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Effect of *Lagenaria siceraria* and *Trigonella foenum graecum* on Lipid Absorption and Excretion for Modulation of Lipid Profile

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ABSTRACT

Background: Hyperlipidemia is a metabolic disorder characterized by elevated serum total cholesterol, low density and decreased high-density lipoprotein levels. *Lagenaria siceraria* contains saponins and dietary fibers which are helpful in faster removal of fatty acids and in promotion of bile acid formation. Similarly *Trigonella foenum graecum* contains steroidal saponins and soluble fibers which have been attributed to anti-hyperlipidemic activity. **Objective:** The objective of the study was to evaluate anti-hyperlipidemic activity of the combinatorial extract of *Lagenaria siceraria* and *Trigonella foenum graecum.* **Materials and Methods:** In the present study, hyperlipidemia was induced in rats by a single dose of Triton WR-1339 (300 mg kg^{-1}). The treatment group comprised of ethanolic extracts of *Lagenaria siceraria* fruit (200 mg kg^{-1}), *Trigonella foenum graecum* extract (200 mg kg^{-1}), combinatorial extract (100 and 100 mg kg^{-1}) and Atorvastatin (10 mg kg^{-1}). Activity was confirmed by biochemical analysis of blood plasma. **Results:** It was observed that there was a significant decrease ($p<0.001$) in triglyceride, total cholesterol and LDL plasma concentration and an increase in plasma HDL levels. The combinatorial extract effectively modulated the lipid profile in hyperlipidemia induced rats. **Conclusion:** The combinatorial extract of *Lagenaria siceraria* fruit and *Trigonella foenum graecum* can potentially serve as therapeutic agents in the treatment of coronary artery diseases.

Key words: Hyperlipidemia, triglyceride, cholesterol, HDL, LDL

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INTRODUCTION

Hyperlipidemia is characterized by elevated serum total cholesterol, low density and decreased high-density lipoprotein levels. It is a metabolic disorder and is closely related to coronary heart disease and diabetes[1].

According to global survey on non-communicable diseases in 2010, approximately 2.8 million people die per annum of being overweight or obese which is also one of the risk factor for developing cardiovascular diseases and around 2.6 million people die globally every year due to raised cholesterol which develops heart diseases and stroke[2].

Hyperlipidemia is a condition, characterized by abnormal elevation of lipid such as (triglyceride and cholesterol) and lipoproteins such as (LDL, VLDL) levels in the blood. Elevated plasma triglyceride concentrations contribute to increased risk of metabolic cardiovascular diseases, both directly and due to these elevations. This is associated with risk factors like obesity, proinflammatory markers, metabolic syndrome, biomarkers and type II diabetes mellitus.

Cholesteryl esters and triglycerides, water insoluble lipids, are the core components and apoproteins, phospholipids and unesterified cholesterol, water soluble components, are located on the surface and perform regular functions[3]. But due to defect in the lipid metabolism whether genetically or due to sedentary lifestyle, there is a disturbance in the lipoprotein lipase activity or due to absence of the surface Apoprotein C-II, the end result is hyperlipidemia[4].

Statins and fibrates are the well-established treatments for hyperlipidemias and the prevention of vascular events. However, if both i.e., statins and fibrates

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are used together as previous studies have made known, it results in development of rhabdomyolysis if gemfibrozil is taken originally with lovastatin and recently, even with cerivastatin. Again, clofibrate causes cholesterol gallstones and inhibition of glucuronidation of statins. Other alternative of niacin also causes problems with patient compliance.

Despite of several available interventions to counteract the disease, epidemiological data are witnessing the growing trend of the problem, reflecting both the multiple etiologies as well as scarce compliance of patients to established therapies[5].

Lagenaria siceraria fruit (LSF); Molina commonly known as bottlegourd, has composition of all the essential constituents like choline, vitamin B complex, fibers and proteins like lagenin-A novel ribosome-inactivating protein and also as a fair source of vitamin C, β-carotene, cucurbitacins and saponins. Since the saponins can be very helpful in faster removal of free fatty acids from the circulation that causes in turn a decrease in total cholesterol by enhancing the lipoprotein lipase activity (LPL) as reported in the previous studies. In addition to this LSF also contains the soluble dietary fibers such as pectin which helps in promotion of the bile acid formation and in the inhibition of cholesterol absorption or their excretion in stool[6-11].

Another such anti-hyperlipidemic plant *Trigonella foenum graecum* (TFG) usually called as fenugreek or Methi belongs to Leguminosae family has been used in foods or as a medicine since ages. Fenugreek seeds contain proteins rich in lysine and L-tryptophan, infrequent chemical constituents such as saponins, fenugreekine, coumarin, nicotinic acid, sapogenins, phytic acid, scopoletin, trigonelline and mucilaginous fiber which are thought to account for many of its presumed therapeutic effects[12-16].

The present study was done with an objective to study the effect of the combinatorial extract for the enhanced anti-hyperlipidemic activity in triton induced model of hyperlipidemia. The activity was evaluated by estimation of biochemical parameters: Cholesterol, triglycerides, LDL and HDL plasma levels.

MATERIALS AND METHODS

Standardization of plant material: From the local market of Mumbai, India, in the months of February-March, the fruits of *Lagenaria siceraria* and the seeds of *Trigonella foenum-graecum* were procured. For authentication, the specimen samples were sent to Ramnarain Ruia College, Matunga (E), Mumbai.

Extraction procedures: The *Lagenaria siceraria* extract was prepared by initially defatting the fruits using petroleum ether (60%) and subsequently extracted with ethanol (95%) using soxhlet apparatus and finally concentrated using rotary evaporator. The dried seeds of *Trigonella foenum-graecum* were used to prepare the extract. This was done by heating at reflux with 95% ethanol for 6-12 h. The mixture was filtered by suction filtration and then the filtrate was concentrated by rotary evaporator.

Characterization of *Lagenaria siceraria* and *Trigonella foenum-graecum* extract: Phytochemical tests were performed on the extracts of *Lagenaria siceraria* and *Trigonella foenum-graecum*, to check the presence of different valuable phytoconstituents like carbohydrates, alkaloids, glycosides, steroids, phenolics, flavonoids and proteins. This was done with the aid of standard phytochemical models[17-20].

The identification of compounds was carried on and confirmed using TLC. A mobile phase consisting of ethyl acetate: Chloroform: Formic Acid (70:29.5:0.5) was developed for *Lagenaria siceraria* extract and the Rf (Retention factor) values obtained were compared to the reported Rf values in Ayurvedic Pharmacopoeia of India. Similarly for *Trigonella foenum-graecum* extract the composition of mobile phase for the TLC optimized based on higher resolution was N-butanol: acetic acid: water (3:1:0.5).

***In vitro* model for evaluation of antioxidant activity using DPPH (1, 1–dipheny-2-picryl hydrazyl) method:** The evaluation of free radical scavenging activities of the two extracts along with the combinatorial extract was carried out using DPPH method. Ascorbic acid served as standard for comparison. Different concentrations of standard and extract were tested for their antioxidant activity. Absorbance was measured at 517 nm[21].

Animal studies

Toxicity studies: Acute oral toxicity studies were carried out on female albino mice in accordance with OECD guidelines 423. The mice were divided into three groups consisting of three mice each. *Lagenaria siceraria* fruit extract at dose 2 g kg^{-1} was tested for toxicity and similarly, *Trigonella foenum-graecum* extract at dose 2 g kg^{-1} and combinatorial extract (1 g and 1 g kg^{-1}) were tested. The treatments were given once and the animals were observed over a period of 14 days.

Induction of hyperlipidemia: The Wistar albino rats were obtained from Haffkine Institute, Parel, Mumbai. All the animals were acclimatized for a week prior to commencement of the study while maintaining the required humidity and temperature conditions as stated by the guidelines (temperature 25±2°C and humidity 75±5%) (CPCSEA approval number: CPCSEA/IAEC/SPTM/P-56/2011). Hyperlipidemia was induced in Wistar albino rats by single intraperitoneally injection of freshly prepared solution of triton WR 1339 (300 mg kg^{-1}) in 0.5% CMC (Carboxy Methyl Cellulose); after overnight fasting for 18 h[22].

Experimental design: The rats were divided into six groups, each group consisting of six animals. The experimental design and schedule of treatment was followed as:

Group I: Normal control
Group II: Positive control (hyperlipidemic rats)
Group III: Atorvastatin tablets (10 mg kg^{-1}); p.o. (standard drug)
Group IV: *Lagenaria siceraria* fruit extract (200 mg kg^{-1}); p.o.
Group V: *Trigonella foenum-graecum* extract (200 mg kg^{-1}); p.o.
Group VI: Combinatorial extract (100 and 100 mg kg^{-1}); p.o.

The standard and experimental treatment was given 24 and 48 h post-hyperlipidemia induction.

Biochemical analysis of plasma: At the end as well as at the initiation of the experiment, rats were kept fasting overnight, they were anaesthetized under light ether and blood samples were collected from retro orbital plexus using glass capillary in heparin coated tubes at 0, 24 and 48 h time interval. Plasma was separated by centrifugation at 4000 rpm for 5 min at 4°C and was then analyzed for total cholesterol, triglycerides, LDL and HDL using ERBA semi-auto analyser[21].

Statistical analysis: One way analysis of variance (ANOVA) followed by Bonferroni's test were applied for comparison of values for control and treated groups. All the values were expressed in terms of Mean±SEM (n = 6). Significant difference between control and experimental groups were assessed by student's t-test. ***$p<0.001$ were considered as statistically significant. The statistical analysis was carried out by the Graph Pad 3.0 software.

RESULTS AND DISCUSSION

Characterization of *Lagenaria siceraria* fruit (LSF) and *Trigonella foenum graecum* (TFG)

Physiochemical parameters: Foreign organic matter, ash values and extractive values were found to be within Pharmacopoeial limits (Ayurvedic Pharmacopoeia of India).

In *Lagenaria siceraria*, the total ash was 5.92% w/w and acid insoluble ash 0.19% w/w. The alcohol soluble extract was 12.7% w/w while water soluble extract was found to be 28.9% w/w. The moisture content was found to be 4.1% w/w. In *Trigonella foenum graecum*, foreign matter was found to be 1.18% w/w. The total ash was 3.95% w/w and acid insoluble ash 0.25% w/w. The alcohol soluble extract was 13.6% w/w while water soluble extract was found to be 36.2% w/w. The moisture content was found to be 3.9% w/w. The physicochemical standards of both the plant materials were found to be within the Pharmacopoeial limits.

Characterization of extracts by phytochemical tests: Tests for LSF extract confirmed the presence of carbohydrates, steroids, flavonoids, glycosides and phenolic compounds. Carbohydrates, flavonoids, glycosides and alkaloids were found to be present in the TFG extract.

Preliminary phytochemical screening was performed for all the herbal extracts in order to determine the organic as well as inorganic chemical constituents present in the plant extract. The tests indicated the presence of carbohydrates, steroids, flavonoids, phenolic compounds, glycosides in the LSF extract and the TFG extract in addition to these constituents was found to have alkaloids.

***In vitro* evaluation of antioxidant activity using DPPH (1, 1-dipheny-2-picryl hydrazyl) method:** The radical scavenging action of combinatorial extract was observed to have a significant increase than the herbal extracts used alone (Table 1).

Acute oral toxicity studies: The two extracts LSF and TFG alone as well as the combinatorial extract did not cause any mortality or symptoms of toxicity through the 14-day period.

Table 1: DPPH free radical scavenging activity of ascorbic acid standard solution

Concentration ($\mu g\,mL^{-1}$)	Inhibition Mean±SD (%)
5	28.46±1.06
10	41.17±0.65
15	52.87±1.28
20	66.10±0.42
25	74.51±0.97

Table 2: DPPH free radical scavenging activity of combinatorial extract of LSF and TFG

Concentration (μg mL^{-1})	LSF extract inhibition (%)	TFG extract inhibition (%)	Combinatorial extracts inhibition (%)
	----------(Mean±SD)----------		
100	16.07±0.67	12.87±0.75	43.09±0.77
200	21.41±0.69	23.88±0.97	38.04±0.35
250	29.08±1.31	29.07±0.60	42.09±0.33
300	34.56±1.44	37.52±1.22	48.07±0.86
350	42.21±0.95	39.16±1.13	55.34±0.99
400	50.90±0.57	46.95±0.61	61.54±0.59
450	54.03±0.89	54.59±0.76	68.74±0.47
500	60.56±0.51	64.58±0.59	73.76±1.43

In accordance to OECD 423 guidelines, the dose of 2 g kg^{-1} was selected. No mortality or any sign of toxicity were observed for the extracts given alone or in the combinatorial extract. Thus the extracts and their combinatorial extract were found to be safe as there was no mortality and no significant change in weight or feed-water intake was observed.

Antihyperlipidemic activity of combinatorial extract of LSF extract and TFG extract in triton induced hyperlipidemia: The acute administration of triton WR-1339 (300 mg kg^{-1}) caused marked increase ($p<0.001$) in the plasma levels of cholesterol, triglycerides (TG), Low Density Lipoprotein (LDL) 356.52±11.03, 1254.5±11.08 and 299.48±8.74 mg dL^{-1} at 24 h. In addition to this, there occurred significant reduction in High Density Lipoprotein (HDL) levels in hyperlipidemic animals as compared with normal control group. The HDL levels after 24 and 48 h time points were elevated in the animals treated with combinatorial extract as compared to positive control animals at the same time points (Table 2).

Effect of combinatorial extract of *Lagenaria siceraria* fruit extract and *Trigonella foenum graecum* extract on plasma lipid profile in rats: On treating the triton WR-1339 induced hyperlipidemic rats with LSF extract (200 mg kg^{-1}), TFG extract (200 mg kg^{-1}) and the combinatorial extract (200 mg kg^{-1}), caused decrease ($p<0.001$) in the levels of triglyceride (TG), total cholesterol, Low Density Lipoproteins (LDL) and increase in High Density Lipoproteins (HDL) as compared to the positive control (triton induced) animals (Fig. 1a-d).

Triton WR-1339, a nonionic surfactant is used to block the clearance of triglyceride rich lipoproteins and hence aid in development of hyperlipidemia in several animals. These acute models are highly used in evaluation of the newer herbal or synthetic anti-hyperlipidemic drugs. In this study, triton-1339 was administered i.p., in rats to facilitate elevation of plasma cholesterol, triglyceride, LDL and reduction of plasma HDL levels. This action is generally due to association of surfactants with triglycerides in the plasma which reduces their rate of hydrolysis by the enzyme, lipoprotein lipase or clearing factor lipase, thus interfering with the uptake of triglycerides from the circulation by the extra-hepatic tissues. It has been studied that the triton induced significant increase in the plasma cholesterol and triglyceride levels is due to increase in VLDL secretions by liver along with a significant reduction of LDL and VLDL catabolism[23].

Lagenaria siceraria is accounted for its cardio protective, antihyperlipidemic, antioxidant and antihyperglycemic, analgesic, anti-inflammatory, immunomodulatory and hepatoprotective functions in humans[24]. Saponins are among the major constituents in LSF extract, it can be very helpful in faster removal of free fatty acid from circulation that causes in turn a decrease in total cholesterol by enhancing the lipoprotein lipase activity (LPL) as reported in the previous studies. Saponins are known to increase the permeability of intestinal cells *in vitro* and also to inhibit active mucosal transport and increase absorption of substances. Across the small intestines of rats they are known to lower the transmural potential difference. Saponins have hemolytic activity which can be attributed to their affinity towards aglycone moiety for membrane sterols especially cholesterol, thus forming a insoluble complex[25]. LSF contains the parts of soluble dietary fibers such as pectin which helps in promotion of the bile acid formation and their excretion in the stool or in the blockage of cholesterol absorption.

TFG seeds are also responsible for the anti-hyperlipidemic activity due to the presence of steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin), alkaloids (mainly trigonelline) and free amino acids. TGF seeds are an important source of diosgenin, a precursor for the production of steroidal drugs and hormones such as testosterone, glucocorticoids and progesterone. These steroidal sapogenins are effective mediators for the management of hypocholesterolemia. Isoleucine, an amino acid is known to modulate the secretion of insulin[26].

The protective efficacy of the TGF seeds extract against artherogenesis was demonstrated by a significant

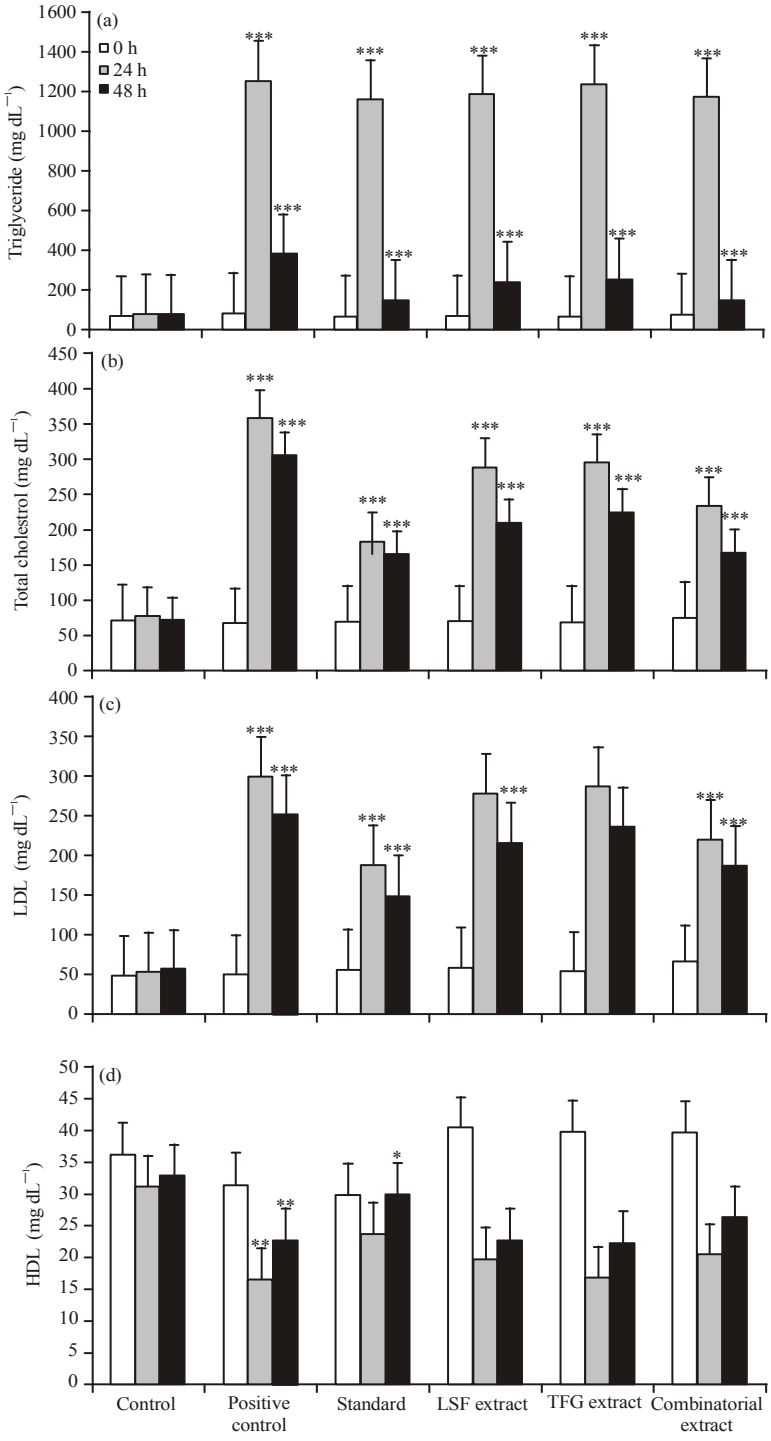

Fig. 1(a-d): Effect of combinatorial extract of LSF extract, TFG extract on plasma (a) Triglyceride, (b) Cholesterol, (c) LDL and (d) HDL in triton induced hyperlipidemia. Values are Mean ± S.E.M. of six rats ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$ versus control hyperlipidemic + *Lagenaria siceraria* extract, *Trigonella foenum graecum* extract, their combinatorial extract and standard drug (10 mg kg^{-1}) were compared with the hyperlipidemic (positive) control

reduction in the triglyceride and total cholesterol level after 24 and 48 h of triton injection in TGF extract treated group. As a result the efficiency of TGF extract in lipid regulating can be taken advantage in prevention of plaque formation leading to artherogenesis and congestive heart failure. High content of soluble fiber in fenugreek can be attributed to its anti-hyperlipidemic activity. The soluble fiber acts by decreasing the gastric emptying rate and thus delays the absorption of lipids from small intestine. The result of the study reveal that the seed extract of fenugreek can effectively control the blood levels in dyslipidemic conditions by interfering with biosynthesis of cholesterol and utilization of lipids[27].

In this study, it was observed that the combinatorial extract of LSF extract and TFG extract at the dose of 200 mg kg^{-1} significantly decreased ($p<0.001$) the levels of triglyceride (TG), Low Density Lipoproteins (LDL), cholesterol and showed an increase in High Density Lipoproteins (HDL) levels. The observations suggested that the cholesterol lowering activity of the combinatorial extract can be a result of the enhanced catabolism of LDL cholesterol through its hepatic receptors for the final elimination in the form of bile acids. Even Lecithin-cholesterol acyltransferase (LCAT) and tissue lipases are activated in the biological system. From the results of this study it can be concluded that the elevated levels of triglycerides, LDL and total cholesterol were significantly decreased by using the combinatorial extract of LSF extract and TFG extract than using the two extracts alone. Thus the antihyperlipidemic activity as well as anti-hyperlipidemic activity of this combinatorial extract can be attributed to the presence of polyphenols and flavanoids for their diverse pharmacological actions which also includes anti-artherogenic activity.

CONCLUSION

The present data revealed the medicinal use of the combinatorial extract of *Lagenaria siceraria* fruit and *Trigonella foenum graecum* can effectively modulate triton induced hyperlipidemia. Further studies to explore the exact mechanism attributing to the effect are needed to ascertain the potential of the combinatorial extract as an antihyperlipidemic agent.

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