RESEARCH ARTICLE

Antihyperlipidemic activity of isolated constituents from the fruits of *Lagenaria siceraria* in albino rats

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Hyperlipidemia is defined as increase in the lipid content (groups of fat or fat like substances along with their lipoprotein counterpart) in blood. Abundant evidence are there to proof the link between hyperlipidemia and atherosclerosis. *Lagenaria siceraria* commonly known as Bottle gourd, which is official in Ayurvedic Pharmacopoeia of India, and having composition of variety of essential phytoconstituents, so that the fruits are traditionally used for their cardioprotective, cardiotonic, general tonic, diuretic, aphrodisiac, antidote to certain poisons and scorpion strings, alternative purgative, and cooling effects. In the present study fruit juice was obtained by crushing the fresh fruits of *L. siceraria* in the juicer and was subsequently dried in the oven at 40°-50°C. The parent dried juice extract was then fractionated by using the solvents according to polarity in ascending order i.e. by using chloroform: acetic acid, methanol, pyridine, and water. Each fraction was dried in oven at 40°-50°C. Thin layer chromatography (TLC) used active fraction obtained by column chromatography for further isolation. The solvent system developed on trial and error basis was n-butanol: methaol: water (6:2:2). Four spots were obtained and were named as LSN-I, LSN-II, LSN-III and LSN-IV. Isolated spots were collected by using preparative TLC the isolated compounds were tested for Antihyperlipidemic activity and compounds LSN-I, LSN-II, LSN-III has shown significant results. The study exhibited that elevated levels of blood cholesterol, triglycerides, LDL, were significantly reduced and decreased HDL was significantly increased by the administration of fractions of *L. siceraria* fruit juice.

Key words: Atherosclerosis, hyperlipidemia, *Lagenaria siceraria*

INTRODUCTION

Hyperlipidemia is a highly predictive risk factor for atherosclerosis, coronary artery disease, and cerebral vascular diseases.[1] Atherosclerosis (*sclrero-hardening*) of arteries is a generalized disease of the arterial network known as a progressive and silent killer disease characterized by the formation of lesions called atherosclerosis plaques in the walls of large and or medium sized coronary arteries and which reduces blood flow to the myocardium - called coronary artery disease (CAD).[2] Abundant evidence links hyperlipidemia to atherosclerosis. Clinical trials showed conclusively that lowering serum cholesterol reduces morbidity and mortality from CAD in patients with established CAD and also reduces new CAD events and mortality in patients without established CAD.[3] Condiments, medicinal plants, fruits used in day-to-day preparation of food in Indian kitchens have been identified as hypolipidaemic in Ayurveda.[4] *Lagenaria siceraria* is commonly known as bottle gourd, (Calabash, Doodhi, and Lauki in Hindi) and Kadoo in Marathi) which is official in Ayurvedic Pharmacopoeia of India.[5]

L. siceraria fruits are traditionally used for its cardioprotective, cardiotonic, general tonic, diuretic, aphrodisiac, antidote to certain poisons and scorpion stings, alternative purgative, and cooling effects. It cures pain, ulcers, and fever and is used for pectoral-cough, asthma and other bronchial disorders.[6,7]

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The fresh fruits of *L. siceraria* were collected in the months of August-December from the local market of Wardha, Maharashtra state, India, and authenticated by the authority of the botany department, Nagpur University, Nagpur. A voucher specimen (specimen No. 9012) was submitted at Institute's herbarium department for future reference.

Extraction of Plant Material

The fruit juice was obtained by crushing the fresh fruits of *L. siceraria* in the mixer (juicer) and subsequently dried in the oven at 40-50°C.

Fractional Extraction of Parent Extract

The parent dried juice extract was then fractionated by using the solvents according to polarity in ascending order i.e. by

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using chloroform: acetic acid, methanol, pyridine, and water [Table 1]. Each fraction was dried in oven at 40-50C.

Column Chromatographic Isolation and Purification of Methanolic Extract

1. Column : Glass
2. Dimension : Length 45 cm, diameter 3 cm
3. Stationary phase : Silica gel
4. Sample : Methanolic extract
5. Mobile phase : Gradient elution

The Methanolic fraction of parent extract was subjected for isolation over the silica gel (mesh size- 100-200) column. Previously, the slurry of silica gel was prepared with the mobile phase. The column was washed with the mobile phase for sufficient period of time. Then Methanolic fraction was loaded over the silica gel. The mobile phase was passed continuously with constant flow rate (10 ml/min.). The fractions were collected at regular intervals of time, evaporated at temperature <40-50°C and subjected for evaluation of antihyperlipidaemic activity.

Isolation of Compounds by Thin Layer Chromatography (TLC)

Active fraction obtained by column chromatography was used for further isolation by TLC. The solvent system developed on trial and error basis was n-butanol: methaol: water (6:2:2). Four spots were obtained and were named as LSN-I, LSN-II, LSN-III and LSN-IV. Isolated spots were collected by using preparative TLC.

Evaluation of Antihyperlipidaemic Activity of the Isolated Compound

Diagnostic kit

Diagnostic Kits used for estimation of Triglyceride, Cholesterol, and HDL-C were obtained from Merck Ltd.

Hyperlipidemia Inducer

Triton-X-100 was used for induction of Hyperlipidemia in experimental rats.

Animals and Treatments

Adult male albino rats, weighing 180-200 g, bred in the animal house of the Institute of Pharmaceutical Education and Research, Wardha, were used. The animals were housed in polypropylene cages in room temperature with a 12 h day-light cycle. During the whole experimental period, animals were fed with a balanced diet and water *ad libitum*. All the animal experiments were performed with prior approval from the Institutional Animal Ethical Committee (Registration No.535/0/a/CPCSEA/Jan 2002) of the Institute of Pharmaceutical Education and Research, Wardha, Nagpur University.

Induction of Hyperlipidemia

Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h.[8,9]

Dose Preparation and Administration of Extracts

The isolated compounds viz. LSN I, LSN II, LSN III, LSN IV of *L. siceraria* were dissolved in distilled water administered orally to the animals by gastric intubation, thrice a day with an interval of 3 h, just after administration of Triton-X-100 *i.p.*

Protocol for Antihyperlipidaemic Activity

In the experiment a total number of 42 rats (36 hyperlipidemia rats, six normal) were used. The rats were divided into seven groups of six each.

Group I : Normal distilled water treated *p.o.*
Group II : Triton-X-100 (100 mg/kg) *i.p.*
Group III: Triton-X-100 (100 mg/kg) *i.p.* + Lovastatin (10 mg/kg) *p.o*
Group IV: Triton-X-100 (100 mg/kg) *i.p* + LSN I *p.o*
Group V : Triton-X-100 (100 mg/kg) *i.p* + LSN II *p.o*

Table 1: Fractionation of parent extracts

Solvent	Polarity index	Fractionation	% Yield
Chloroform + glacial acetic acid (1:1)	5.15	Soxhelation	4.5
Methanol	5.2	Soxhelation	51
Pyridine	5.3	Maceration	12
Water	9.0	Maceration	27.5

Table 2: Effect of isolated compounds (LSN) on total cholesterol level of triton-induced hyperlipidemia in rats

Groups	Treatments	Cholesterol (mg/dl)
I	Normal (Normal saline)	74.51 ± 0.87
II	Triton control (100 mg/kg)	285.55 ± 1.85
III	Triton + standard (10 mg/kg)	186.49 ± 1.23
IV	Triton + LSN - I (10 mg/kg)	261.82 ± 1.02**
V	Triton + LSN - II (10 mg/kg)	270.71 ± 0.91**
VI	Triton + LSN - III (10 mg/kg)	283.00 ± 1.77*
VII	Triton + LSN - IV (10 mg/kg)	283.66 ± 1.75^{ns}

**$P < 0.01$, very significant; *$P < 0.05$, significant; and $^{ns}P > 0.05$, non-significant. One-way ANOVA followed by Dunnett's test

Table 3: Effect of isolated compounds (LSN) on Triglyceride level of Triton-induced hyperlipidemia in rats

Groups	Treatments	Triglycerides (mg/dl)
I	Normal (Normal saline)	69.07 ± 0.93
II	Triton control (100 mg/kg)	169.20 ± 0.70
III	Triton + standard (10 mg/kg)	89.54 ± 0.86
IV	Triton + LSN - I (10 mg/kg)	147.19 ± 2.52**
V	Triton + LSN - II (10 mg/kg)	153.24 ± 2.00**
VI	Triton + LSN - III (10 mg/kg)	166.75 ± 0.89**
VII	Triton + LSN - IV (10 mg/kg)	167.74 ± 1.02^{ns}

** $P < 0.01$, very significant and $^{ns}P > 0.05$, non-significant. One-way ANOVA followed by Dunnett's test

Table 4: Effect of isolated compounds (LSN) on HDL-c level of Triton-induced hyperlipidemia in rats

Groups	Treatments	HDL-c (mg/dl)
I	Normal (Normal saline)	28.73 ± 1.47
II	Triton control (100 mg/kg)	18.66 ± 1.19
III	Triton + standard (10 mg/kg)	27.93 ± 1.42
IV	Triton + LSN - I (10 mg/kg)	22.99 ± 0.63**
V	Triton + LSN - II (10 mg/kg)	21.94 ± 0.67**
VI	Triton + LSN - III (10 mg/kg)	20.37 ± 0.78**
VII	Triton + LSN - IV (10 mg/kg)	17.80 ± 0.80^{ns}

** $P < 0.01$, very significant and $^{ns}P > 0.05$, non-significant. One-way ANOVA followed by Dunnett's test

Table 5: Effect of isolated compounds (LSN) on LDL level of Triton-induced hyperlipidemia in rats

Groups	Treatments	LDL-c (mg/dl)
I	Normal (Normal saline)	59.59 ± 2.24
II	Triton control (100 mg/kg)	300.73 ± 2.23
III	Triton + standard (10 mg/kg)	176.46 ± 1.40
IV	Triton + LSN - I (10 mg/kg)	268.26 ± 1.67**
V	Triton + LSN - II (10 mg/kg)	279.44 ± 1.30**
VI	Triton + LSN - III (10 mg/kg)	295.99 ± 2.08**
VII	Triton + LSN - IV (10 mg/kg)	298.75 ± 2.13^{ns}

** $P < 0.01$, very significant and $^{ns}P > 0.05$, non-significant. One-way ANOVA followed by Dunnett's test

Table 6: Effect of isolated compounds (LSN) on VLDL level of Triton-induced hyperlipidemia in rats

Groups	Treatments	VLDL-c (mg/dl)
I	Normal (Normal saline)	13.81 ± 0.18
II	Triton control (100 mg/kg)	33.83 ± 0.14
III	Triton + standard (10 mg/kg)	17.90 ± 0.17
IV	Triton + LSN - I (10 mg/kg)	29.43 ± 0.50**
V	Triton + LSN - II (10 mg/kg)	30.64 ± 0.4**
VI	Triton + LSN - III (10 mg/kg)	33.35 ± 0.15*
VII	Triton + LSN - IV (10 mg/kg)	33.54 ± 0.20^{ns}

** $P < 0.01$, very significant; * $P < 0.05$, significant; and $^{ns}P > 0.05$, non-significant. One-way ANOVA followed by Dunnett's test

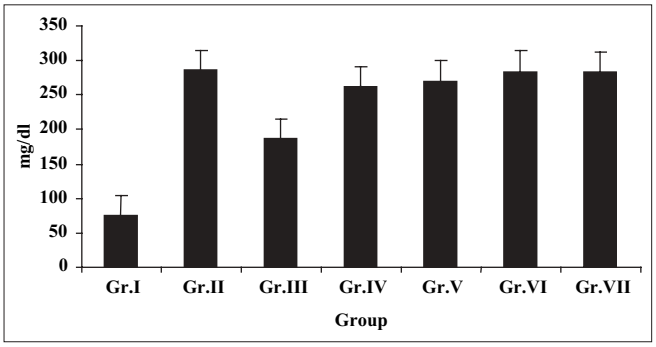

Figure 1: Effect of isolated compounds (LSN) on Cholesterol level of Triton-induced hyperlipidemia in rats

Group VI: Triton-X-100 (100 mg/kg) *i.p* + LSN III *p.o*
Group VII: Triton-X-100 (100 mg/kg) *i.p* + LSN IV *p.o*

Statistical Analysis

All the values are expressed in terms of Mean ± S.E. M. of (n = 6)

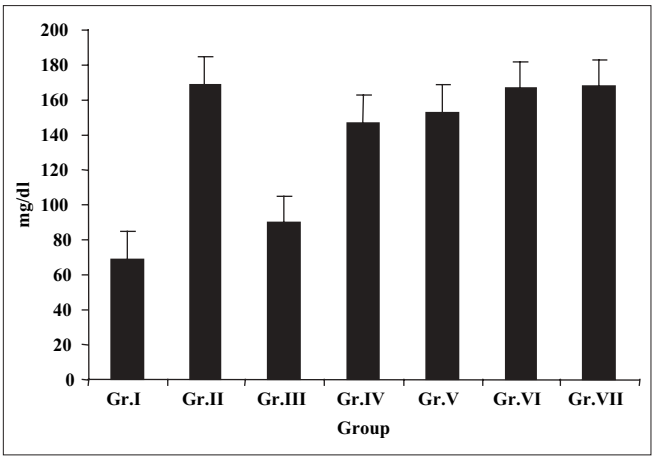

Figure 2: Effect of isolated compounds (LSN) on Triglyceride level of Triton-induced hyperlipidemia in rats

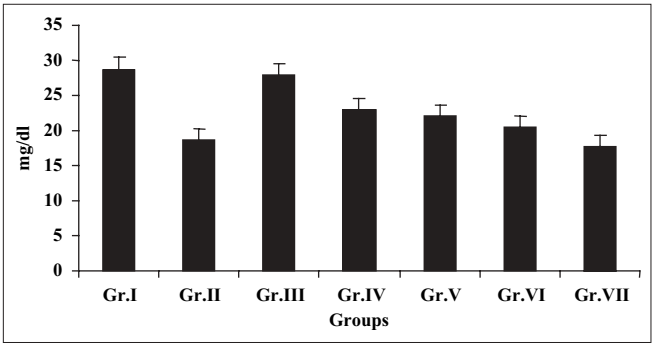

Figure 3: Effect of isolated compounds (LSN) on HDL-c level of Triton-induced hyperlipidemia in rats

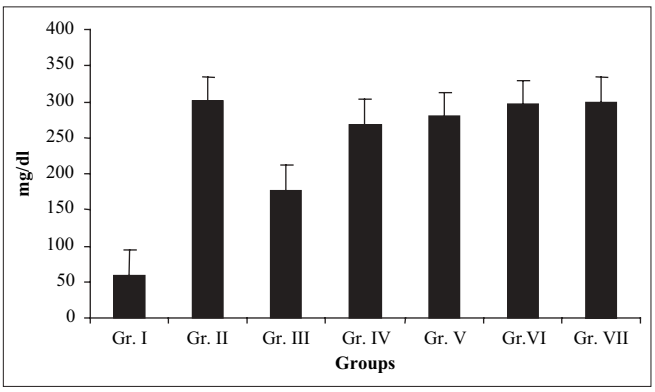

Figure 4: Effect of isolated compounds (LSN) on LDL level of Triton-induced hyperlipidemia in rats

The Triton control was compared with normal.

The experimental results were compared with Triton control.

DISCUSSION

The study was undertaken in order to evaluate the antihyperlipidaemic activity of different isolated compounds

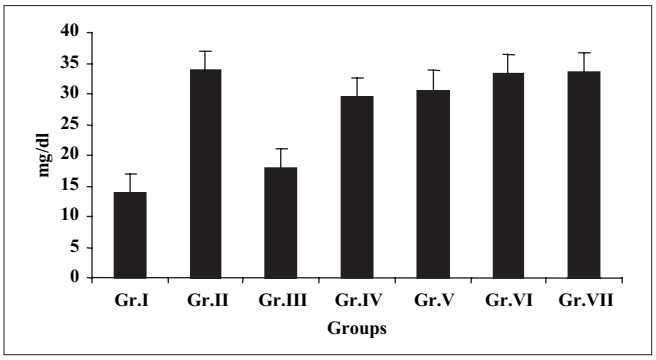

Figure 5: Effect of isolated compounds (LSN) on VLDL level of Triton-induced hyperlipidemia in rats

of *L. siceraria* fruits. The compounds were isolated from the active methanolic fraction of parent extract of *L. siceraria* fruit juice using column chromatography and TLC. The isolated compounds were tested for antihyperlipidaemic activity and compounds LSN-I, LSN-II, LSN-III showed significant results [Figs. 1-5 and Tables 2-6]. The study exhibited that elevated blood cholesterol, triglycerides, LDL, and decreased HDL which occur in hyperlipidemia, was significantly reduced by the administration of fractions of *L. siceraria* fruit juice. This finding provides some biochemical basis for the use of fruit, fruit juice or fruit extracts in the management of patients with hyperlipidemia. This also helps to place the *L. siceraria* (Mol.) Stand fruit among the list of antihyperlipidaemic agents and having potential to be popularized as household remedy with preventive and curative effect against hyperlipidemia and its consequences.

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