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Antihyperlipidaemic and antiatherosclerotic activity of different fractions of *Lagenaria siceraria* stem and leaves juice in hyperlipidaemic rats

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ABSTRACT

We have investigated different extract of *Lagenaria siceraria* for their potential of antihyperlipidaemic and antiatherosclerotic effect. These activities were tested by using the triton and cholesterol induced hyperlipidaemia. Fractions were tested at two-dose levels 250mg/kg and 500 mg/kg body weight. The results of the study revealed that by triton, total cholesterol and triglycerides level were significantly increased ($P<0.0001$) in rats as compared to normal control. Rats with dried petroleum ether and methanolic extracts (250 mg/kg) caused significant reduction ($P<0.01$) in total cholesterol and triglycerides compared to triton WR 1339. In cholesterol induced hyperlipidaemia, at the end of 9th day there were significant increase in above parameters of cholesterol-administered rats excepting HDL-c (which decreased significantly) compared to normal control ($P<0.0001$). Besides reducing TC, TG, LDL-c, VLDL-c, TC: HDL, LDL: HDL and atherogenic index, petroleum ether and methanolic extracts increased HDL-c level significantly compared to cholesterol treated rats ($P<0.01$). Based on this study, we confirmed that fractions of stem and leaves of *Lagenaria siceraria* is a potentially useful for the treatment of hyperlipidaemia and atherosclerosis.

KEYWORDS

Lagenaria siceraria;
Cucurbitaceae;
Antihyperlipidaemia;
Triton and cholesterol-induced hyperlipidaemia.

INTRODUCTION

Increased plasma lipid level, mainly total cholesterol (TC), triglycerides (TG), and low density lipoproteins (LDL) along with decrease in high density lipoproteins (HDL) are known to cause hyperlipidaemia which is core in initiation and progression of arteriosclerosis impasse. Therefore, prime consideration in the therapy for hyperlipidaemia and arteriosclerosis is to attenuate the elevated plasma levels of TC, TG and LDL along with increase in HDL lipids levels[1,2].

Lagenaria siceraria Stand. belongs to family cucurbitaceae, is a common vegetable known for India. *Lagenaria siceraria* (LS) is a climber stem and leaves traditionally used for its cardioprotective, cardiotonic, general tonic and diuretic properties. Presently Ayurvedic practiceners of India are recommending juice of LS for the treatment of high lipid level and atherosclerosis. Prominent effects of juice have been reported by beneficiaries; even cases of angioplasty and bypass surgery avoidance are available[19].

Literature studies on LS have shown the presence of falconoids[2], cucurbitacin saponin[3], flavone c glycosides[2]. LS fruits have been reported to possess an-

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tioxidant activity[5], hypolipidemic and antihyperlipidemic effects in normocholesterolemic and triton-induced hypolipidemic rats[6-7]. Early work shows that ribonucleolytic activity of lagenin isolation and identification from LS[8].

Survey has revealed a number of uses of different parts of this plant. The survey of literature revealed that no antihyperlipidemic effect has been carried out on different extracts of stem and leaves juice of LS. This investigation is an attempt to find out the possible effects of five different extracts from LS stem and leaves in triton-induced hyperlipidaemic rats as well as in cholesterol induced hyperlipidaemic rats, by measuring different parameters of blood-lipid profile, *viz*. TC, TG, LDL and HDL.

MATERIAL AND METHODS

Chemicals and plant material

Triton WR 1339 (Tyloxapol) was received as gift sample from HiMedia laboratory, Bombay. Standard drug, Gemfibrozil was gift sample from Panacea Biotech, New Delhi. Cholesterol and coconut oil was supplied by Sameer chemical, Nagpur, Maharastra. Enzymatic kits**,** JK diagnostics were used for determination of different biochemical parameters.

The plant of *Lagenaria siceraria* (Molina) Standley stem and leaves were collected from maharaj bagh region of Nagpur. It was authenticated by Dr. M. N. Dongarwar, department of botany, Nagpur University, Nagpur. A voucher specimen (289/2, dated September 10, 2013) was deposited at the herbarium of above department.

Removal of stem and leaves juice

Juice was obtained using juicer (without the addition of water and peeling). Total 7-kilograms of stem and leaves were treated to get around 3000 ml of juice. Juice was filtered through fine cloth. Color of the fresh juice was light green with turbidity.

Fractionation of juice

Juice was fractionated (liquid-liquid extraction) in separating funnel successively with organic solvents in ascending order of their polarity. The juice was concentrated and then extracted with further solvents petroleum ether, benzene, solvent ether, chloroform and methanol. Solvents removed by evaporators and weight determined.

Animal

The study was carried out in Sprague–Dawley albino rats of either sex (160-200 g). All animals were housed in group of 5 and maintained under standard condition (12/12 h light/dark cycle, 25±2^{0}C) with free access to pellet rat diet (Gold-Mohor Brand, India) and water ad libitum. Institutional Animal Ethics Committee, constituted under the guidelines of CPCSEA, Ministry of Environment, Govt. of India, New Delhi, approved all the animal experimental protocol.

Acute toxicity study in rats

The petroleum ether, benzene, solvent ether, chloroform and methanolic extract was administrated at increasing doses of 125, 250, 500, 750, 1000 and 2000 mg/kg bw by oral route. After administration of the extracts, the animals were observed continuously for 24 h (with special attention given during the first 2 h). Changes in the normal activity of rats due to acute toxicity were monitored. The lethal dose that killed 50% of the mice was estimated after 24 h, applying the method of[9]. Effective dose has been decided on the bases of above study.

Evaluation of hyperlipidaemia

Triton-induced hyperlipidaemic protocol method A

For this protocol total eight groups of rats (six rats each group) were formed. 16 h fasted rats were employed in the study. Before starting of experiment, for baseline readings, blood was withdrawn from retro orbital plexus (using a fine capillary) of ether-anaesthetized rats. Serum was obtained from blood by centrifugation (150x g, 10 minutes) and then analyzed for total triglycerides and total cholesterol.

Hyperlipidaemia was induced by single i.p. injection of Triton WR 1339 (350mg/kg, 07 % solution in normal saline). All the test fractions were administered by gavage route (only once in entire experiment) immediately after triton administration. Fractions were tested at two-dose levels 250 mg/kg and 500 mg/kg-body weight. Rats were not fed but had free access to water during the experiment period (48 h).

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Cholesterol induced hyperlipidaemic method B

The method described by[14] was employed in the study. In this method hyperlipidaemia was induced by cholesterol fractions of juice which were effective in triton developed hyperlipidaemia were tested. At the start and at the end of protocol (9th day) animals were fasted for 12 h and blood was withdrawn and serum was estimated for lipid profile (HDL-c, LDL-c, VLDL-c, total triglycerides, total cholesterol, TC: HDL, LDL: HDL, atherogenic index)

Blood collection and biochemical analysis

Serum sample were analysed for the estimation of TC, TG, LDL, HDL, atherogenic index, TC: HDL, LDL: HDL and atherogenic index as per reported method[14].

Evaluation of weight gain

A significant weight gain at the end of 9th day was found in cholesterol administered animals compared to normal control animal but weight gain was significantly less in petroleum ether and methanolic groups compared to cholesterol administer rats.

Determination of fecal fat

During the last three days of the experiment feces were collected every morning, dried and preserved for analysis[13]. Fat was determined using gravimetery method. A weigh amount of dried feces (homogenized) was extracted with petroleum ether. The extract was evaporated to dryness and the residue was weighed. This was repeated till constant weight was obtained. The weight was taken to represent the amount of fat present in the specimen.

Preliminary phytochemical screening

Petroleum ether and methanolic fractions were showed antihyperlipidaemic and antiatherosclerotic activities; therefore to know the principles present in them, they were tested for primary and secondary metabolites.

Thin layer chromatography

Petroleum ether and methanolic fractions of LSSL juice showed antihyperlipidaemic and antiatherosclerotic activities therefore they were subjected to thin layer chromatography to find out the probable number of compounds present in them. The adsorbent used for thin layer chromatography was silica gel G. A number of developing solvent systems were tried, for each fraction but the satisfactory resolution was obtained for petroleum ether hexane: acetone (90:10) and for methanolic chloroform: ethyl acetate (90:10).

Statistical analysis

Data were statistically analyzed as means ± S.E.M. and expressed as extremely significant compared to normal control ($p<0.0001$); Student's *t test*, and significant compared to triton group ($p<0.01$); one way ANOVA followed by Dennett's *t test.*

RESULT AND DISCUSSION

Fractionation of juice

Preliminary phytochemical screening showed the presence of steroids, tannins and flavonoids, so primary role of these secondary metabolites in above activities cannot be ruled out (result not shown).

Thin layer chromatography

TLC of the petroleum ether extract on precoated silica gel GF_{254} plates using hexane: acetone (90: 10) showed under UV 365 nm, three fluorescent zones at R_f value 0.45, 0.75 and 0.67 respectively (brown, light green and green). Methanolic extract on precoated silica gel GF_{254} using chloroform: ethyl acetate (90:10) showed less than 370 nm, three fluorescent zones at R_f value 0.35, 0.69, and 0.73 (light brown, pink and light pink respectively). On spraying with sulphuric acid: anisaldehyde reagent and heating the TLC plate for 10 min at 105 C, three spots were appeared at R_f value 0.45, 0.65 and 0.57 (greenish, purple and light brown respectively) and at R_f value 0.35, 0.61 and 0.76 (brown, purple and light brown).

Toxicological studies

The oral administration of the petroleum ether, benzene, solvent ether, chloroform and methanolic extract was administrated at increasing doses of 125, 250, 500, 750 and 1000 mg/kg bw by oral route did not produce mortality in rats up to 2000 mg/kg bw. The single dose LD50 was found to be 5000 mg/kg bw.

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Triton-induced hyperlipidaemic protocol

By triton used as standard, total cholesterol and triglycerides level were significantly increased (P<0.0001) in rats as compared to control rats. Rats with dried petroleum ether and methanolic extracts (250 mg/kg) caused significant (P<0.01) fall in total cholesterol and triglycerides (compared to triton group). Remaining different test extracts and residue, remained after successive extraction, failed (even at 500 mg/kg) to show any change in above parameters (TABLES 1 and 2).

Benzene, solvent ether, chloroform and residue were not effective at 250 mg/kg so all were tested at 500mg/kg b. w., keeping all the experimental conditions same excepting dose (TABLE 2).

Cholesterol induced hyperlipidaemia

To support above findings and to estimate other lipid related and atherosclerosis parameters, e.g. LDL-c, HDL-c, VLDL-c, TC: HDL, LDL: HDL and atherogenic index, one more antihyperlipidaemic protocol (cholesterol induced) was executed. This study also revealed the beneficial effects of petroleum ether and

TABLE 1 : Effect of successive fractions of stem and leaves juice of *Lagenaria siceraria* on synthesis (24 h) and excretory (48 h) phases of triton-induced hyperlipidaemia in rats (250 mg/Kg)

Group	Total cholesterol (mg/dl)			Total triglycerides (mg/dl)		
	Initial	After 24 h*	After 48 h*	Initial	After 24 h*	After 48 h*
control	86±2.258	86±1.924	85.4±1.887	94.2±2.657	92.4±2.227	89±1.897
Triton	77.8±1.393	335.8±9.635[a]	155.8±1.908[a]	65.6±1.806	982±8[a]	216±4.848[a]
Petroleum ether	77.8±0.969	105±7.029[b]	94.2±2.577[b]	84.6±2.112	238±10.265[b]	103±2.51[b]
Benzene	77.4±2.731	327.2±7.768	156.8±2.8	65±1.817	983±4.359	208.8±6.078
Solvent ether	83±0.836	333±8.888	158.2±2.596	72.2±2.131	988.8±6.119	213.2±5.229
Chloroform	86.4±1.6	335.8±9.635	155.8±.908	71.2±1.393	988.8±4.641	208±3.619
Methanolic	91.6±1.4	75.2±6.461[b]	71.4±5.819[b]	71.2±1.393	283.2±11.586[b]	89±9.742[b]
Residue	102.2±1.463	321.4±7.859	152.6±2.249	68.4±1.077	980±9.121	214.4±4.611

*After triton administration, N=5, values are mean ± S. E. M.; a: extremely significant compared to normal control (p<0.0001); Student's t test; b: significant compared to triton group (p<0.01); one way ANOVA followed by Dennett's t test

TABLE 2 : Effect of successive fractions of stem and leaves juice of *Lagenaria siceraria* on synthesis (24 h) and excretory (48 h) phases of triton-induced hyperlipidaemia in rats (500 mg/kg)

Group	Total cholesterol (mg/dl)			Total triglycerides (mg/dl)		
	Initial	After 24 h*	After 48 h*	Initial	After 24 h*	After 48 h*
Normal Control	86±2.258	86±1.924	85.4±1.887	92.4±2.65	92.4±2.227	89±1.897
Triton	77.8±1.393	335.8±9.635[a]	155.8±1.908[a]	65.6±1.806	982±17.889[a]	216±4.848[a]
Benzene	77.4±2.731	334±9.274	158±2.55	65±1.817	984.2±6.393	212.2±6.127
Solvent ether	83±0.836	333.8±7.459	186.8±28.561	72.2±2.131	987.6±11.664	209.8±6.996
Chloroform	86.4±1.6	328.6±12.824	161.2±3.513	71.2±1.393	956.2±15.383	209.4±6.997
Residue	102.2±1.463	334.6±8	159.8±3.541	68.4±1.077	958.8±16.951	213.8±4.883

*After triton administration, N=5, values are mean ± S. E. M.; a: extremely significant (p<0.0001) compared to normal control; Student's t test

methanolic extracts. Values were not significant compared to standard. At the end of 9th day there were significant increase in above parameters of cholesterol-administered rats excepting HDL-c (which decreased significantly) compared to control rats (P<0.0001) (TABLE 3)

Besides reducing total cholesterol, total triglycerides, LDL-c, VLDL-c, TC: HDL, LDL: HDL and atherogenic index, petroleum ether and methanolic extracts increased HDL-c level significantly compared to cholesterol treated rats (P<0.01) (TABLE 4). A significant weight gain (at the end of 9th day) was found in cholesterol administered animals compared to normal control animal but weight gain was significantly less in petroleum ether and methanolic groups compared to cholesterol administer rats. In rats fed high cholesterol

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diet (Method B), fractions reduced the lipid levels by virtue of increased fecal fats (results not shown).

Fractions showed therapeutic effect as they can reduce high levels of lipids by increasing latter's metabolism (catabolism) and eventual excretion. Because of significant reduction in atherogenic risk ratio (TC: HDL and atherogenic index) fractions could also be said antiatherosclerotic.

Triton induced hyperlipidaemia[4,12,16] occurs in 2 phases. The initial phase was increase of lipid levels, the maximum reaching 24 h after the administration of triton, is referred to as the synthesis phase. From the 24th h, the lipid levels decrease, almost reaching normal levels by the end of 48th h, this is referred to as excretory phase. The biphasic nature of triton induced hyperlipidaemia is helpful in understanding the mode of

TABLE 3 : Effect of different extracts of stem and leaves juice of *Lagenaria siceraria* on cholesterol induced hyperlipidaemia in rats (250 mg/kg)

Group	Total cholesterol		Total triglycerides		HDL –c		LDL-c	
	Initial	At the end of 9th day	Initial	At the end of 9th day	Initial	At the end of 9th day	Initial	At the end of 9th day
Normal Control	117±1.924	116.2±1.855	107.6±3.796	108±3.742	24.6±2.694	24.6±2.421	70.8±2.289	69.6±1.720
Cholesterol	118.6±2.356	155.2±2.672[a]	106.2±2.557	150.2±2.835[a]	26.2±1.934	17.8±1.960[a]	71±2.881	107.4±3.203[a]
Standard (Gemfibrozil)	117.4±1.965	120.2±2.853[b]	108±4.012	121.2±3.153[b]	25.8±2.354	37.2±1.158[b]	70±2.168	58.8±2.888[b]
Petroleum Ether	118.8±2.375	127.2±3.007[b]	111±2.074	124.2±2.782[b]	22.6±2.249	30.2±2.835[b]	74.2±4.283	72±4.626[b]
Methanolic	117.4±1.965	124.8±4.42[b]	108.8±0.583	122.2±3.992[b]	26.6±2.750	38.4±1.288[b]	69±2.828	61.8±4.587[b]

a: extremely significant compared to normal control (p<0.0001); Student's t test; b: significant compared to cholesterol group (p<0.01); one way ANOVA followed by Dunnet's t test; Values are mean ± S.E.M. of five rats

TABLE 4 : Effect of successive fractions of stem and leaves juice of *Lagenaria siceraria* on cholesterol induced hyperlipidaemia in rats (250mg/kg)

Group	VLDL-c		LDL: HDL		TC: HDL		Atherogenic index	
	Initial	At the end of 9th day	Initial	At the end of 9th day	Initial	At the end of 9th day	Initial	At the end of 9th day
Normal Control	21.6±0.748	21.6±0.748	3.024±0.367	2.946±0.310	4.944±0.444	4.874±0.392	3.944±0.444	3.854±0.381
Cholesterol	21±0.447	30±.547[a]	2.794±0.303	6.326±0.708[a]	4.632±0.38	9.086±0.868[a]	3.632±0.38	8.086±0.868[a]
Standard (Gemfibrozil)	21.6±0.748	24.2±0.663[b]	2.586±0.249	1.588±0.108[b]	4.68±0.377	3.244±0.142[b]	3.676±0.38	2.244±0.142[b]
Petroleum Ether	22±0.316	24.6±0.6[b]	3.52±0.6168	2.56±0.47[b]	5.542±0.732	4.412±0.561[b]	4.456±0.731	3.414±0.563[b]
Methanolic	22±0.316	24.8±0.489[b]	2.75±0.396	1.628±0.16[b]	4.61±0.485	3.268±0.197[b]	3.62±0.492	2.268±0.197[b]

a: extremely significant compared to normal control (p<0.0001); Student's t test; b: significant compared to cholesterol group (p<0.01); one way ANOVA followed by Dunnet's t test; Values are mean ± S. E.M. of five rats

action of the antihyperlipidaemic agents. Drugs interfering with lipid biosynthesis or uptake will be active in the synthesis phase, while drugs interfering with lipid excretion and metabolism will be active in the excretory phase. The antihyperlipidaemic activity of methanolic and petroleum ether fractions were evident in both synthesis and excretory phases of triton induced hyperlipidaemia in rats.

In the present study, method B also revealed the beneficial effects of petroleum ether and methanolic extracts. At the end of 9th day there were significant increase in lipid parameters of cholesterol-administered rats except HDL-c (which decreased significantly) compared to control rats (P<0.0001). Besides reducing TC, TG, LDL-c, VLDL-c, TC: HDL, LDL: HDL and atherogenic index, petroleum ether and methanolic extracts increased HDL-c level significantly compared to cholesterol treated rats (P<0.01). A significant weight gain at the end of 9th day was found in cholesterol administered animals compared to control animal but weight gain was significantly less in petroleum ether and methanolic groups.

Because of significant reduction in atherogenic risk ratio (TC: HDL and atherogenic index) fractions could also be said antiatherosclerotic.

Increased metabolism could be due to an increase in HDL cholesterol, indicating that fractions may be mobilizing cholesterol from extra hepatic tissue to the liver

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where it is catabolised.

During the study, albino rats did not show any mortality or other adverse effect when the rats fed gavage with LSSLE at the dose of 250-500 mg/kg, hence it is safe. The results concluded that the LSSLE have definite antihyperlipidaemic and hence antiatherosclerotic potential, therefore this can be used for the treatment of coronary artery diseases.

ABBREVIATIONS

AI : atherogenic index
LDL-c : low density lipoprotein cholesterol friction
VLDL-c : low density lipoprotein cholesterol friction
HDL-c : high density lipoprotein cholesterol friction
LS : *Lagenaria siceraria*
LSSLE : *Lagenaria siceraria* stem and leaves
CHD : coronary heart diseases

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