INTRODUCTION
Atherosclerosis underlies the leading reason of mortality and morbidity globally. Initiation of atherosclerosis involves a decade-long expansion of the arterial intima, which occurs due to an intricate interaction of circulatory factors and various cell types in the vessel wall including endothelial cells, lymphocytes, monocytes, and smooth muscle cells [1]. Cardiovascular disease (CVD) causes 1 of every 5 deaths in past years [2]. Current allopathic drug treatment for atherosclerosis involves statins (HMG-CoA reductase inhibitors), fibrates, niacin, and bile acid resins. Among these remedies, the statin is the most widely and reliably used drug as it directly lowers the serum cholesterol level. While toxicological studies suggest no adverse effect on renal and liver function tests, hematological parameters were also observed in a normal range. Histological analysis showed that cholesterol administration caused a narrowing of the aortal lumen while treatment with 70% EtOH and atorvastatin decreased the plaque size and restored the luminal size of the aorta to normal.

Conclusion: The present study suggests that commonly used culinary spice cumin seed possesses hypolipidemic and cardioprotective effect with a positive effect on serum biochemistry, histology, and hematology.

Keywords: Atherosclerosis, Hyperlipidemia, Cumin seed, Cardioprotective.

ABSTRACT

Objective: The present study is related with the assessment of anti-atherosclerotic efficacy of 70% EtOH extract of cumin seed in diet-induced hyperlipidemic rabbits.

Methods: Rabbits were rendered hyperlipidemic by oral administration of cholesterol for 15 days. Then, the animals were treated with 70% EtOH (ethanolic extract) of cumin seed extract for 45 days (Group III). Another set of animals was treated with atorvastatin, the standard drug for 45 days. At the end of experimental period, the serum biochemical, hematological, and histological analysis of thoracic aorta was done.

Results: The cumin seed extract showed contain hypolipidemic effect by reducing plasma cholesterol, low-density lipoproteins, and triglycerides level. While toxicological studies suggest no adverse effect on renal and liver function tests, hematological parameters were also observed in a normal range. Histological analysis showed that cholesterol administration caused a narrowing of the aortal lumen while treatment with 70% EtOH and atorvastatin decreased the plaque size and restored the luminal size of the aorta to normal.

Conclusion: The present study suggests that commonly used culinary spice cumin seed possesses hypolipidemic and cardioprotective effect with a positive effect on serum biochemistry, histology, and hematology.

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METHODS

Extraction of plant material
Dried and cleaned seeds of the C. cyminum (Linn.) were bought from the local market. 70% of ethanolic extract was prepared with the use of Soxhlet apparatus for 24 h. The extract was treated under low pressure and temperature and then distilled to remove excess of ethanol from the extract. After complete removal of ethanol from the extract, it was dried to obtain the brown-colored sticky extract. The extract was stored in desiccation for future use in experiments.

Experimental animals
New Zealand white rabbits weighing between 1 and 1.25 kg were obtained from the certified institute. Animals were acclimatized for 10 days before the onset of the experiment. Animals were kept in clean, metallic wire gauge cages in a room with 12:12 h light-dark cycle, 20–25°C temperature, and 40–50% relative humidity and were fed with standard pellet diet and fresh green vegetables and drinking water. The experimental protocol was approved by the Institutional Animal Ethical Committee (Reg No.: 1646/GO/Re/12/CPCSEA).

Experimentation

Induction of hyperlipidemia
Rabbits were rendered hyperlipidemic by the oral dose of cholesterol powder at the dose of 500 mg/kg.b.wt./day dissolved in 5 ml of coconut oil for 15 days.
Dose regimen of atorvastatin
Atorvastatin (Atorlip-10, Cipla) was used as a standard hypolipidemic drug. It was orally administered at the dose of 0.25 mg/kg.b.wt./day dissolved in 5 ml of distilled water.

Preparation of plant drug
70% of ethanolic extract (500mg/kg b.wt.) was then given to the experimental animals by mixing it in 5 ml distilled water. The dose of the extract was determined by LD₅₀ test.

Experimental design
The experimental period was of 60 days and divided into four groups (n=5).
- Group I: Intact control
- Group II: Hyperlipidemic control
- Group III: 70% EtOH of cumin seed treatment group
- Group IV: Atorvastatin (Atorlip-10) treatment group.

Assessment of hematology
Blood was collected by direct cardiac puncture at the end of experimental period. The collected blood was stored in EDTA vials at −20°C. Hematological assessments of hemoglobin, TRBC, hematocrit, mean corpuscular volume, mean cell hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, total leucocyte count, platelet count, plateletcrit, mean platelet volume, and platelet distribution width were examined using hematology analyzer through standard methods [10].

Assessment of serum biochemistry
At the end of experimental period, animals were sacrificed under prolonged anesthesia and direct cardiac puncture. Serum was separated by centrifugation of blood at 3000 rpm for 15 min and stored at −20°C for further need. Serum samples were analyzed using Biochem Auto-analyzer RX-50 (Microlab Instrument) for total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TGs) using commercial diagnostic kits (Siemens Healthcare Diagnostics, USA). Low-density lipoprotein (LDL)-cholesterol and very-LDL (VLDL-C) were calculated by Friedewald’s formula [11]. TC/HDL and LDL/HDL were also calculated as ischemic indices to access the atherogenic risk. The toxicity profile was also performed by standard kits [12].

Histopathology of the aorta
The thoracic aorta was cut (2–3 cm length) and excised from the heart. The tissue was cleaned in 0.9% saline and kept in 10% formalin fixative. The processed aorta was then ultrasectioned (5–6 μm thickness). Sectioned tissues were stained with hematoxylin and eosin stain and examined under the light microscope for histopathological observations.

Statistical analysis
All biochemical parameters were expressed as the mean±standard error of the mean. One-way ANOVA was analyzed using Tukey’s multiple comparison tests. GraphPad Prism-7 was used for data analysis, and graphical representation was made using MS Excel-2007. Level of significance was expressed as p<0.05.

RESULTS
Effect on lipid profile
A drastic increase in serum TC was observed when high cholesterol diet was administered to the animals, and this increase in TC was decreased significantly 85% (p≤0.01) in animals treated with 70% EtOH of cumin seed extract. While highly significant (p≤0.001) increase in HDL-C concentration was observed in the hyperlipidemic group, this level came back down when treated with 70% EtOH cumin seed extract and statin. Similarly, in the case of LDL-C, 96%, in VLDL-C, 39.61%, and in TG, 64.08% reduction were observed in cumin seed extract-treated groups which are comparable with statin treatment. CHOL/HDL and HDL/LDL ratio of both 70% EtOH cumin seed extract and statin-treated groups were comparable with the control group. Total lipid concentration also showed high significant (83.69%) increase in case of the hyperlipidemic group, while other two groups showed significant decrease, and 74.78% in cumin extract treated and 81.88% in atorvastatin-treated groups were observed.

Effect on serum biochemistry
Serum biochemical analysis suggests that there is a considerable increase in the level of blood sugar in the hyperlipidemic group while cumin seed extract and atorvastatin-treated animals showed almost normal values near to the control group. Some of the other parameters of the liver functional test and the renal function test did not show any considerable variation throughout the experimental period in all the four groups.

Effect on hematology
Observations suggest that no noticeable variation was observed in hematological parameters. All the parameters were near the control values.

Effect on aortic plaque formation (Figs. 1 and 2, Tables 1 and 2)
Histopathological study of ascending aorta shows plaque formation with the presence of foam cells, collagen, and lipid deposition causing thickened intimal lining, resulting in narrowing of the arterial lumen,

**Fig. 1:** Graphical representation of the effect of 70% EtOH extract of cumin seed-treated hyperlipidemic rabbits. (Mean of five values±standard error of the mean)

**Fig. 2:** Photomicrographs (H and E stain, 100×) of thoracic aorta of treated rabbits – (a) intact control: Thoracic aorta showing normal histology with aortic wall consisting of tunica adventitia, tunica media, and tunica intima. (b) Hyperlipidemic: Thoracic aorta exhibiting endothelium with the formation of atherosclerotic plaque which has thickened intima with large number of foam cells. (c) Cumin extract treated: Aorta showing normal histoarchitecture with restoration in endothelial wall integrity. (d) Atorvastatin: Restored histoarchitecture of thoracic aorta
Table 1: Serum lipid profile of cholesterol-fed rabbits treated with 70% EtOH of cumin seed extract

| Treatment groups         | TC (mg/dl) | HDL-C (mg/dl) | LDL-C (mg/dl) | VLDL-C (mg/dl) | TG (mg/dl) | TC/HDL | HDL/LDL | Total lipid (mg/dl) |
|-------------------------|------------|---------------|---------------|----------------|------------|--------|---------|-------------------|
| Group I: Intact control | 79.8±9.63  | 27.3±2.01     | 11.1±1.36     | 19.4±4.16      | 198.6±0.1  | 2.9±0.31| 2.45±0.2 | 388.7±30.16       |
| Group II: Hyperlipidemic control | 100.8±10.16 | 27.3±2.01     | 11.1±1.36     | 35.4±4.76      | 299.2±22.51| 10.6±1.3 | 1.25±0.16 | 2379.8±210.16     |
| Group III: Cumin seed extract | 145.7±13.12 | 34.2±6.15     | 95.2±8.9      | 102.6±10.14    | 9.2±0.87  | 33.3±2.67| 0.33±0.01 | 59.9±7.12        |
| Group IV: Atorvastatin  | 113.35±9.01 | 27.05±1.97    | 24.6±2.2      | 26.9±2.61      | 7.1±0.43  | 24.6±2.2 | 3.1±0.43 | 431.37±41.12      |

Values expressed as mean±SEM (n=5). Groups II to IV compared with Group I, where \( p\leq0.05 \), \( \leq0.01 \), \( \leq0.001 \) and \( \text{non-significant}=h \). SEM: Standard error of the mean, TC: Total cholesterol, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, TGs: Triglycerides.

DISCUSSION

while treatment with 70% EtOH extract of cumin seed showed a significant reduction in plaque size and restonction of arterial wall integrity. These findings were comparable to atorvastatin-treated groups.

CONCLUSION

The present study proves that the plant C. cymnum L. has an anti-atherosclerotic effect due to its capacity to reduce ox-LDL and LDL concentration in serum, and both the elements play a central role in developing atherosclerosis. The plant also possesses antioxidant, hypoglycemic, anticancerous, and carminative effect.

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AUTHORS' CONTRIBUTIONS

The complete research work was suggested and mentored by Prof. Ashok Purohit. All the experimental work was performed by Harshlata Chouhan. Authors drafted and approved the final manuscript.

CONFLICTS OF INTEREST

The author declares no conflict of interest.
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