour light/ 12 hour dark cycle, and 27 ± 2^{o}C and were fed with standard laboratory pellets (Amrut rat feed, Chakan oil mills Ltd, Pune, India) and tap water *ad libitum*.

Experimental design

The animals were randomly divided into four groups (n = 6). The rats in Group 3 and Group 4 were exposed to chronic cigarette smoke for a period of 90 days and subsequently assigned to a control group (Group 3, CSE) and a treatment group (Group 4, CSE + ASSG). The rats in Group 3 and Group 4 were exposed to cigarette smoke as described earlier [17]. Briefly, the rats were kept in a polypropylene cage with a lid made of polythene paper. A lighted cigarette was placed in a flask connected to the cage, and air was supplied into the flask for 10 min by a small air pump. A length of 5.9 cm of each cigarette was allowed to be burned by clamping the butt when it was placed in a flask.

Each rat was subjected to inhalation of cigarette smoke seven times a day at regular intervals of one hour (from 11 AM to 5 PM). The rats in Group 1 (Control) and Group 2 (ASSG alone) were treated identically but without exposure to cigarette smoke. The rats under different groups were treated for 3 weeks by oral gavage and the dose of ASSG was kept as 1000mg kg^{-1} of body weight.

At the end of experimental period, the animals were sacrificed by cervical decapitation; blood was collected and centrifuged to obtain serum. Lung, liver, kidney and heart were excised immediately, washed in ice cold saline and then homogenized in Tris-HCl buffer (0.05 M, pH 7.4).

Biochemical analysis

Total lipids were extracted from tissues according to Folch et al [18] using chloroform - methanol mixture ($CHCl_3$:CH_3OH) (2:1 v/v). The total cholesterol was estimated by the method of Allain [19]. Triglycerides were estimated by the method of Werner et al [20]. Phospholipid content was determined by the method of Zilversmit et al [21] and liberated phosphorus was estimated by the method of Fiske and Subbarow [22]. HDL-C was separated by adding phosphotungstic acid and magnesium chloride to the fresh samples to precipitate other lipoproteins and the HDL-C was estimated by the method of Allain [19]. The concentration of LDL-C was calculated by using the Friedwald formula [23] and VLDL-C was calculated by dividing the triglyceride value (in mg/dl) by 5.

Statistical analysis

Results are expressed as mean ± SD (n = 6). The observed differences were analyzed for statistical significance by One-way of the analysis of variance with Tukey's multiple comparison as post test

Results

Table 1-3 shows the levels of total lipids, total cholesterol, triglycerides and phospholipids in serum, lung, heart, liver and kidney of control and experimental rats. Total lipids, total cholesterol and triglycerides in the serum, lung, heart, liver and kidney of Group 3 rats (CSE) was significantly higher while phospholipids was significantly lower as compared with that observed in Group 1 rats (Control). Group 4 rats (CSE + ASSG) showed significant decrease in the levels of total lipids, total cholesterol and triglycerides whereas significant increase in the levels of phospholipids as compared with that observed in Group 3 rats. Significant changes were not observed in Group 2 rats (ASSG + alone) as compared with Group 1 rats.

Table 4 represents the concentration of lipoproteins in the control and experimental rats. The levels of HDL-C were significantly decreased in Group 3 rats with a significant increase in LDL-C, VLDL-C, LDL/HDL and TC/HDL ratios when compared to Group 1 rats. Group 4 rats showed significantly higher HDL-C concentrations and significantly lower in LDL-C, VLDL-C, LDL/HDL and TC/HDL ratios as compared with that observed in Group 3 rats. No significant alterations were seen between Group 1 and Group 2 (ASSG alone treated) rats.

Table 1: Effect of *S. grandiflora* on total lipids, total cholesterol, triglycerides and phospholipids in serum

Parameters	Total lipids (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	Phospholipids (mg/dl)
Control	256.42 ± 14.72	112.77 ± 6.02	61.42 ± 3.04	96.29 ± 5.74
ASSG alone	254.52 ± 12.73	110.28 ± 6.24	60.95 ± 3.14	96.13 ± 5.17
CSE	439.51 ± 14.93 *	143.01 ± 6.19 *	97.53 ± 3.42 *	67.42 ± 5.23 *
CSE+ASSG	321.73 ± 12.43 *	114.21 ± 6.18 *	74.61 ± 3.00 *	90.18 ± 4.97 *

Values are expressed as mean ± SD (n=6), Statistical comparison are made between Control vs ASSG alone and CSE; CSE vs CSE+ASSG. Statistical significance at * $p < 0.001$

Table 2: Effect of *S. grandiflora* on total lipids, total cholesterol, triglycerides and phospholipids in lung and heart

Parameters (mg/g tissue)	Total lipids	Total cholesterol	Triglycerides	Phosphplipids
Lung				
Control	15.52 ± 1.04	5.93 ± 0.39	4.22 ± 0.42	4.03 ± 0.22
ASSG	15.43 ± 1.24	5.62 ± 0.43	4.17 ± 0.39	4.23 ± 0.24
CSE	24.23 ± 1.07*	12.46 ± 0.62 *	11.45 ± 0.37 *	2.13 ± 0.20*
CSE+ASSG	19.26 ± 1.23 *	7.92 ± 0.41 *	7.21 ± 0.41 *	3.89 ± 0.26 *
Heart				
Control	17.57 ± 1.14	3.27 ± 0.41	3.63 ± 0.23	3.13 ± 0.17
ASSG	17.56 ± 1.38	3.22 ± 0.38	3.54 ± 0.32	3.24 ± 0.26
CSE	33.19 ± 2.11 *	8.13 ± 0.32 *	8.23 ± 0.27 *	1.52 ± 0.10 *
CSE+ASSG	23.01 ± 1.24 *	4.73 ± 0.36 *	4.62 ± 0.24 *	2.92 ± 0.31 *

Values are expressed as mean ± SD (n=6), Statistical comparison are made between Control vs ASSG alone and CSE; CSE vs CSE+ASSG. Statistical significance at * $p < 0.001$.

Table 3: Effect of *S. grandiflora* on total lipids, total cholesterol, triglycerides and phospholipids in liver and kidney

Parameters (mg/g tissue)	Total lipids	Total cholesterol	Triglycerides	Phospholipids
Liver				
Control	30.12 ± 1.97	7.69 ± 0.30	10.41 ± 0.73	9.57 ± 0.42
ASSG	30.10 ± 1.26	7.21 ± 0.33	10.07 ± 0.52	9.65 ± 0.47
CSE	41.14 ± 2.29 *	19.27 ± 0.61 *	23.13 ± 0.78 *	4.62 ± 0.36 *
CSE+ASSG	32.24 ± 2.01*	9.16 ± 0.48 *	12.44 ± 0.63 *	8.43 ± 0.44 *
Kidney				
Control	21.34 ± 1.35	6.65 ± 0.29	8.23 ± 0.42	4.76 ± 0.33
ASSG	20.78 ± 1.29	6.03 ± 0.34	8.15 ± 0.51	4.77 ± 0.29
CSE	37.47 ± 1.42 *	13.24 ± 0.52 *	19.38 ± 0.47 *	2.34 ± 0.13 *
CSE+ASSG	26.52 ± 1.67 *	7.33 ± 0.42 *	11.72 ± 0.31 *	4.08 ± 0.27 *

Values are expressed as mean ± SD (n=6), Statistical comparison are made between Control vs ASSG alone and CSE; CSE vs CSE+ASSG. Statistical significance at * $p < 0.001$

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Table 4: Effect of *S. grandiflora* on LDL, VLDL, HDL, LDL/HDL and TC/HDL in serum.

Parameters (mg/dl)	Control	ASSG alone	CSE	CSE+ASSG
LDL	47.18 ± 2.36	46.21 ± 2.31	94.27 ± 3.44*	49.59±2.48*
VLDL	12.28 ± 1.04	12.19 ± 0.76	19.51 ± 1.09*	14.92 ± 0.92*
HDL	27.15 ±1.36	27.05 ± 1.40	18.54± 0.93*	23.02 ± 1.15*
LDL/HDL	1.74 ± 0.09	1.71 ± 0.09	5.08 ± 0.23 *	2.15 ± 0.11*
TC/HDL	4.15 ± 0.16	4.08 ±0.15	7.71 ± 0.34*	4.96 ± 0.23 *

Values are expressed as mean ± SD (n=6), Statistical comparison are made between Control vs ASSG alone and CSE; CSE vs CSE+ASSG. Statistical significance at * $p < 0.001$

Discussion

In the present study, the effect of *S. grandiflora* leaves were investigated on the alterations of lipid and lipoproteins in cigarette smoke exposed rats. The present results demonstrated that exposure to cigarette smoke increased concentration of total cholesterol in serum, lung, heart, liver and kidney of rats. This result is suggesting that chronic cigarette smoke exposure could enhance the cholesterol synthesis. This observation was consistent with earlier report [8]. Cigarette smoke increased the production of cholesterol, through the induction of HMG- CoA reductase activity which is acting as a rate limiting step enzyme in cholesterol biosynthesis [24]. Administration of ASSG decreased the concentration of total cholesterol in cigarette smoke exposed rats. This might be attributed to saponin, grandiflorol and vitamin C [10]. Saponin act by binding with bile acids and cholesterol then removed these fatty substances from the body. Grandiflorol is a long chain fatty alcohol; it affects the cholesterol biosynthesis in liver and reduced the complication of coronary heart disease [25]. Vitamin C which is present in *S. grandiflora* enhanced the cholesterol degradation process result in decreased levels of total cholesterol in cigarette smoke exposed rats. This result correlated with perivious report [24].

Cigarette smoke exposure significantly increased the triglycerides levels [26].The present study also observed increased concentration of triglycerides in cigarette smoke exposed rats. This might be attributed to nicotine. Kavitharaj and Vijayammal [27] also reported that chronic administration of nicotine was found to produce enhanced synthesis of triglycerides.

The increased level of triglycerides is an independent risk factor for coronary heart disease [28]. Administration of ASSG was reduced the levels of triglycerides to near normal in cigarette smoke exposed rats. In addition, the present study observed significant reduction in the levels of phospholipids in serum, lung, heart, liver and kidney of cigarette smoke exposed rats. Chitra et al [8] also reported that phospholipids level was significantly decreased in cigarette smoke exposed rats. This might be due to cigarette smoke generated free radicals, attacked on phospholipids result in the reduction of phospholipids. ASSG administration was ameliorated the levels of phospholipids in cigarette smoke exposed rats. This might be due to antioxidant compounds like β-carotene and vitamin C which are present in *S. grandiflora*.

Cigarette smoke exposure altered the concentration of lipoproteins. This might be due to cigarette smoke induced reduction of lipoprotein lipase (LPL) and lecithin cholesterol acyltransferase activity (LCAT) [29]. Lipoprotein lipase is involved in the uptake of VLDL-C (TG rich lipoproteins). In the present study, VLDL-C level was significantly increased in cigarette smoke exposed rats. This result is correlated with the decreased activity of LPL. The increased VLDL-C leads to the high concentration of LDL-C. This process was reflected in the preset study as elevated level of LDL-C in cigarette smoke exposed rats. Earlier studies also reported that LDL-C level was significantly increased in cigarette smokers [30,31]. The elevated LDL-C is highly susceptible to oxidation. Oxidized LDL-C plays an important role in the development of atherosclerotic process and endothelial dysfunction [32,33].

Moreover, the present study observed diminished level of HDL-C in cigarette smoke exposed rats. This result was reliable with earlier reports [26,30]. Besides, Vella et al [34] also reported that HDL-C level was significantly decreased in cigarette smokers. This might be due to increased VLDL-C leads to decreased levels of HDL-C by the poorer availability of phospholipids remnants from VLDL-C to HDL-C formation and decreased activity of LCAT [35]. Due to lipoproteins alteration, lipids might be accumulated in the tissues. Hence, in the present study, the total lipids level was significantly elevated in cigarette smoke exposed rats. In addition, the present study observed elevated levels of TC/HDL-C and LDL-C/HDL-C ratio in cigarette smoke exposed rats. These results also predictors of coronary risk [31, 36]. Administration of ASSG normalized the above alterations in cigarette smoke exposed rats. This might be due to hypolipidemic and antioxidant property of *S. grandiflora*.

In conclusion, *S. grandiflora* has a hypolipidemic action against cigarette smoke induced hyperlipidemia. These results provide further support to the traditional medicinal use of *S. grandiflora* for the treatment of cigarette smoke induced hyperlipidemia associated diseases.

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