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Hypolipidemic and antihyperlipidemic effects of *Lagenaria siceraria* (Mol.) fruit extracts

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Bottle gourd [(*Lagenaria siceraria* (Mol.) Stand.] fruit is ascribed with many therapeutic effects. The present study was undertaken to explore the antihyperlipidemic effect of four different extracts *viz.* petroleum ether, chloroform, alcoholic and aqueous extracts from bottle gourd in Triton-induced hyperlipidemic rats and their hypolipidemic effects in normocholesteremic rats. The study is comprised preliminary phytochemical screening of the extracts. Oral administration of the extracts, at doses of 200 and 400 mg/kg body weight in rats, dose-dependently inhibited the total cholesterol, triglycerides, low-density lipoproteins level, and significantly increased the high density lipoproteins level. However, petroleum ether extract did not show the significant effects. Both the chloroform and alcoholic extract exhibited more significant effects in lowering total cholesterol, triglycerides and low density lipoproteins along with increase in HDL as compared to the others. Preliminary phytochemical screening revealed the presence of flavonoids, sterols, cucurbitacin saponins, polyphenolics, proteins, and carbohydrates. The results obtained suggest marked antihyperlipidemic and hypolipidemic activity of the extracts.

Keywords: Antihyperlipidemic effect, Fruit extracts, Hypolipidemic effect, *Lagenaria siceraria*

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

Lagenaria siceraria fruit is a common vegetable known as bottle gourd or lauki, a pubescent or trailing herb with bottle, oval or dumbbell shaped fruit which is traditionally used for its cardioprotective, cardiotonic, general tonic and diuretic properties along with antidote to certain poisons and scorpion stings. The fruit is considered as a good source of choline, vitamin B complex, fibers, and proteins, and also as a fair source of vitamin C, β-carotene, cucurbitacins and saponins[2-5]. Methanolic fruit extract showed superoxide inhibition in XA/XOD medium[6]. HPLC analysis of methanolic extract from *L. siceraria* showed the presence of flavones-C glycoside[7]. A novel protein Lagenin is also isolated from the seeds of *L. siceraria*[8]. Fruits are reported to contain more soluble dietary fibers (SDF) than insoluble cellulose fibers (ICF); SDF are having profound effect in lowering cholesterol, which indicates that pectin may be a predominant component of SDF from *L. siceraria* fruits[3]. In addition, Sannoumaru, *et al*.[9] reported that semi-purified dietary fibers isolated from *L. siceraria* fruits have an effect on fecal excretion of steroids.

However, despite such interesting health virtue of this fruit a perusal of literature reveals that no scientific study has been carried out to screen the pharmacological activity of the extracts from the same in terms of their antihyperlipidemic or hypolipidemic effects. This investigation is an attempt to find out the possible effects of four different extracts from *L. siceraria* fruit (LSFE) in Triton-induced hyperlipidemic rats as well as in normochlolesteremic rats, by measuring the changes in different parameters of blood-lipid profile, *viz.* TC, TG, LDL and HDL.

Several studies showed that systematic administration of Triton WR1339 (ionic surfactant) in fasted rats causes elevation in plasma lipid level. Initially, there is sharp increase in lipid level reaching a peak two to three times the control value by 24 hr after the administration of Triton injection, phase I (synthetic phase), this hyperlipidemia falls off within

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next 24 hr i.e. 48 hr after Triton administration, phase II (excretion phase). This increase in plasma lipids by Triton is thought to be due one of the following mechanisms; as due to increased hepatic synthesis of cholesterol or Triton physically alters very low density lipoproteins (VLDL) rendering their removal from the blood. Drugs interfering with cholesterol synthesis were shown to be active in phase I, while drugs interfering cholesterol excretion and metabolism were active in phase II[10-12]. The method employed of Triton-induced hyperlipidemia is rather simple and rapid for the evaluation of test substance and can be considered as the useful method for preliminary screening of antihyperlipidemic drugs[9]. It provides the supportive assessment in preliminary screening with advantage of simplicity and rapidity[10].

Materials and Methods

Collection and extraction—The fresh fruits of *L. siceraria* were collected from the local farms of Wardha District and authentified by the Department of Botany, Nagpur University, Nagpur. The fruits were swarfed coarsely powdered and shade exsiccated. The coarsely powdered plant material was then successively extracted in a Soxhlet with petroleum ether, chloroform, and 90% (v/v) alcohol and then cold macerated with water to obtain the respective extracts viz; PTE, CHE, ALE and AQE. All the extracts were filtered, distilled and the last traces of solvents were removed *in vacuo*. The yields obtained were 1.5, 2.1, 12.5, and 27.5% (w/w) of dried, coarsely powdered plant material for petroleum ether (PTE), chloroform (CHE), alcohol (ALE) and water (AQE), extracts respectively. The extracts were administered in the form of suspension p.o. at two dose levels of 200 and 400 mg/kg body weight to rats as 1 % (w/v) sodium carboxy methyl cellulose (SCMC) for evaluating their hypolipidemic and antihyperlipidemic effects.

All the extracts were subjected to preliminary phytochemical screening for the detection of various phytoconstituents. All chemicals used were of AR grade. Triton was purchased from SD Fines Chemical and Kits for lipid profile analyses were purchased from a local supplier, viz; TC and HDL (Span Diagnostics), TG (Diassays, Germany). Male Albino rats of the Sprague-Dawley strain (average weight 150 g), bred and maintained in our Institute's Animal House were used for the study after prior scrutinization and approval from Institutional Animal Ethical Committee (IAEC). Antihyperlipidemic potential of LSFE in Triton-induced hyperlipidemic rats and in normal rats was evaluated as per the method described by Moss[10], Vogel[13] and Hirsch, *et al*[14].

Effects in Triton-induced hyperlipidemic rats—The antihyperlipidemic effects of the above extracts were evaluated in 45 Triton-induced hyperlipidemic rats starved for 18 hr. The rats were divided into 9 groups of 5 each and then injected, ip, with Triton at a dosage of 100 mg/kg body weight except rats of Group I, which served as normal vehicle treated and Group II as control treated with 1% SCMC, po. Group III-IX were treated daily with PTE, CHE, ALE and AQE extracts in two divided doses of 200 and 400 mg/kg, respectively immediately after the Triton injection by ip administration. Blood samples were collected after 6, 24 and 48 hr of Triton injection to evaluate the lipid profile.

Effect on normochlolesteremic rats—The hypolipidemic effects of the extracts were evaluated in 9 groups of a total of 45 normochlolesteremic rats fasted for 18 hr and these studies were carried out as described for antihyperlipidemic effects. The rats were treated orally for 7 days with the divided doses of 200 mg and 400 mg/kg, po. After the end of the stipulated period of drug treatment, all animals were starved for 20 hr and blood samples were collected from the puncture of retro-orbital plexus and analyzed for blood lipid profile.

Data analysis— Data were statistically analyzed as mean ±SE and expressed as non-significant $P>0.05$, just significant $P<0.05$ and significant $P<0.01$ as the case may be using ANOVA followed by Dunnett's *t*-test and unpaired *t*-test with Welch correction.

Total cholesterol was determined by one-step method of Wybenga and Pillegi[11] based on the reaction between cholesterol and cholesterol reagent (ferric chloride, ethyl acetate and sulphuric acid). HDL from blood was determined by a two-step method i.e. initial separation of HDL from blood using a precipitating agent and then the precipitated HDL was determined by using colorometric reaction with cholesterol reagent[15]. Triglyceride (TG) was determined colorimetrically by enzymatic reaction using glycerol-3-phosphate oxidase. Enzymatic splitting of lipoprotein lipase along with reaction between 4-aminoantipyrin, 4-chlorophenol and H_2O_2 under catalytic action of peroxidase generates quinimine which is used as internal indicator in this colorimetric determination[16]. LDL was

determined by using Friedelwald's formula — LDL=TC− (TC−VLDL)[17].

Results and Discussion

The preliminary phytochemical screening revealed the presence of sterols and fixed oil in petroleum ether extract; cardiotonic aglycones and sterols in chloroform extract; flavonoids, saponins, and polyphenolics in alcoholic extract; proteins, soluble dietary fibers, polyphenolics, and pectins in the aqueous extract. Results on lipid profile are given in Tables 1-3. All the extracts, except petroleum ether extract (results not shown), showed decrease in blood lipids in hyperlipidemic rats when compared with their respective controls. Among the extracts only mere or less polar ones i.e. CHE, ALE and AQE showed significant (P<0.05) decrease in TC, TG and LDL along with an increase in HDL level, dose-dependently at the tested dose (400 mg/kg body weight), when administered in Triton-induced hyperlipidemic rats (Tables 1 and 2). Evaluation in normochlolesteremic rats showed the same results as mentioned above (Table 3).

The antihyperlipidemic potential of LSFE can be proved, as all the above three extracts from *L. siceraria* fruits showed decreases in TC, TG and LDL along with an increase in HDL in biphasic model of Triton-induced hyperlipidemic rats as well as in normochlolesteremic rats. The lipid lowering effects of CHE may be due to its content of plant sterols[18] (campesterol, fucosterol, etc.) and fixed oil, which is considered as good source of mono- and poly-unsaturated fatty acids and cardiac aglycones. Plant sterol reduces the absorption of cholesterol and thus increases the fecal excretion of steroids that results in decrease of body lipids[19]. Secondary plant metabolites such as flavonoids, saponins and polyphenolics from polar extracts may be responsible for the antihyperlipidemic activity. Flavonoids from ALE may augment the activity of lecithin acyl transferase (LCAT), which regulates blood lipids. LCAT plays a key role in the incorporation of free cholesterol into

Table 1—Effect of LSFJEs on total cholesterol and triglyceride levels in Triton-induced hyperlipidemic rats
[Values, expressed as mg/dl, are mean ±SE of 5 animals in each group]

Group(s)/	Cholesterol			Triglyceride		
Treatment	6 hr	24 hr	48 hr	6 hr	24 hr	48 hr
I. Normal	68.71±1.32	69.58±0.64	70.17±1.36	62.71±1.62	59.86±0.60	63.82±1.04
II. Control	103.78±2.49^{x}	189.26±3.56^{z}	98.26±2.10^{x}	98.14±1.40^{x}	140.39±2.36^{x}	87.60±3.12^{y}
III. AQE 200	101.71±2.43^{ns}	185.06±3.39^{ns}	95.22±1.70^{ns}	96.39±2.41^{ns}	138.52±2.66^{ns}	85.96±1.87^{ns}
IV. AQE 400	95.57±1.62^{b}	163.78±2.48^{a}	87.24±2.46^{a}	88.85±1.22^{b}	121.69±1.68^{a}	82.83±1.52^{ns}
V. ALE 200	102.15±2.71^{ns}	185.41±2.74^{ns}	95.54±1.61^{ns}	96.74±1.61^{ns}	137.77±1.72^{ns}	84.94±1.06^{ns}
VI. ALE 400	95.57±1.62^{b}	169.83±2.47^{a}	86.18±1.26^{a}	93.63±2.70^{ns}	125.50±2.82^{a}	83.36±0.91^{ns}
VII. CHE 200	100.07±2.18^{ns}	185.60±3.61^{ns}	95.39±1.50^{ns}	95.93±1.49^{ns}	95.39±1.56^{a}	84.26±1.34^{ns}
VIII. CHE 400	92.91±1.54^{a}	168.69±2.95^{a}	83.83±1.22^{a}	86.62±2.45^{a}	120.01±1.56^{b}	81.74±1.92^{ns}

In statistical analysis, the results of Group I are compared with Group II (unpaired *t* test) and the extract treated groups have been compared with their respective control i.e. Group II (ANOVA followed by Dunnett's *t*-test).
$^{a}P < 0.01$; $^{b}P < 0.05$; $^{ns}P > 0.05$ (Dunnett's *t*-test).
$^{x}P < 0.0001$; $^{y}P < 0.001$; $^{z}P < 0.067$ (Unpaired *t*-test).

Table 2— Effect of LSFJEs on HDL and LDL levels in Triton-induced hyperlipidemic rats
[Values, expressed as mg/dl, are mean ±SE of 5 animals in each group]

Group(s)/	HDL			LDL		
Treatment	6 hr	24 hr	48 hr	6 hr	24 hr	48 hr
I. Normal	26.34±1.99	25.76±1.31	25.59±1.68	54.75±2.46	59.19±0.63	57.33±1.69
II. Control	20.86±1.67^{z}	16.48±1.47^{y}	21.47±1.58^{z}	102.36±1.75^{x}	178.03±0.67^{x}	94.24±1.45^{x}
III. AQE 200	19.27±2.32^{ns}	19.13±1.94^{ns}	21.70±1.83^{ns}	101.15±1.84^{ns}	176.40±1.89^{ns}	90.02±1.26^{ns}
IV. AQE 400	22.88±2.35^{ns}	23.41±2.13^{b}	23.29±1.29^{ns}	91.42±1.72^{a}	162.42±2.77^{a}	80.43±1.89^{a}
V. ALE 200	19.58±0.96^{ns}	18.98±1.52^{ns}	20.45±1.32^{ns}	101.42±1.87^{ns}	176.69±2.12^{ns}	92.07±1.85^{ns}
VI. ALE 400	22.72±2.16^{ns}	22.30±1.60^{ns}	23.86±1.53^{ns}	94.40±1.28^{b}	167.00±1.95^{a}	78.34±1.48^{a}
VII. CHE 200	22.33±1.37^{ns}	21.85±1.42^{ns}	22.11±1.95^{ns}	97.87±1.23^{ns}	181.18±1.68^{ns}	87.75±1.72^{b}
VIII. CHE 400	23.78±1.51^{ns}	24.40±1.63^{b}	25.28±1.04^{ns}	86.49±1.58^{a}	167.08±2.47^{a}	74.98±1.65^{a}

In statistical analysis, the results of Group I are compared with Group II (unpaired *t* test) and the extract treated groups have been compared with their respective control i.e. Group II (ANOVA followed by Dunnett's *t*-test).
$^{a}P < 0.01$; $^{b}P < 0.05$; $^{ns}P > 0.05$ (Dunnett's *t*-test).
$^{x}P < 0.0001$; $^{y}P < 0.001$; $^{z}P < 0.067$ (Unpaired *t*-test).

Table 3— Effect of LSFJEs on blood lipid profile in normocholesteremic rats
[Values, expressed as mg/dl, are mean ±SE of 5 animals in each group]

Group(s)/	Blood lipid profile				Atherogenic index
Treatment	Cholesterol	Triglyceride	HDL	LDL	
I. Control	74.32±1.58	62.34±1.58	26.34±1.39	60.44±1.96	2.821
II. AQE 200	71.97±1.71^{ns}	59.53±1.55^{ns}	28.21±1.15^{ns}	55.82±1.35^{ns}	2.551
III. AQE 400	67.44±1.53^{b}	56.96±1.49^{ns}	31.40±1.25^{b}	47.35±1.77^{a}	2.147
IV. ALE 200	71.85±1.36^{ns}	59.13±1.42^{ns}	29.30±1.01^{ns}	54.35±1.74^{ns}	2.452
V. ALE 400	66.63±1.52^{a}	55.76±1.25^{b}	35.62±1.39^{a}	45.15±1.32^{a}	1.870
VI. CHE 200	70.79±1.58^{ns}	57.46±1.07^{ns}	30.16±1.48^{ns}	50.83±1.63^{a}	2.347
VII. CHE 400	65.74±0.54^{a}	54.43±1.43^{a}	33.56±1.21^{a}	42.30±1.64^{a}	1.958

In statistical analysis the extract treated groups have been compared with their respective control.
$^{a}P < 0.01$; $^{b}P < 0.05$; $^{ns}P > 0.05$ (ANOVA followed by Dunnett's *t*-test).

HDL (this may increase HDL) and transferring it back to VLDL and LDL which are taken back later in liver cells. Several studies have showed that increase in HDL-C is associated with decrease in CAD[12,19,20]. Saponins also act as antihyperlipidemics by one of the following mechanisms as by binding with cholesterol in intestinal lumen, so that cholesterol is less readily absorbed or bile acids causing reduction in its extra hepatic circulation and increasing metabolism of cholesterol to sterols through their fecal excretion. Increase in bile acid excretions offset by enhanced synthesis from cholesterol in the liver consequently lowers the plasma cholesterol. Saponins are also reported to increase the lipoprotein lipase activity (LPL), which is considered as helpful in faster removal of free fatty acid from circulation that causes in turn a decrease in total cholesterol[19,21,22]. It has also been ascertained that parts of soluble dietary fibers such as pectin from the fruits of *L. siceraria* may have cholesterol-lowering effect. Soluble dietary fiber contents are having the beneficial effect in the promotion of bile acid formation and their excretion in the stool or in the blockage of cholesterol absorption[3,9,23].

Plant proteins are considered as less hyperlipidemic than animal proteins. The exact action mechanism by which proteins act as antihyperlipidemics is not yet clear but the following mechanism may be considered. In plant proteins, especially the ratio of lysine:arginine (L:A) is less than 2, which is important for the control of the progression in hyperlipidemia and arteriosclerosis. L:A ratio of *L. siceraria* proteins is only 0.45, which is less than that reported for the well documented plants viz; L:A ratios of soy proteins, garlic proteins, and coconut proteins are 0.84, 0.7, and 0.86, respectively[24,25]. Thus on the basis of results of the present study, it can be concluded that LSFE have a definite potential as reported for several traditional antihyperlipidemic drugs. Further researches on the fractionation of extracts, isolation, purification and characterization of active constituents responsible for the antihyperlipidemic activity and their mechanisms are in progress.

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