Ameliorative Efficiency of Coriandrum sativum Seed Extract on Atherosclerosis and Oxidative Stress in Male Albino Hyperlipidemic Rabbits.

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ABSTRACT

Coriandrum sativum is widely distributed and mainly cultivated for the seeds. The present study was aimed to evaluate the serum lipid parameters, faecal biochemistry, antioxidant parameters and histopathological study of aorta in the high fat diet induced atherosclerotic rabbits after administering the methanolic extract of the Coriandrum sativum seeds at the dose level of 250 mg/kg.b.wt/day. The animals were divided into Control group, High fat diet group for 60 days, High fat diet group for 120 days, High fat diet for 60 days then no treatment for next 60 days, High fat diet for 60 days then treated with 70% methanolic extract of C. sativum (250mg/kg.b.wt/day) for next 60 days, High fat diet + C. sativum seed extract for 120 days treated groups. Administration of the C. sativum seed extract significantly reduced the serum lipid parameters like Total cholesterol, triglycerides, Phospholipids, LDL and VLDL-Cholesterol along with increase in HDL ratio after treatment. Oral administration of C. sativum resulted in a significant increase in excretion of cholesterol and phospholipids when compared with atherodiet fed rabbits. It is also observed that there was drastic reduction in level of lipid peroxidation whereas GSH content and catalase activity were elevated after the treatment with 70% methanolic extract of C. sativum. The Plant extracts also significantly prevented the atheromatic changes and plaque formation in the aorta and favored increased fecal cholesterol and phospholipids output. All the above parameters indicting that seeds of this plant may contain the active constituents that may be effective in treatment of hyperlipidemia and atherosclerosis.

Key words: Atherosclerosis, Coriandrum sativum, High fat diet, Lipid peroxidation, HDL ratio

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INTRODUCTION

Diseases are generally considered to be the consequences of severe disorders and abnormalities in body homeostasis [1]. The major examples of such debilitating conditions are cardiovascular diseases, diabetes mellitus, and kidney stone diseases. Among them, cardiovascular diseases, especially atherosclerosis, stand as one of the leading cause of death in both the developed and developing countries.

This complex disease can be described as an excessive inflammatory, fibro fatty, proliferate response to damage of the artery wall. Many believe that it can be induced from simple dysfunction of endothelial lining as occurs with hyperlipidemia, hypertension or cigarette smoke [2]. Atherosclerosis is considered a process involving the interplay of inflammation and oxidative stress. Oxidations of low-density lipoprotein (LDL), and the subsequent uptake by macrophages in the vascular wall, are important steps in the development of atherosclerosis [3]. A small part of the oxidized LDL (OxLDL) particles escapes uptake by macrophages and returns to the blood stream or may leak from atherosclerotic plaques. Thus, measuring circulating levels of OxLDL may contribute to the estimation of cardiovascular disease (CVD) risk [4].

In many developing countries most hyperlipidemic individuals use medicinal plants as folk medicine to treat hyperlipidemia and atherosclerosis. Therefore there is a strong interest locally to search for natural hyperlipidemic substances derived from medicinal plants. The prophylactic and therapeutic effect of plant foods and extracts in reducing cardiovascular disease has been reviewed. A vast number of these plants are to receive attention in this regard and have been shown to lower plasma lipid levels, some examples are Gacinia cambogia, Zingiber officinale and Emblica officinalis [5].

The wide use of these herbal plants had now leaded to carry out research in institutions and universities on the potential benefits of herbal drugs. Our present study is also an attempt towards this direction. Coriandrum sativum (Common name: Coriander), belonging to family Umbelliferae, is a herb that is widely cultivated in India and is recognized for its carminative and cooling properties [6]. It was shown that coriander extracts have phenolic compounds and flavonoids, suggesting that these compounds contribute to the antioxidants activity. Phenolic substances such as flavonoids, cumarins, cinnamic acid and caffeic acids are believed to have antioxidant properties, which may play an important role in protecting cells and any organ from oxidative degeneration [7]. Coriander has been reported to exhibit several pharmacological effects such as as antifertility [8], antihyperglycemic [9], hypotensive [10] and digestive stimulant [11].

In the light of aforementioned medical properties of coriander, this study was carried out to investigate the possible protective properties of coriander extracts on oxidative stress and hyperlipidemia related biochemical, antioxidant parameters and histopathological study in aorta of cholesterol fed rabbit.
MATERIALS AND METHODS

Collection and Authentication of Plant Material

_Coriandrum sativum_ belongs to family Umbelliferae and commonly known as “Dhania”. The Plant was acquired from local market of Jaipur, Rajasthan state, India and authenticated by the authority of Department of Botany, University of Rajasthan, Jaipur. A voucher specimen number (no.) (RUBL20879) was submitted at Institute’s herbarium department for future reference.

Extraction of Plant Material

Coriander seeds were powdered and extracted with 70% methanol for 24 to 36 hours by soxhlet extraction method. Then methanol was separated under reduced pressure to obtain solid mass.

Animal Model

New Zealand white male rabbits (weights 1.50-2.0 kg.) maintained on a control pellet diet and water _ad libitum_ were used for the study.

Experimental Design

The rabbits were divided into the following groups:

**Group1 (G1):** Control- Placebo treated for 120 days.

**Group2 (G2):** Atherodiet + Cholesterol feeding from day 1-60 (atherogenic diet + 500 mg chol./kg.b.wt./rabbit/day in 5ml coconut oil).

**Group3 (G3):** Cholesterol feeding for 120 days (atherogenic diet + 500 mg chol./kg.b.wt./rabbit/day in 5ml coconut oil).

**Group4 (G4):** Cholesterol feeding for 60 days (atherogenic diet + 500 mg cholesterol/kg.b.wt./rabbit/day in 5 ml coconut oil/day), then no treatment for next 60 days. (i.e. from day 61-120).

**Group5 (G5):** Cholesterol feeding for 60 days (atherogenic diet + 500 mg chol./kg.b.wt./rabbit/day in 5ml coconut oil/day) then treated with 70% methanolic extract of _C. sativum_ (250mg/kg.b.wt/day) for next 60 days i.e. from day 61-120.

**Group6 (G6):** Cholesterol feeding (atherogenic diet + 500 mg chol./kg.b.wt./rabbit/day in 5ml. coconut oil) + 70% methanolic extract of _C. sativum_ (250mg/kg.b.wt/day) from day 1-120 (Concurrent treatment).
Blood, aorta and Faecal Collection

At the end of the experiment all the rabbits were sacrificed and blood was collected through cardiac puncture. Serum was separated by centrifugation and stored at -20°C until analysis. The heart together with the aorta (2–3 cm length) was excised from each animal. The aorta was cut at the origin and removed from the heart. A 2 mm section of the aorta of each animal was soaked in a 10% (v/v) formocalcium solution for H & E staining. The aorta sections were processed for normal histological section. The tissue samples were ultra sectioned (5–6 μm thickness), stained with haematoxylin and eosin (H&E) and examined under a light microscope for observation of structural abnormality. During last week of experiments total faecal matter of control, hyperlipidaemic and the treated rabbits was collected daily and assayed for total cholesterol [12] and phospholipids [13].

Parameters Studied

Following biochemical parameters have been estimated in serum and aorta-

**Serum Biochemistry**

Total cholesterol [12], Triglyceride [14], Phospholipids [13], LDL-Cholesterol [15], VLDL-Cholesterol [15], HDL Ratio [16], Atherogenic Index

**Faecal Biochemistry**

Total cholesterol [12], Phospholipids [13]

**Oxidative stress and antioxidant parameters in aorta**

Lipid peroxidation (LPO) [17], Catalase [18], Glutathione [19], Superoxide dismutase (SOD) [20]

**Histopathological Studies**

Ascending aorta was examined for histological changes.

**Statistical Analysis**

The data obtained from the above experiments were subjected to statistical analysis. Data were represented as Mean±SEM. The differences were compared for statistical significance by "t-test" by using SPSS software (16.0 version) and they were considered non significant at P ≤ 0.05, significant at P ≤ 0.01 and highly significant at P ≤ 0.001. Graphical representation of data has been done using Microsoft Excel 2007.

**RESULTS**

**Serum Biochemistry** (Table: 1)
Hypercholesterolaemic rabbits depicted significant increase (P≤ 0.001) in the total serum cholesterol, triglyceride and phospholipids when compared to control group. The study hibited that elevated serum total cholesterol, triglyceride and phospholipids which occur in hyperlipidemia, was significantly reduced by the administration of C. sativum seed extract. The levels of LDL-cholesterol (an important atherogenic factor) and VLDL-cholesterol were increased significantly after cholesterol feeding for 60 and 120 days. In comparison with cholesterol fed group, the group which consumed C. sativum seed extract alone and concurrent group showed significantly reduction in these parameters. HDL ratio which is considered as an important risk predictor reduced significantly in hyperlipidaemic rabbits after cholesterol feeding for 60 and 120 days. C. sativum extract administration to hyperlipidaemic groups showed significant improvement in HDL ratio. Atherogenic Index showed significant elevation in hypercholesterolaemic rabbits, which was lowered after C. sativum extract treatment.

Table 1: Effects of C. sativum on serum lipid profile in rabbits

<table>
<thead>
<tr>
<th>IDENTIFICATION GROUP</th>
<th>Total Cholesterol</th>
<th>Total phospholipids</th>
<th>Triglyceride</th>
<th>LDL Cholesterol</th>
<th>VLDL Cholesterol</th>
<th>HDL Ratio</th>
<th>Atherogenic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Placebo treated) from day 1-120</td>
<td>G1</td>
<td>128.00 ± 8.90</td>
<td>121.62 ± 8.99</td>
<td>76.21 ± 7.57</td>
<td>58.52 ± 3.83</td>
<td>14.46 ± 1.56</td>
<td>51.94</td>
</tr>
<tr>
<td>Atherodiet + Cholesterol feeding* from day 1-60</td>
<td>G2</td>
<td>702.65 ± 23.49</td>
<td>442.56 ± 12.90</td>
<td>398.90 ± 28.34</td>
<td>419.68 ± 21.83</td>
<td>67.52 ± 6.45</td>
<td>24.25</td>
</tr>
<tr>
<td>Atherodiet + Cholesterol feeding* from day 1-120</td>
<td>G3</td>
<td>1206.00 ± 45.55</td>
<td>641.30 ± 22.05</td>
<td>731.15 ± 30.63</td>
<td>750.45 ± 16.65</td>
<td>96.93 ± 7.59</td>
<td>15.42</td>
</tr>
<tr>
<td>Atherodiet + Cholesterol feeding* from day 1-60 + No treatment for next 60 day</td>
<td>G4</td>
<td>505.50 ± 18.21</td>
<td>384.14 ± 18.00</td>
<td>290.72 ± 14.00</td>
<td>324.30 ± 14.11</td>
<td>48.38 ± 2.45</td>
<td>29.25</td>
</tr>
<tr>
<td>Atherodiet + Cholesterol feeding* + C. sativum** from day 1-60 + C. sativum** from day 61-120</td>
<td>G5</td>
<td>232.15 ± 7.05</td>
<td>256.18 ± 21.35</td>
<td>174.12 ± 15.85</td>
<td>105.66 ± 9.90</td>
<td>28.84 ± 4.43</td>
<td>45.71</td>
</tr>
<tr>
<td>Atherodiet + Cholesterol feeding* + C. sativum** from day 1-120 (concurrent feeding)</td>
<td>G6</td>
<td>352.36 ± 14.40</td>
<td>345.63 ± 19.17</td>
<td>245.96 ± 12.00</td>
<td>202.72 ± 20.00</td>
<td>43.38 ± 3.77</td>
<td>36.55</td>
</tr>
</tbody>
</table>

*Cholesterol feeding –500mg/ kg.b.wt in 5 ml coconut oil / day
**C. sativum –250mg/ kg.b.wt. / day

<table>
<thead>
<tr>
<th>Values ± 6 determinations</th>
<th>a – P ≤ 0.001 Highly Significant</th>
<th>b – P ≤ 0.001 Highly Significant</th>
<th>c – P ≤ 0.01 Significant</th>
<th>ns – Non Significant</th>
</tr>
</thead>
</table>

Faecal Biochemistry (Fig.1.1 and 1.2)

Rabbits fed on high cholesterol diet showed a significant increment in faecal cholesterol and phospholipids level as compared to control group. Oral administration of C. sativum resulted in a significant increase in excretion of cholesterol and phospholipids when compared with atherodiet fed rabbits.
Oxidative stress and antioxidant parameters in aorta (Fig. 1.3, 1.4, 1.5 and 1.6)

Hypercholesterolaemic animals, (at the end of the study), recorded a significantly elevated levels of LPO compared to control animals maintained on standard pellet diet. Hypercholesterolaemic animals treated with C. sativum extract revealed significantly reduced level of the same, restoring the level closer to untreated normal animals. Figures 1.4 to 1.6 showed the effect of the plant extract on antioxidant enzymes CAT, GSH and SOD respectively. Atherogenic diets feeding to rabbits resulted in a significant decrease in the CAT, GSH and SOD activity of aorta, which was elevated after treatment with C. sativum. Antioxidant enzymes activities were increased in treatment and concurrent groups indicating a positive influence of C. sativum on the enzymatic antioxidant defence in hypercholesterolaemic rabbits.

Figures 1.1-1.6: It shows faecal biochemistry and enzyme activity of C. sativum treated rabbits
Group 1= Control  
a-P ≤ 0.001 Highly Significant  

Group 2= Ath. + Chol. (60D)  
b-P ≤ 0.001 Highly Significant  

Group 3= Ath. + Chol. (120D)  
c-P ≤ 0.01 Significant  

Group 4= Ath. + Chol. (60D) + No treatment (60 D) ns – Non Significant  

Group 5= Ath. + Chol. (1-60D) + C. sativum 250 mg (60D)  

Group 6= Ath. + Chol. + C. sativum 250 mg (120D)  

**Histopathological Studies** (Fig. 2.1-2.6)

The aorta of animals given *C. sativum* seed extracts alone or concurrently with atherogenic diet showed almost normal histology. However, aorta of high cholesterol diet animals showed spaces within the intima tunica and media tunica. These spaces had originally contained fat droplets which were dissolved during the H & E staining procedure. Less cholesterol deposits were seen in the aorta of the concurrent group animals with very little deposits observed in the intima tunica and media tunica region and no cholesterol deposit seen in the adventitia tunica region. Administration of *C. sativum* showed significant protection from aortic lesions. Aortic plaques were almost regressed and lumen size was also restored near to normal.

![Fig. 2.1 Ascending Aorta of control rabbit](image1)

![Fig. 2.2 Ascending Aorta of Atherodiet fed rabbit for 60 days](image2)

![Fig. 2.3 Ascending Aorta of Atherodiet fed for 120 days](image3)

![Fig. 2.4 Ascending Aorta-Atherodiet feeding (60 days) + No treatment (61-120 days)](image4)
DISCUSSION

Lipids play an important role in cardiovascular disease, not only by way of hyperlipidemia and the development of atherosclerosis, but also by modifying the composition, structure, and stability of cellular membranes. Excess lipids in the blood are considered to accelerate the development of arteriosclerosis and are the major risk factor in myocardial infarction [21]. Cardiovascular diseases, particularly atherosclerosis, are considered to be the major causes of medical complications among humans that can lead to death and it has been estimated that atherosclerosis would be the number one cause of deaths worldwide by the year 2020 [22]. This condition is a result of a series of impairments in the normal body functions, most important of which are overproduction of cholesterol, redox imbalance and vascular dysfunction [23].

Natural products always found to be the reliable source for several ailments, their popularity and contribution is undoubtedly worthless. Plants and herbs have a dramatic cholesterol lowering properties without any side effects which are normally associated with synthetic drugs [24]. In the present study, oral administration of methanol extract of seeds of *C. sativum* (250 and mg/kg body weight) showed hypolipidaemic activity.

Cholesterol levels have become the source of health concerns, even though cholesterol is one of the most valuable substances in the human body [25]. A significant elevation in serum cholesterol level after cholesterol feeding in rabbits were probably due to the overproduction of VLDL in the liver or by delayed catabolism of VLDL or both. It leads to elevated concentrations of VLDL remnants and ultimately of LDL [26]. The control of serum cholesterol is the major concern of the modern medicine. Treatment with methanolic extract of *C. sativum* reduced serum cholesterol level. Reduction of total cholesterol levels may be due to fibers content that increases the activity of plasma lecithin cholesterol acyl transferase (LCAT) which enhances hepatic bile acids synthesis and increases degradation of cholesterol to faecal bile acids and neutral sterols [27].

Triglycerides are the major component of most food. It is shown that atherogenic diet elevates serum triglyceride levels essentially by preventing its uptake and clearance by inhibiting catabolising enzymes like lipoprotein lipase (LPL) and LCAT [28]. Apparently the methanolic extract of the seeds of *C. sativum* is able to reduce the inhibition on LPL and
LCAT activity making triglycerides available for uptake and metabolism by tissues. The decrease in serum triglyceride has been attributed to stimulation of the degradation of triglycerides through increased expression and activity of lipoprotein lipase LPL and to decrease hepatic synthesis and secretion of triglycerides [29].

It has been shown that phospholipid level can be increased by atherogenic diet and cholesterol feeding [30]. Oxidized phospholipids accumulate under conditions of oxidative stress during inflammatory conditions such as atherosclerosis; they are also generated in apoptotic and necrotic cells [31]. The reduction in phospholipids level could possibly be due to a higher level of phospholipase that metabolized the blood phospholipids in hypercholesterolemic animals [32].

LDL cholesterol has been clearly associated with the risk of developing coronary heart disease. The oxidative modification hypothesis predicts LDL oxidation as an early event in atherosclerosis, and oxidized LDL as one of the important contributors of atherogenesis [33]. One of the first steps in the development of atherosclerosis is the passage of LDL out of the arterial lumen into the arterial wall. Plasma LDL is transported across the intact endothelium and becomes trapped in the ECM ( Extracellular Matrix) of the sub endothelial space where it is subjected to oxidative modifications to produce highly oxidized and aggregated LDL, referred to as OxLDL (Oxidized LDL). OxLDLs are believed to be the most atherogenic forms of LDL. Some natural antioxidants present in dietary sources, although in a small amount, can contribute powerfully to increasing the oxidative resistance of LDL [34]. The results obtained in this study indicate that oral administration of methanolic extract of C. sativum decreases the level of LDL in a model of experimental atherosclerosis. The LDL-cholesterol lowering could result from a reduced LDL- synthesis and/ or an increased LDL metabolism [35].

VLDL Cholesterol is assembled in the liver from triglycerides, cholesterol, and apolipoproteins. VLDL is converted in the bloodstream to LDL. VLDL are produced by the liver and some VLDL remnants seem to promote atherosclerosis similar to LDL [36]. In the present study, there was elevation in serum VLDL level in response to high cholesterol diet as compare to normal control group. VLDL cholesterol levels reduced by increasing the fractional catabolic rate of LDL cholesterol and lower the content of cholesterol in the VLDL and LDL particles by increasing the liver LDL receptors activity [37].

HDL Ratio (HDL-Cholesterol/ Total Cholesterol) is most important indicator of CHD at both high and low serum cholesterol level [38]. In the present study cholesterol feeding to rabbits caused a significant reduction in the HDL-ratio which was improved dose-dependently after treatment with C. sativum. It has been suggested that HDL functions to transport cholesterol from peripheral tissue to the liver. These results are consistent with earlier reports [39, 40] which have clearly established a correlation between dietary lipids and serum lipid profile.

Universally, Atherogenic index of plasma (AIP) has been used by some practitioners as a significant predictor of atherosclerosis [41]. This ratio represents an Atherogenic index, which is an important prognostic marker for cardiovascular disease such as atherosclerosis. Indeed, the risk of myocardial infarction increases considerably when this ratio is higher than five and it should ideally be four or less [42]. The rabbits fed with high cholesterol diet
exhibited significant increase in Atherogenic index. Treatment with methanolic extract of *C. sativum* shows significant decrease in Atherogenic index. These results are similar in the work done by other workers [43, 44].

Atherodiet fed rabbits showed significant increment faecal excretion of cholesterol and phospholipids whereas rabbits treated with *C. sativum* extract excreted more faecal cholesterol and phospholipids contents in faeces. The increased faecal excretion of neutral sterols probably included nondietary as well as dietary sources of neutral sterols. *C. sativum* L. seed oil constitutes a good source of sterols mainly of stigmasterol and β-sitosterol which exhibit an inhibitory effect on the absorption of dietary cholesterol [45]. Studies in humans and animals clearly documented the ability of dietary plant sterols to inhibit cholesterol absorption and reduce plasma LDL cholesterol concentration [46].

Lipid peroxidation is the parameter most often employed for assessing oxidative damage in the human body [47]. In this process LDL-C and other lipid containing molecules may becomes oxidized in the blood stream exerting adverse effects on a variety of processes like inhibiting antithrombin III activity, producing procoagulant activity, enhancing platelet aggregation, modulating vascular responses and acting as mitogen [48]. In the present study, after oral administration of *C. sativum* caused a significant reduction of lipid peroxidation (TBARS) in aorta. The ability of the methanolic extract to inhibit the process of lipid peroxidation in vivo may be due to the free radical scavenging activities of its phytochemical components [49]. In addition, anti-lipid peroxidative activity of the extracts may be due to the presence of anti-lipidemic agents [50].

Glutathione is a small tripeptide protein synthesized in the liver. It is a potent antioxidant with high redox potential and it also serves as a co-factor for several oxidative stress detoxifying enzymes (glutathione peroxidase and glutathione transferase) [51]. It can be assumed that the reduction in tissue glutathione levels was as a result of increased oxidative stress and lipid peroxidation occasioned by the high cholesterol diet (48). Extract treated hypercholesterolemic animals, showed a significantly elevated level of GSH. It is possible that extract might have reduced the extent of oxidative stress, leading to lesser GSH degradation or increase in the biosynthesis of GSH [52].

SOD and CAT considered as primary enzymes since they are involved in the direct elimination of reactive oxygen species. SOD and CAT are present in all oxygen-metabolizing cells and their function is to provide a defence against the potentially damaging reactivity’s of superoxide and hydrogen peroxide [53]. Decrease in the SOD and the catalase levels in the high cholesterol fed animals is again attributed to increased oxidative stress on cholesterol feeding in these animals. These findings are similar to other studies [54, 55]. *C. sativum* extract treated hypercholesterolaemic animals had significantly elevated levels of SOD and CAT, reversing the ill effects of hypercholesterolemia. The currently noted elevated levels of both SOD and CAT levels could be due the influence of flavonoids and polyphenols of *C. sativum* [50].

Aorta of cholesterol fed animals exhibited atheromatous plaque as compared to normal control group. The lipids deposited in the atherosclerotic lesions are mostly derived from plasma LDL, which is modified by oxidative processes, resulting in an enhanced uptake
by the scavenger receptor of macrophages leading to foam cell formation [56]. Dietary antioxidants should therefore inhibit atherogenesis by inhibiting oxidation and accumulation of LDL in macrophages [57, 58]. In the C. sativum treated group plaques were decreased significantly compared to the high-cholesterol diet group and compared to concurrent group. It may be due to reduced formation of foam cells. The inhibition of intimal thickening by C. sativum may be due to its antioxidant and anti-inflammatory properties [59].

CONCLUSION

The result of this study implies that the cardio protective effect of C. sativum seeds in high cholesterol diet induced atherosclerotic rabbits by preserving the membrane integrity and restoring the activities of enzymes to near normal levels. This might be due to the antioxidant effect of Coriandrum sativum fruits, and hence C. sativum fruits seem to be promising tools to explore as therapeutic agent in cardiovascular diseases.

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REFERENCES


