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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.

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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 hyperlipoprotenemia.[1] 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.[2] 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".[3] 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.[4-7] The present study has been taken up to evaluate the hypolipidemic activity of hydroalcoholic extract of *Cardiospermum halicacabum* L. 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[8]

High-fat diet or atherodiet was prepared as per the method of Bopanna et al,[8] 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[9-13]

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[14]

The Atherogenic Index was calculated as per the standard protocol.

Atherogenic Index = (LDL + VLDL)/HDL

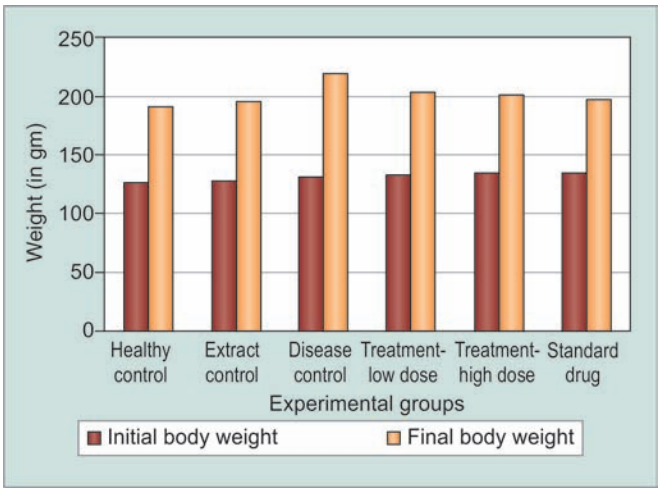
Coronary Risk Index[15]

Coronary Risk Index was calculated by the method of Alladi et al.[15]

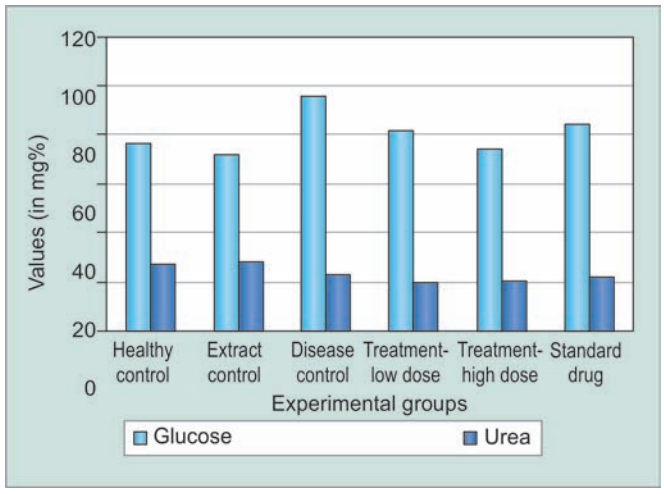
Coronary Risk Index = (Total cholesterol)/HDL

Table 1: Experimental design and treatment plan of the study

Group	*Name of group*	*Details*
I	Healthy control group (HC)	Standard diet
II	Extract control group (EC)	Standard diet + *C. halicacabum* extract 250 mg/kg bwt
III	Disease control group (DC)	Atherodiet (High-fat diet)
IV	Treatment group—Low dose (LD)	Atherodiet + *C. halicacabum* extract 250 mg/kg bwt
V	Treatment group—High dose (HD)	Atherodiet + *C. halicacabum* extract 500 mg/kg bwt
VI	SD group	Atherodiet + Atorvastatin 5 mg/kg bwt



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

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.

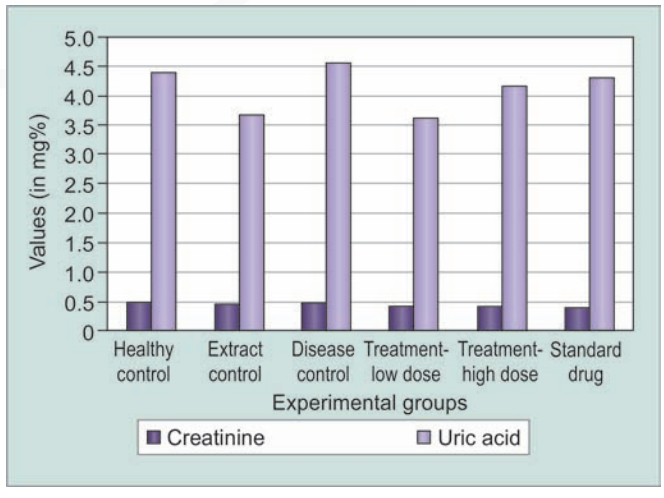
Statistical Analysis

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

Table 1: Lipid profile, total lipids, phospholipids, AI, and CRI parameters in Wistar Albino rats during efficacy study

Groups	Biochemical parameters—lipid profile								
	Cholesterol (mg/dL)	*HDL (mg/dL)*	*LDL (mg/dL)*	*VLDL (mg/dL)*	*Triglyceride (mg/dL)*	*Total lipid (mg/dL)*	*Phospholipids (mg/dL)*	*Atherogenic index (AI)*	*Coronary risk index (CRI)*
Healthy control group	75.66 ± 4.43	30.50 ± 2.46	26.0 ± 4.07	19.16 ± 0.74	96.66 ±4.02	676.0 ± 13.15	138.50 ± 7.96	1.52 ± 0.17	2.52 ± 0.17
Test extract group	77.0 ± 6.45^{a}	38.50 ± 3.07^{a}	18.66 ± 3.98^{a}	19.83 ± 1.57^{a}	95.0 ± 5.93^{a}	638.50 ± 9.44^{a}	141.83 ± 12.18^{a}	1.00 ± 0.09^{a}	2.00 ± 0.09^{a}
Disease control group	176.16 ± 7.86^{a**}	27.66 ± 1.38^{a}	110.50 ± 8.97^{a**}	38.0 ± 1.69^{a**}	189.66 ± 7.85^{a**}	849.83 ± 5.70^{a**}	194.33 ± 9.25^{a**}	5.45 ± 0.45^{a**}	6.45 ± 0.45^{a**}
Treatment group—low dose (250 mg/kg bwt)	120.83 ± 5.41^{a*b**}	33.50 ± 3.77^{ab}	61.16 ± 5.61^{a**b**}	26.16 ± 1.10^{a*b**}	130.66 ± 5.28^{a**b**}	823.83 ± 19.53^{a**b}	174.16 ± 6.63^{a*b}	2.81 ± 0.41^{a*b**}	3.81 ± 0.41^{a*b**}
Treatment group—high dose (500 mg/kg bwt)	92.83 ± 4.22^{ab**}	34.50 ± 3.54^{ab}	34.33 ± 5.18^{ab**}	24.0 ± 1.03^{ab**}	119.83 ± 4.95^{ab**}	675.50 ± 20.55^{ab**}	149.83 ± 8.05^{ab**}	1.82 ± 0.29^{ab**}	2.82 ± 0.29^{ab**}
SD	81.33 ± 3.41^{ab**}	34.66 ± 3.47^{ab}	24.50 ± 1.23^{ab**}	22.16 ± 1.72^{ab**b**}	111.50 ± 9.03^{ab**}	687.33 ± 10.20^{ab**}	146.50 ± 3.29^{ab**}	1.43 ± 0.18^{ab**}	2.43 ± 0.18^{ab**}

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, disease control respectively. Values with superscript ^{a*}/^{a**}indicate significant difference compared with healthy control; ^{b*}/^{b**}indicate significant difference compared with disease control at $p < 0.05$ and $p < 0.01$ respectively

control group but there were significant improvement of the same as compared with disease control group. The lipid profile of the treatment group—high dose (500 mg/kg bwt) showed that there were significant improvement as compared with disease control and the values are normal and comparable with healthy control. The Atorvastatin-treated SD group showed significant decrease of lipid profile as compared with disease control group and the values are in normal range in healthy control. These details have been documented in Table 1 and Graphs 4 to 6.

The evaluations of liver function tests, including total protein, albumin, globulin, SGOT, SGPT, ALP and total protein levels, were found to be normal in all the groups, and there were no significant changes observed when compared with healthy control (Table 2 and Graphs 7 and 8).

Histopathology

No major histopathological changes were observed in liver, kidney, and heart tissue samples when compared with healthy control (Figs 1 to 6). So, the study proved the safety of the test extract at the prescribed dosages.

CONCLUSION

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.

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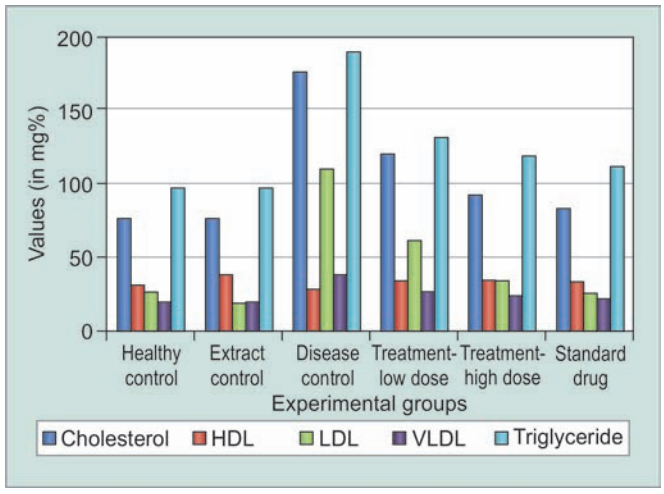
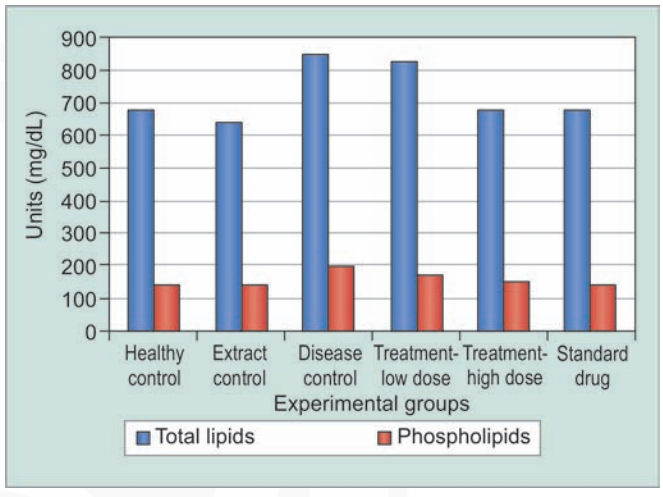
Table 2: Liver function parameters in Wistar Albino rats during efficacy study

	Biochemical parameters—liver function test							
Groups	*Total protein (gm/dL)*	*Albumin (gm/dL)*	*Globulin (gm/dL)*	*A/G*	*SGOT (U/L)*	*SGPT (U/L)*	*ALP (U/L))*	*Total bilirubin (mg/dL)*
Healthy control group	6.46 ± 0.13	3.0 ± 0.12	3.46 ± 0.15	0.86 ± 0.07	142.0 ± 2.94	51.50 ± 2.81	113.33 ± 12.63	0.26 ± 0.03
Test extract group	6.36 ± 0.12[a]	2.88 ± 0.07[a]	3.48 ± 0.16[a]	0.85 ± 0.06[a]	138.83 ± 7.54[a]	51.33 ± 2.39[a]	108.66 ± 9.17[a]	0.26 ± 0.02[a]
Disease control group	6.10 ± 0.13[a]	2.75 ± 0.06[a]	3.35 ± 0.10[a]	0.85 ± 0.02[a]	141.0 ± 11.50[a]	52.33 ± 5.12[a]	155.83 ± 26.25[a]	0.33 ± 0.03[a]
Treatment group—low dose (250 mg/kg bwt)	6.01 ± 0.11[ab]	2.78 ± 0.07[ab]	3.23 ± 0.06[ab]	0.86 ± 0.02[ab]	132.50 ± 11.19[ab]	46.0 ± 4.10[ab]	142.16 ± 20.03[ab]	0.36 ± 0.04[ab]
Treatment group—high dose (500 mg/kg bwt)	6.26 ± 0.32[ab]	2.88 ± 0.15[ab]	3.38 ± 0.20[ab]	0.85 ± 0.05[ab]	126.83 ± 8.32[ab]	44.83 ± 4.11[ab]	144.83 ± 29.74[ab]	0.25 ± 0.03[ab]
SD	6.18 ± 0.16[ab]	2.73 ± 0.10[ab]	3.45 ± 0.09[ab]	0.80 ± 0.02[ab]	133.16 ± 5.93[ab]	44.16 ± 3.67[ab]	144.16 ± 27.76[ab]	0.26 ± 0.03[ab]

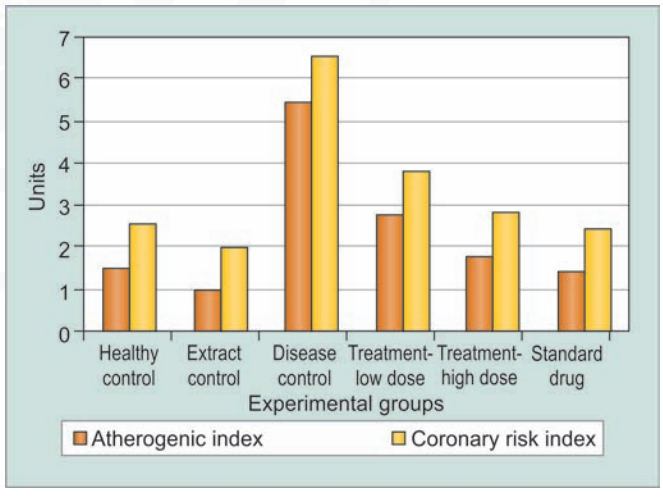
Values are expressed as mean ± standard error of mean, n = 6 per group. Values with superscript [a, b] indicate no significant difference ($p > 0.05$) when compared with healthy control and disease control respectively. Values with superscript [a]*/[a]**indicate significant difference compared with healthy control; [b]*/[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. hallicacabum* L. extract on total lipids and phospholipids



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

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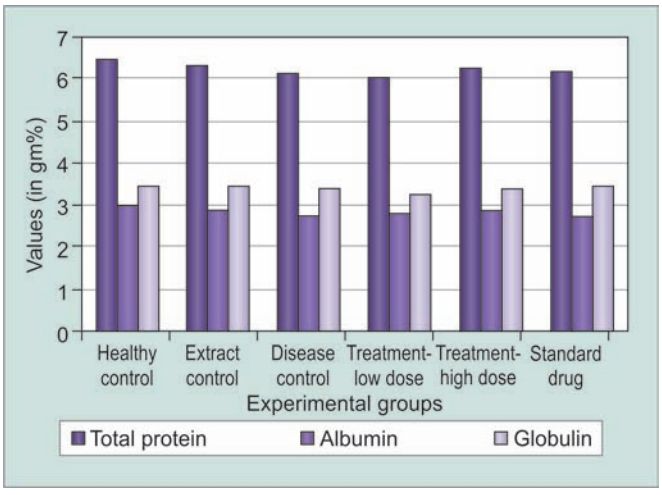
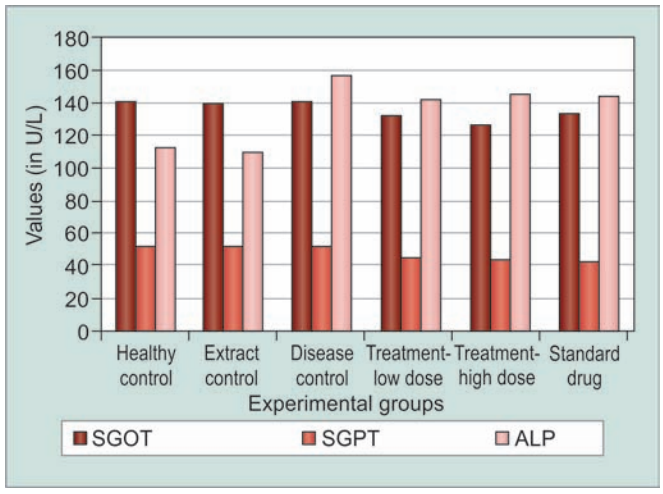


Graph 7: Effect of *C. halicacabum* L. extract on levels of serum proteins



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

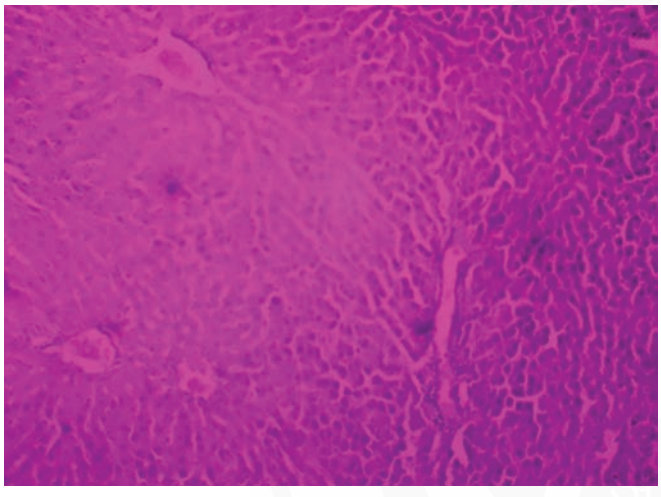


Fig. 1: Healthy control. Section of liver tissue of male rat showing normal portal triads and hepatic veins

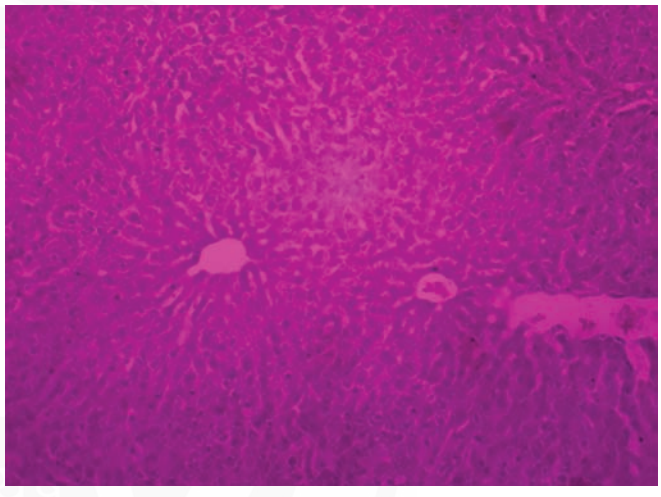


Fig. 2: Test extract group high dose. Section of liver of male rat showing normal portal triads and hepatic veins. Hepatocytes appear normal and they are arranged in cords. Kupffer cells and sinusoidal spaces are normal

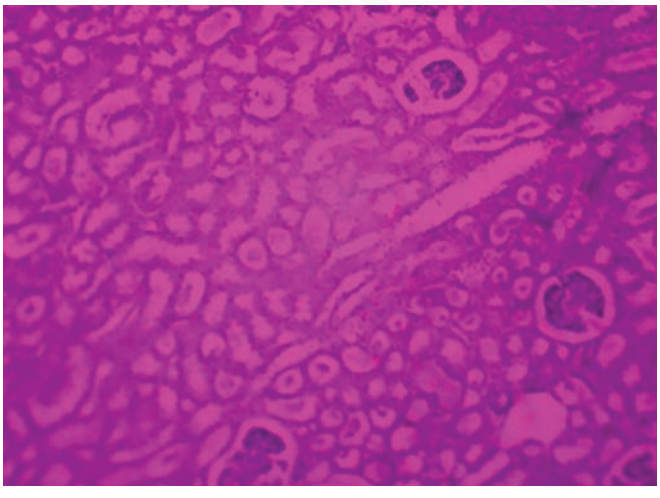


Fig. 3: Healthy control. Section of kidney tissue of male rat showing normal glomeruli. Afferent and efferent arterioles appear normal

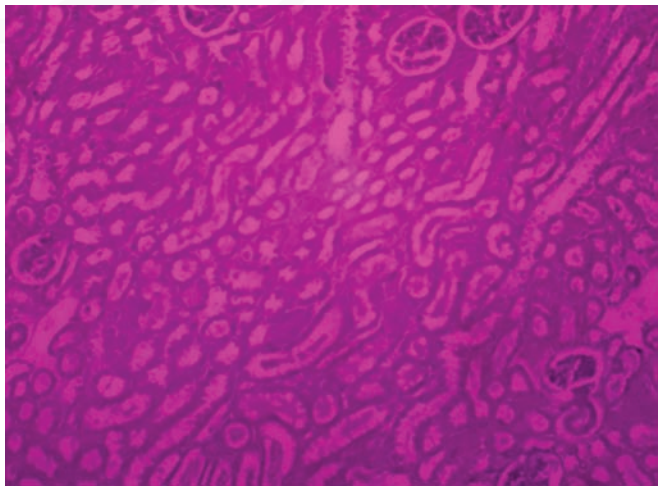


Fig. 4: Test extract group high dose. Section of kidney of male rat showing normal glomeruli and Bowman's capsule. Renal tubules are normal. Interstitial tissue also appear normal

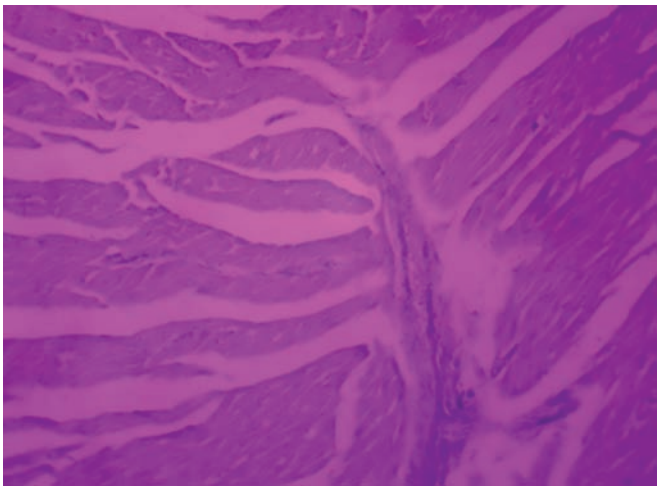


Fig. 5: Healthy control. Section of heart tissue of male rat showing normal endocardium, myocardium, and pericardium

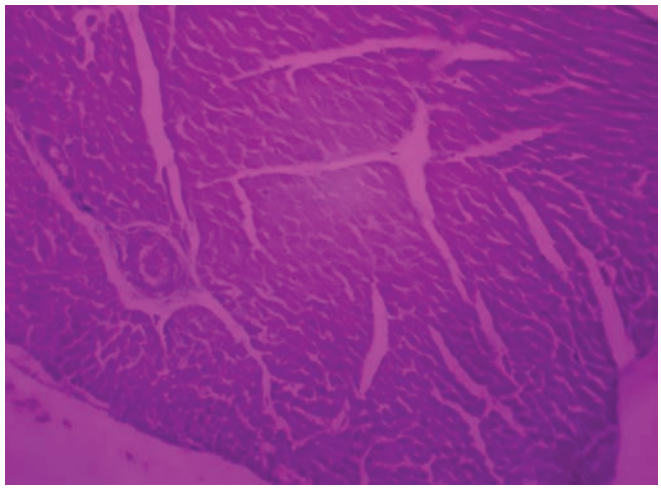


Fig. 6: Test extract group high dose. Section of heart tissue of male rat showing normal endocardium, myocardium, and pericardium

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हिन्दी सारांश

एथेरोडाइट प्रेरित विस्टार अल्बिनो रेट्स में कार्डियोस्पर्मम हेलीकाकाबम एल. पत्र की हाइपोलिपिडेमिक गतिविधि का मूल्यांकन

उद्देश्यः हाइपरलिपिडिमिया को कोरोनरी हृदय रोगों के प्रसार एवं गंभीरता के लिए सबसे बड़ा जोखिम कारक माना गया है। वर्तमान अध्ययन कार्डियोस्पर्मम हेलिकाकाबम एल. पत्र के हाइड्रोएल्कोहोलिक निस्सार की हाइपोलिपिडेमिक गतिविधि के मूल्यांकन के लिए किया गया है। दक्षिण भारत के स्थानीय चिकित्सकों द्वारा विभिन्न रोगों की चिकित्सा के लिए इस पादप का उपयोग बहुतायत से किया जाता है।

कार्य प्रणालीः विस्टार रेट्स में एथेरोडाइट प्रेरित हाइपरलिपिडिमिया पर परीक्षण किया गया परीक्षण अवधि 28 दिन तथा समूह छः रखे गए यथा स्वस्थ नियंत्रण समूह, निस्सार नियंत्रण समूह, रोग नियंत्रण समूह, कम औषध मात्रा चिकित्सा समूह, अधिक औषध मात्रा चिकित्सा समूह एवं मानक औषध समूह। मानक प्रोटोकॉल का उपयोग करते हुए एथेरोजेनिक इण्डेक्स व कोरोनरी रिस्क इएडेक्स के साथ लिपिड प्रोफाईल आदि जैवरसायनिक परीक्षण एवं अन्य चयापचय मापदण्डों का मूल्यांकन किया गया। जन्तुओं के महत्त्वपूर्ण अंगों का ऊतकविकृतिविज्ञानीय अध्ययन भी किया गया।

परिणामः अध्ययन से ज्ञात हुआ कि रोग नियंत्रण समूह की तुलना में विस्टाररेट्स में एथेरोडाइट प्रेरित हाइपरलिपिडिमिया पर कार्डियोस्पर्मम हेलीकाकाबम एल. पत्र के हाइड्रोएल्कोहोलिक निस्सार की महत्त्वपूर्ण हाइपोलिपिडेमिक गतिविधि है। ऊतक विकृतिविज्ञानीय अध्ययन भी निस्सार की सुरक्षा के साक्ष्य रहे।

निष्कर्षः अध्ययन का निष्कर्ष निकला कि कार्डियोस्पर्मम हेलीकाकाबम के हाइड्रो एल्कोहोलिक निस्सार की निर्धारित मात्रा में महत्वपूर्ण हाइपोलिपिडेमिक गतिविधि है।

आतुरीय प्रासंगिकताः जैसा कि इस पादप ने हाइपोलिपिडिमिक गतिविधि के परिप्रेक्ष्य में आशाजानक प्रभाव दिखाया। हाइपरलिपिडिमिया की चिकित्सा के लिए आयुष चिकित्सा पद्धतियों के औषध योग तथा एकल औषध के रुप में नवीन उत्पाद के विकास के लिए अग्रिम अध्ययन किया जाना चाहिए।

मुख्य शब्दः एथेरोडाइट कार्डियोस्पर्मम हेलीकाकाबम, हाईपोलिपिडेमिक गतिविधि।