



# Hypolipidemic and Anti-Atherogenic Activity of Aqueous Extract of Leaves of *Lagenaria Siceraria* in Wistar Rats

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## Abstract

This study was carried out to evaluate the hypolipidemic activity of aqueous extract of leaves of *Lagenaria siceraria* (Bottle gourd) in experimentally induced hyperlipemic Wistar rats. Aqueous extract of leaves of *Lagenaria siceraria*, orally at the dose of 200 and 400 mg/kg, was given to High Cholesterol Diet (HCD) induced hyperlipemic rats. After eight weeks of dosing, the possible antihyperlipidemic activity was assessed by investigating serum lipid levels. Atorvastatin (10 mg/kg p.o.) was used as a standard drug. Results showed that aqueous extract of leaves of *Lagenaria siceraria* (AELS) caused a significant ( $P<0.01$ ) reduction in serum levels of Total Cholesterol (TC), TriGlycerides (TG), Low-Density Lipoprotein Cholesterol (LDLC) and Very Low-Density Lipoprotein Cholesterol (VLDLC) and Atherogenic Index Serum (AIS). The results also demonstrated a significant ( $P<0.01$ ) increase in High-Density Lipoprotein Cholesterol (HDLC). Our observations of the study indicate that the AELS has a significant antihyperlipidemic potential.

**Keywords:** *Lagenaria siceraria*, hypolipidemic, aqueous extract, high cholesterol diet, serum lipids

## 1. Introduction

Coronary Artery Disease (CAD), an aftereffect of atherosclerosis, is one of the five diseases having highest morbidity and mortality rate in the world. Hyperlipidaemia is the major risk factor in the induction and promotion of atherosclerosis and Coronary Artery Disease (CAD) [1–3]. Standard drugs such as statins used to treat hyperlipidemia cause adverse drug reactions [4]. There is no drug, which halts the oxidation reaction of Low-Density Lipoprotein Cholesterol (LDLc) as it is considered to play principal role in the pathogenesis of atherosclerosis and CAD [5]. Studies have indicated that a decrease in the levels of LDLc or total cholesterol, reduce the risk of atherosclerosis and CAD [6, 7]. Further, there is established opposite relation between the risk

of atherosclerosis and coronary artery disease and the levels of HDLC [8]. Therefore, it is required to explore the newer lipid lowering drugs with better safety profile and having potential to inhibit the oxidation and production of LDLc. Traditional herbal drugs have proven to be a better choice when compared to modern synthetic drugs. These drugs have a few or no untoward and are claimed to be safer ones. *Lagenaria siceraria* contains many vital constituents, which are important for better health of humans [9]. The leaves of *Lagenaria siceraria* are claimed to possess hypolipidemic potential in the folklore medicinal system. Phytochemical screening of the leaves has shown that the leaves contain various compounds, including phytosterols, saponins, flavonoids, tannins and phenolic compounds [10]. This study was carried out to evaluate the hypolipidemic activity of aqueous extract of

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leaves of *Lagenaria siceraria* in experimentally induced hyperlipemic Wistar rats.

## 2. Materials and Methods

### 2.1 The Collection of Leaves

The fresh leaves of *Lagenaria siceraria* were collected from the local farms of Malir District, Karachi. The sample was authenticated by an herbalist of the Department of Herbal Extracts, Al Saudia Tibi Foundation, Pakistan. Voucher specimen (BPHL. 1607) was deposited in the institute for future reference.

### 2.2 Preparation of the Extract

Shade dried leaves were powdered and kept in a conical flask filled with distilled water and chloroform (9:1) for one week at room temperature (25°–30°C) with gentle shaking every day. It was then filtered with a sterile piece of cotton cloth. The mixture was dried through evaporation. A green coloured extract was obtained and stored in a refrigerator at 4–8°C for subsequent use.

### 2.3 Drugs and Chemicals

Atorvastatin (PZ0001 SIGMA) was purchased from Sigma–Aldrich. HDL and LDL/VLDL quantitation kit (MAK045 SIGMA) and cholesterol quantitation kit (MAK043 SIGMA) were also obtained from Sigma–Aldrich. Triglycerides kit (SKU # 72LS100-40) was purchased from Span Diagnostics. All other chemicals used in the study were of AR grade and obtained from Span Diagnostics.

### 2.4 Preparation of HCD

High Cholesterol Diet was prepared using cholesterol (100 g), cholic acid (50 g) in 1 litre of coconut oil and supplementation of 50 g egg yolk [11–13].

### 2.5 The Selection of Animals

Male albino rats (Wistar strain) weighing between 150–200 g were used in this research. The specifications given in Helsinki Resolution 1964 were followed during animal handling. This research was approved by the ethical committee of the Board of Advanced Studies and Research University of Karachi vide Resol. No. 12 (68) dated 13, 15, 20 & 24-06-2011.

### 2.6 Determination of Acute Oral Toxicity

The extract in the dose range of 100–1000 mg p.o. was given to different groups of rats. Litchfield & Wilcoxon Method was used to determine the acute toxicity.

### 2.7 Dosing

The dose of the drug was calculated according to body weight of the animals and then the required dilution was prepared in distilled water. The dosing of the drug was done daily in normal doses according to the body weight of the animals.

### 2.8 Methodology

Thirty male albino rats (Wistar strain) weighing 150–200 g were procured from animal house of University of Karachi, Pakistan. Before administration of the drug, the animals were kept in the laboratory for one week for conditioning period. They were kept individually in polypropylene cages under controlled conditions at room temperature (25°–30°C) with 12/12 hours light–dark cycle. The rats were given a standard rat diet and water *ad libitum*. The rats were divided into five groups viz.:

- |                   |  |
|-------------------|--|
| <b>Group I:</b>   | Normal control   |
| <b>Group II:</b>  | High Cholesterol Diet Control  |
| <b>Group III:</b> | High Cholesterol Diet + Atorvastatin (10 mg/kg p.o.)   |
| <b>Group IV:</b>  | High Cholesterol Diet + Aqueous extract of leaves of <i>Lagenaria siceraria</i> (200 mg/kg p.o.) |
| <b>Group V:</b>   | High Cholesterol Diet + Aqueous extract of leaves of <i>Lagenaria siceraria</i> (400 mg/kg p.o.) |

There were six rats in each group. For the induction of hypercholesterolemia, high cholesterol diet (1 ml/100 g) was given daily through intragastric route to all rats except group I. Blood samples were taken through retro orbital venous plexus for the confirmation of induction of hypercholesterolemia. After the confirmation of induction of hypercholesterolemia at serum cholesterol range 130–140 mg/dl, group II continued to receive high cholesterol diet for next 20 consecutive days. Group III, group IV and group V received high cholesterol diet for 10 consecutive days and from 11th to 20th day they received atorvastatin (10 mg/kg p.o.), aqueous extract (200 mg/kg p.o.) and

aqueous extract (400 mg/kg p.o.) respectively along with high cholesterol diet. After final dose, the rats were not given any food for the next eight hours and then blood samples were taken through retro orbital venous plexus under light diethyl ether anaesthesia. Blood samples were centrifuged for ten minutes at 3000 rpm. Serum was analysed using commercial kits through colorimeter for levels of total cholesterol, triglycerides, LDL cholesterol, VLDL cholesterol and HDL cholesterol [14, 15].

### 2.8.1 Serum Lipid Profile Estimation

Total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and VLDL cholesterol were estimated through cholesterol quantitation kit (MAK043 SIGMA), auto span gold triglycerides kit (SKU # 72LS100-40) and HDL and LDL/VLDL quantitation kit (MAK045 SIGMA) respectively. The Atherogenic Index Serum (AIS) was calculated through the following formula [16].

Atherogenic Index Serum = Total Cholesterol / HDL cholesterol

### 2.8.2 Statistical Analysis

All values are mean  $\pm$  SD. The values were compared by taking the mean of all of them and the significance of difference between mean was determined by student's t-test. Values of  $P < 0.01$  were considered as significant. IBM SPSS statistics 20 (version 20.0) was used for statistical analysis.

## 3. Results

High cholesterol diet rats showed remarkable elevations in their lipid profile. The rats were then divided into experimental and control groups. Changes in lipid profiles of experimental and control groups are shown in Table 1. The results of this study demonstrate that serum TC, serum TG, and serum LDLc were increased significantly ( $P < 0.01$ ) when compared with normal diet control rats. Nevertheless, there was a significant decrease in the levels of serum TC and serum TG of all AEELS treated and atorvastatin treated rats in a dose dependent manner when compared with cholesterol diet control rats.

The levels of serum LDLc were raised when compared with normal diet control rats. However, there was highly significant decrease in the levels of serum LDLc of all AEELS treated and atorvastatin treated rats in a dose dependent manner when compared with cholesterol diet control rats. There was a significant decrease in levels of serum HDLc of high cholesterol diet rats in comparison to rats on a normal diet. However, there was a significant increase in the levels of serum HDLc of all AEELS treated and atorvastatin treated rats in a dose dependent manner in comparison to rats on high cholesterol diet (Table 1).

The Atherogenic Index Serum (AIS) of high cholesterol diet control rats was significantly increased in comparison to normal diet control rats. However, AIS of all AEELS treated and atorvastatin treated rats was

**Table 1:** Effect of aqueous extract of leaves of *Lagenaria siceraria* on the lipid profile of high cholesterol diet hyperlipemic Wistar rats

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	LDLc (mg/dl)	VLDLc (mg/dl)	HDLc (mg/dl)
Normal Control	47.19 $\pm$ 8.51	53.62 $\pm$ 4.46	13.04 $\pm$ 1.01	10.71 $\pm$ 2.97	23.40 $\pm$ 4.64
HCD Control	137.88 $\pm$ 6.14 <sup>a</sup>	213.9 $\pm$ 11.9 <sup>a</sup>	82.36 $\pm$ 0.68 <sup>a</sup>	42.78 $\pm$ 1.13 <sup>a</sup>	12.74 $\pm$ 7.75 <sup>a</sup>
HCD + ATV	49.07 $\pm$ 6.60 <sup>b</sup>	139.92 $\pm$ 4.00 <sup>a,b</sup>	34.04 $\pm$ 0.9 <sup>a,b</sup>	27.98 $\pm$ 1.85 <sup>a,b</sup>	17.04 $\pm$ 2.41 <sup>a,b</sup>
HCD + AEELS 200 mg/kg.	75.15 $\pm$ 8.02 <sup>a,b*</sup>	160.0 $\pm$ 11.03 <sup>a,b*</sup>	30.51 $\pm$ 0.8 <sup>a,b*</sup>	32.00 $\pm$ 0.97 <sup>a,b*</sup>	12.64 $\pm$ 2.56 <sup>a,b*</sup>
HCD + AEELS 400 mg/kg.	70.82 $\pm$ 7.59 <sup>a,b*</sup>	153.82 $\pm$ 8.99 <sup>a,b*</sup>	24.45 $\pm$ 1.0 <sup>a,b*</sup>	30.76 $\pm$ 2.81 <sup>a,b</sup>	15.60 $\pm$ 3.56 <sup>a,b</sup>

Values are mean  $\pm$  Standard deviation

<sup>a</sup> $P < 0.01$ : Significant difference when compared with normal diet control rats

<sup>b</sup> $P < 0.01$ : Significant difference when compared with cholesterol diet control rats

\* $P < 0.01$ : Significant difference when compared with standard drug (atorvastatin)

decreased in a dose dependent manner in comparison to rats on high cholesterol diet (Table 2).

A significant increase in body weight was detected in the HCD control group when compared with normal diet control rats. The body weight of normal diet control rats increased by 2.3% and 4.2% after 4<sup>th</sup> week and 8<sup>th</sup> week respectively. While the weight of the HCD control group increased by 8.5% and 25.9% after 4<sup>th</sup> week and 8<sup>th</sup> week respectively. However, there was a highly significant decrease in the weight of atorvastatin, AELS 200 and AELS 400 treated groups. After four weeks, the weight of atorvastatin, AELS 200 and AELS 400 treated groups was decreased by 4.4%, 5.1%, and 4.8% respectively. Similarly, at the end of the eighth week, the weight of atorvastatin, AELS 200 and AELS 400 treated groups was decreased by 2.6%, 4.4% and 4.1% respectively (Table 3).

## 4. Discussion

Phytochemical screening of the leaves of *Lagenaria siceraria* showed that the aqueous extract of the plant

contains saponins, proteins and amino acids, flavonoids, phenolic compounds and tannins [10]. Table 1 shows the effects of AELS on the lipid profile of the hyperlipemic rats. The results show that *Lagenaria siceraria* possesses the significant antihyperlipidemic potential, which may be due to secondary metabolites as saponins, flavonoids, and phenolic compounds present in the leaf extract. Flavonoids present in the extract may enhance Lecithin Acyl Transferase (LCAT) activity. LCAT is responsible for the regulation of blood lipids. LCAT is the principal enzyme responsible for incorporation of cholesterol into HDLc. This may result in increase in HDLc and transferring it back into LDLc and VLDLc. Later, these are taken back in hepatocytes. Literature shows that there is an inverse relation between the incidence of Coronary Artery Disease (CAD) and the levels of HDLc [17–19]. Saponins are also antihyperlipidemic in nature. They exert their antihyperlipidemic effect through different mechanisms. Saponins decrease the intestinal absorption of cholesterol by binding with it and thus increasing its faecal elimination. There are certain

**Table 2:** Effect of aqueous extract of leaves of *Lagenaria siceraria* on the atherogenic index of high cholesterol diet hyperlipemic Wistar rats

Groups	Atherogenic Index Serum (%)
Normal Control	2.01 ± 0.39
High Cholesterol Diet Control	10.82 ± 2.38 <sup>a</sup>
High Cholesterol Diet + Atorvastatin	2.88 ± 0.24 <sup>a,b</sup>
High Cholesterol Diet + <i>L. siceraria</i> 200 mg/kg.	5.95 ± 1.02 <sup>a,b*</sup>
High Cholesterol Diet + <i>L. siceraria</i> 400 mg/kg.	4.54 ± 0.47 <sup>a,b*</sup>

Values are mean ± Standard deviation

<sup>a</sup>P < 0.01: Significant difference when compared with normal diet control rats

<sup>b</sup>P < 0.01: Significant difference when compared with cholesterol diet control rats

\*P < 0.01: Significant difference when compared with standard drug (atorvastatin)

**Table 3:** Effect of aqueous leaf extract of *Lagenaria siceraria* on body weight of high fat diet hyperlipemic Wistar rats

Groups	Increase in the body weight of the rats	
	4 <sup>th</sup> week	8 <sup>th</sup> week
Normal Control	2.3 %	4.2 %
HCD Control	8.5 %	25.9 %
HCD + ATV	4.4 %	2.6 %
HCD + AELS 200 mg/kg.	5.1 %	4.4 %
HCD + AELS 400 mg/kg.	4.8 %	4.1 %

The values indicate increase in the body weight in percentage.

reports indicating that saponins also increase the activity of LipoProtein Lipase (LPL). This activity increases the elimination of circulating free fatty acids, which in turn results in reduced levels of TC [17, 19, 20]. It has been found that plant proteins are less hyperlipimic in nature than animal proteins, which may be due to the fact that the ratio of lysine:arginine (L:A) is less than 2 in plant proteins. This is important in the control of the progression of hyperlipidaemia. In *Lagenaria siceraria* proteins (L:A) ratio is 0.45, which is very less than that of soya proteins (0.84), garlic proteins (0.7) and coconut proteins (0.86) [21]. Another possible reason for the hypolipidemic effect of AEELS is that the lipid lowering effect of *Lagenaria siceraria* might be related to the antioxidant properties of its constituents [22]. It has been reported that inhibition of endogenous oxidation of cholesterol exerts hypolipidemic effect through reducing the concentration of LDLc [23, 24].

## 5. Conclusion

The results of our study indicated that AEELS exerted a marked hypolipidemic effect on the plasma lipids of laboratory animals. After oral administration of AEELS the levels of serum total cholesterol, triglycerides, LDLc and AI decreased highly significantly. Our results showed that AEELS might be preventive in hyperlipidaemia and atherosclerosis and decrease the risk of Coronary Artery Disease (CAD). Further studies of the isolated constituents of the leaves may be helpful in the discovery and development of safer and more powerful hypolipidemic agents.

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