RESEARCH ARTICLE

Evaluation of Hypolipidemic Activity of *Cardiospermum halicacabum* L. Leaf in Atherodiet-induced Wistar Albino Rats

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ABSTRACT

**Aim:** Hyperlipidemia has been ranked as one of the greatest risk factors contributing to prevalence and severity of coronary heart diseases. The present study has been taken up to evaluate hypolipidemic activity of hydro alcoholic extract of *Cardiospermum halicacabum* L. leaf, as this plant is widely used by the local healers of South India for treatment of various illnesses.

**Materials and methods:** The experiment was carried out in atherodiet-induced hyperlipidemia in Wistar Albino rats. The experiment duration was 28 days and it comprised six groups, such as healthy control, extract control, disease control, treatment group low dose, treatment group high dose, and standard drug (SD). The biochemical investigations including lipid profile with Atherogenic Index and Coronary Risk Index, and other metabolic parameters were evaluated using standard protocols. Histopathology studies for vital organs of animals were also carried out.

**Results:** The study showed that the hydroalcoholic extract of *C. halicacabum* L. leaf has significant hypolipidemic activity in the atherodiet-induced hyperlipidemia in Wistar rats when compared with the disease control group. The histopathology study also evidenced the safety of the extract.

**Conclusion:** The study concluded that the hydroalcoholic extract of *C. halicacabum* L. has significant hypolipidemic activity at the prescribed dosages.

**Clinical relevance:** As the plant has shown promising effect in the aspect of hypolipidemic activity, further studies have to be carried out for developing of new product as single drug and in the formulations of AYUSH (Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homoeopathy) system of medicines for management of hyperlipidemia.

Keywords: Atherodiet, *Cardiospermum halicacabum*, Hypolipidemic activity.


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Conflict of interest: None

INTRODUCTION

Lipids play a critical role in almost all aspects of biological life as they are structural components in cells and are involved in metabolic and hormonal pathways. Lipids are naturally occurring molecules from a plant or animal source. Hyperlipidemia is a condition when abnormally high levels of lipids, i.e., fatty acid substances are found in blood. This condition is also called hypercholesterolemia or hyperlipoproteinemia. Hyperlipidemia has been ranked as one of the greatest risk factors contributing for prevalence and severity of coronary heart diseases. Coronary heart disease, stroke, atherosclerosis, and hyperlipidemia are the primary causes of death. The elevation of serum total cholesterol and low-density lipoprotein (LDL) cholesterol has been reported as a primary risk factor for cardiovascular disease. The World Health Organization has reported that cardiovascular diseases are going to be one of the largest cause of death and disability in India by 2020. Hypolipidemic drugs are extensively used for preventing and treatment of cardiovascular diseases. As the long-term consumption of synthetic drugs has been reported for various adverse effects, the researchers have started focusing on plant-based medicines. Many plant species and plant derivatives have been screened and documented for their hypolipidemic action. The advantages of herbal medicines are effectiveness, safety, affordability, and easy acceptability.

*Cardiospermum halicacabum*, L. known as “Balloon vine” or “Love in a puff”, is a climbing plant widely distributed in tropical and subtropical Africa and Asia. It is an annual or sometimes perennial climber, commonly found as...
a weed in farmland. It is mentioned as one among the “Ten Sacred Flowers” of Kerala State in India, collectively known as “Dasapushpam”. It is widely used by local healers of southern part of India for treatment of various illnesses and the plant has also been reported for some biological activities including anticonvulsant, analgesic, and anti-inflammatory activities. The present study has been taken up to evaluate the hypolipidemic activity of hydroalcoholic extract of Cardiospermum halicacabum leaf, in experimental animals.

MATERIALS AND METHODS

Plant Collection and Extraction

The plant Cardiospermum halicacabum L. was collected from the Western Ghat region (Palghat and Thrissur) of Kerala, India. The authentication of the plant was done by Taxonomist, Kerala Forest Research Institute (KFRI), Government of Thrissur, Kerala, India. Voucher specimen is maintained in the Biochemistry Department of National Ayurveda Research Institute for Panchakarma, Cheruthuruthy. The fresh leaves of Cardiospermum halicacabum L. were collected and used for the present study. The hydroalcoholic extract of leaf (one part alcohol and one part water) was prepared as per Ayurvedic Pharmacopoeia of India Part I Vol. VIII. The extract was stored in refrigerator for the experimental use.

Preparation of Atherodiet

High-fat diet or atherodiet was prepared as per the method of Bopanna et al., comprising 2% cholesterol, 0.25% bile salts, and 15% butter and was used for the present study.

Experimental Animals

Wistar Albino rats were procured from the College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India. Animals were acclimatized to the laboratory condition before initiating the experiment. The animal studies were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals.

Institutional Animal Ethics Committee Approval for Toxicity Study

Institutional Animal Ethics Committee’s (IAEC) approval was obtained for the animal experiments vide Proposal No. IAEC/NRIP/2015-16/05 dated 23.01.2016 in the meeting held at National Research Institute for Panchakarma, Cheruthuruthy, Thrissur, Kerala, India.

Efficacy Study Design

A total of 36 Wistar Albino rats of 12 weeks old were randomized and equally divided into six groups. Each group consisted of 6 animals (3 males and 3 females). Control group received standard diet and distilled water; test extract group received 250 mg/kg bwt C. halicacabum L. extract and standard diet; disease control group received only atherodiet; treatment group—low dose received atherodiet with 250 mg/kg bwt C. halicacabum L. extract; treatment group—high dose received atherodiet with 500 mg/kg bwt C. halicacabum L. extract; and SD group received atherodiet with 5 mg/kg bwt Atorvastatin (Table 1).

Sample Collection and Biochemical Studies

After 4 weeks of treatment, body weights were measured and blood samples were collected by retro orbital route after overnight fasting of the animals for the study of biochemical parameters. Serum was evaluated for fasting blood sugar, glycated hemoglobin (HbA1C) level, total lipids, total cholesterol, triglycerides, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, very-low-density lipoprotein (VLDL), and phospholipids level. The liver function tests [(serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP)] and renal function tests (urea, creatinine) were also carried out.

Atherogenic Index

The Atherogenic Index was calculated as per the standard protocol.

Atherogenic Index = (LDL + VLDL)/HDL

Coronary Risk Index

Coronary Risk Index was calculated by the method of Alladi et al.

Coronary Risk Index = (Total cholesterol)/HDL

Table 1: Experimental design and treatment plan of the study

<table>
<thead>
<tr>
<th>Group</th>
<th>Name of group</th>
<th>Details</th>
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<tbody>
<tr>
<td>I</td>
<td>Healthy control</td>
<td>Standard diet</td>
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<td></td>
<td>group (HC)</td>
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<td>II</td>
<td>Extract control</td>
<td>Standard diet + C. halicacabum extract 250 mg/kg bwt</td>
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<td></td>
<td>group (EC)</td>
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<tr>
<td>III</td>
<td>Disease control</td>
<td>Atherodiet (High-fat diet)</td>
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<td></td>
<td>group (DC)</td>
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<tr>
<td>IV</td>
<td>Treatment group—</td>
<td>Atherodiet + C. halicacabum extract 250 mg/kg bwt</td>
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<td></td>
<td>Low dose (LD)</td>
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<td>V</td>
<td>Treatment group—</td>
<td>Atherodiet + C. halicacabum extract 500 mg/kg bwt</td>
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<tr>
<td></td>
<td>High dose (HD)</td>
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<tr>
<td>VI</td>
<td>SD group</td>
<td>Atherodiet + Atorvastatin 5 mg/kg bwt</td>
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</table>
Histopathological Studies
At the end of experiment, animals were sacrificed as per standard euthanasia procedure and the organs liver, kidney, and heart were collected for histopathological examinations.

Statistical Analysis
One-way analysis of variance (ANOVA) with post hoc analysis was carried out. Number of observations (N), group means, standard error mean, and standard deviations were calculated. The data were examined using normality tests. Once the data showed normal distribution, it was tested for heterogeneity of variances. One-way ANOVA was performed once the variances are homogeneous. Dunnett’s test was applied to compare the dose groups over the control arm. If the data were not distributed normally and heterogeneous, the Kruskal–Wallis test was done.

RESULTS AND DISCUSSION
The hypolipidemic activity of hydroalcoholic extract of *C. halicacabum* L. leaf was carried out in Wistar Albino rats using atherodiet. The % body weight gain of the healthy control group showed 50.54% and disease control group is found to be 67.84%. The body weight gain was found to be 52.72 and 49.81% for treatment group—low dose and high dose respectively. The % body weight gain of SD group (Atorvastatin treated) showed 46.55%. The study showed that the body weight gain was found to be less in the extract treated groups and SD group as compared with disease control group. The details of body weight gain have been documented in Graph 1.

The study showed that there are no significant changes in glucose, HbA1C, urea, creatinine, and uric acid levels among treatment groups and SD group when compared with healthy control and disease control (Graphs 2 and 3). The extract control group showed there was no significant changes in the lipid profile as compared with healthy control (p > 0.05). The lipid profile parameters cholesterol, LDL, VLDL, triglyceride levels were found to be significantly (p < 0.05 and p < 0.01) elevated in disease control group when compared with healthy control group. As such, there were significant increase of total lipids, phospholipids, Atherogenic Index, and Coronary Risk Index factors (p < 0.01) in the disease control group. The treatment groups with *C. halicacabum* L. leaf extract, i.e., low dose and high dose, showed significant decrease in level of cholesterol, LDL, VLDL, triglyceride levels, total lipids, phospholipids, Atherogenic Index, and Coronary Risk Index factors as compared with disease control group (p < 0.01). It was noted that the lipid profile parameters of the treatment group—low dose were not attaining the normal range of healthy

**Graph 1:** Effect of *C. halicacabum* L. extract on initial body weight and final body weight

**Graph 2:** Effect of *C. halicacabum* L. extract on levels of glucose and urea

**Graph 3:** Effect of *C. halicacabum* L. extract on levels of creatinine and uric acid
CONTROL

Hyperlipidemia is a condition of elevated levels of lipid profile in the blood. Hyperlipidemic condition increases the risk for cardiovascular diseases and stroke. The research on development of plant-based hypolipidemic drugs is going on all over the world on priority basis. The present study has been carried out for evaluating hypolipidemic activity of hydroalcoholic extract of *C. halicacabum* L. leaf in experimental animals. The experiment was carried out in atherodiet-induced hyperlipidemia in Wistar Albino rats. The biochemical parameters, such as lipid profile with Atherogenic Index and Coronary Risk Index, liver function tests, renal function tests, and other metabolic parameters were evaluated. The study showed that the hydroalcoholic extract of *C. halicacabum* L. leaf has significant hypolipidemic activity in the atherodiet-induced hyperlipidemia in Wistar rats. As the plant has shown significant hypolipidemic activity, it may be used for developing of new product as single drug and/or in the formulations of AYUSH system of medicines for management of hyperlipidemia, after ensuring the clinical trials as per the guidelines.

ACKNOWLEDGMENTS

Authors would like to thank the Director General, Central Council for Research in Ayurvedic Sciences, New Delhi, for the Intra Mural Research Project Grant in the Department of Pharmacology and Toxicology, Central Council for Research in Ayurvedic Sciences.
Table 2: Liver function parameters in Wistar Albino rats during efficacy study

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total bilirubin (mg/dL)</th>
<th>Total protein (gm/dL)</th>
<th>Albumin (gm/dL)</th>
<th>Globulin (gm/dL)</th>
<th>A/G</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
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<tbody>
<tr>
<td>Healthy control group</td>
<td>6.46 ± 0.13</td>
<td>3.0 ± 0.12</td>
<td>2.88 ± 0.07</td>
<td>0.85 ± 0.06</td>
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<tr>
<td>Test extract group</td>
<td>6.36 ± 0.12</td>
<td>2.88 ± 0.07</td>
<td>0.85 ± 0.06</td>
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<tr>
<td>Disease control group</td>
<td>6.10 ± 0.13</td>
<td>2.75 ± 0.09</td>
<td>0.85 ± 0.02</td>
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<tr>
<td>Treatment group—low</td>
<td>6.01 ± 0.11</td>
<td>2.76 ± 0.07</td>
<td>0.86 ± 0.02</td>
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<tr>
<td>Treatment group—high</td>
<td>6.26 ± 0.32</td>
<td>2.88 ± 0.15</td>
<td>0.85 ± 0.05</td>
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<tr>
<td>SD</td>
<td>6.18 ± 0.16</td>
<td>2.73 ± 0.10</td>
<td>0.85 ± 0.02</td>
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</table>

Values are expressed as mean ± standard error of mean, n = 6 per group. Values with superscript a, b indicate no significant difference (p > 0.05) when compared with healthy control and disease control respectively. Values with superscript a*, b* indicate significant difference compared with healthy control; a**, b** indicate significant difference compared with disease control at p < 0.05 and p < 0.01 respectively.

Graph 4: Effect of C. halicacabum L. extract on levels of lipid profile

Graph 5: Effect of C. halicacabum L. extract on total lipids and phosholipids

Graph 6: Effect of C. halicacabum L. extract on Atherogenic index and Coronary Risk Index

and support. The authors also express their gratitude to Laboratory Technicians of Biochemistry and Pathology Department Mr Venugopalan TN, Mrs Ranjini KR, Mr Sanal Gopi CG for their extended help and cooperation in the studies.
Graph 7: Effect of *C. halicacabum* L. extract on levels of serum proteins

Graph 8: Effect of *C. halicacabum* L. extract on levels of liver function marker enzymes

Fig. 1: Healthy control. Section of liver tissue of male rat showing normal portal triads and hepatic veins

Fig. 2: Test extract group high dose. Section of liver of male rat showing normal portal triads and hepatic veins. Hepatocytes appear normal and they are arranged in cords. Kupffer cells and sinusoidal spaces are normal

Fig. 3: Healthy control. Section of kidney tissue of male rat showing normal glomeruli. Afferent and efferent arterioles appear normal

Fig. 4: Test extract group high dose. Section of kidney of male rat showing normal glomeruli and Bowman’s capsule. Renal tubules are normal. Interstitial tissue also appear normal
REFERENCES

हिन्दी सारांश
एप्सेस्रैट प्रेटील विस्तार अल्बियो रेड्स में कार्डियोस्पर्म हेलिकाकाब्य एल. पत्र की हाइपोलिपिड्मिंक गतिविधि का मूल्यांकन

विषयः हाइपरलिपिड्मिया की कोरोनरी इलाज रोगों के प्रसार एवं गंभीरता के लिए सबसे बड़ा जोखिम कारक माना गया है। वर्तमान अध्ययन कार्डियोस्पर्म हेलिकाकाब्य एल. पत्र के हाइपरलिपिड्मिया निस्सार की हाइपोलिपिड्मिंक गतिविधि के मूल्यांकन के लिए किया गया है। दक्षिण भारत के स्वास्थ्य विभिन्न विभागों द्वारा विभिन्न रोगों की विश्लेषण के लिए इस पादप के उपयोग बढ़तावर से किया जा रहा है।

कार्य प्रणाली: विस्तार रेड्स में एप्सेस्रैट प्रेटील हाइपरलिपिड्मिया पर पश्चातवर किया गया परीक्षण अवधि 28 दिन तथा समूह छ रखे गए। यद्यपि, ब्लैड नियंत्रण नियंत्रण, निस्सार नियंत्रण समूह, रोग नियंत्रण समूह, कम औषध भर्ती क्षेत्र, अधिक औषध भर्ती क्षेत्र, कैच अधिक समूह एवं नामक औषध समूह। नामक प्रोटोकॉल का उपयोग करते हुए एप्सेस्रैट इन्टेक्स एवं कोरोनरी रिस्क इन्डेक्स के साथ विनियम प्रोफाइल आदि जीवसाधिक परिक्षण एवं अन्य चयनपद्धति मापदंडो का मूल्यांकन किया गया।

परिणाम: अध्ययन से ज्ञात हुआ कि रोग नियंत्रण समूह की दुनिया में विस्ताररेड्स में एप्सेस्रैट इन्टेक्स हाइपरलिपिड्मिया पर कार्डियोस्पर्म हेलिकाकाब्य एल. पत्र के हाइपरलिपिड्मिया निस्सार की महत्त्वपूर्ण हाइपोलिपिड्मिंक गतिविधि है। उत्कर विकृतविकासी अध्ययन भी निस्सार की दुनिया के साथ रहे।

निषेध: अध्ययन का निषेध निषेध कि कार्डियोस्पर्म हेलिकाकाब्य के हाइपरलिपिड्मिया निस्सार की निर्दिष्ट मात्रा में महत्त्वपूर्ण हाइपोलिपिड्मिंक गतिविधि है।

अभीत भाषाः संपीडिता जैसा कि इस पादप ने हाइपरलिपिड्मिया गतिविधि के परिवर्तन में आशाजनक प्रभाव दिखाया। हाइपरलिपिड्मिया की विश्लेषण के लिए आयुष विशिष्ट पद्धतियों के औषध योग्यता तथा एकल औषध के रूप में नवीन उत्पाद के विकास के लिए अधिम अध्ययन किया जाना चाहिए।

मुख्य शब्द: एप्सेस्रैट कार्डियोस्पर्म हेलिकाकाब्य, हाइपोलिपिड्मिंक गतिविधि.