Effect of *Amomum subulatum* on Oxidative Stress and Serum Lipids in Cholesterol Fed Rabbits

S. C. Joshi*, Gopal Lal Bairwa* and Nidhi Sharma*

*Reproductive Toxicology Unit, Center for advanced studies, Department of Zoology, University of Rajasthan, Jaipur – 302 055 (India)

*E-mail: s_c_joshi2003@rediffmail.com

Received 01 January 2012; accepted 21 January 2012

Abstract

The aim of this study was to find out the effects of a fraction (25:75; CHCl₃:CH₃OH) of *Amomum subulatum* (Zingiberaceae) on antioxidant status and lipid profile in cholesterol fed rabbits. Oral administration of cholesterol (500mg/kg. b.wt./day) for 120 days caused increase in levels of serum cholesterol, phospholipids, triglyceride, Low Density Lipoprotein and Very Low Density Lipoprotein cholesterol but the High Density Lipoprotein-cholesterol ratio was decreased as compared with controls. The change in the enzyme activities were accompanied by an increase in Lipid Per oxidation and reduction in Glutathione and catalase activity. The level of Lipid Per oxidation, Glutathione and catalase in liver were found near to normal in treated animals. The serum cholesterol, triglyceride, phospholipids, Low Density Lipoprotein and Very Low Density Lipoprotein cholesterol were reduced whereas High Density Lipoprotein ratio increased after administration of *A. subulatum*. *A. subulatum* extract feeding increased the faecal excretion of cholesterol and phospholipids. Our study reveals that *A. subulatum* is a potent antihyperlipidaemic agent and provides antioxidant protection against oxidative stress induced by free radicals.

Key words: Cholesterol; antioxidant; atherosclerosis; antihyperlipidaemic; *Amomum subulatum*.

1. Introduction

Atherosclerosis, which characterized by lipid accumulation, inflammation, fibrosis, development of focal plaques and arterial sclerosis is the leading cause of morbidity and mortality in most developed countries [1, 2]. In particular, the disproportion between atheroprotective and atherogenic lipoproteins in plasma is one of the most important contributors towards atherosclerosis. Specifically, elevated Low-density lipoprotein (LDL) and reduced High Density Lipoprotein (HDL) were found to independently predict one's risk for developing cardiovascular disease [3, 4]. Oxidation of LDL is a strong predictor of coronary heart disease [5]. Plants still remain a major source for drug discovery in spite of the great development of synthetic molecules. Consequently, the uses of traditional plant extract in the treatment of various diseases have been flourished [6, 7]. Spices and condiments are widely used in Indian recipes to enhance the flavor and taste of food [8]. The spices and herbs contain potent antioxidant compound that protect LDL from oxidation, is thought to play a key role in pathogenesis of atherosclerosis [9, 10, 11, and 12]. *Amomum subulatum* is one of the world’s very ancient spices and has also been universally used for its health benefits. Medicinally, the seeds of *A. subulatum* are prescribed for treatment of indigestion, vomiting, abdominal pains and rectal disease [13]. The seeds of *A. subulatum* contains the glycosides, Petunidin-3,5-diglucoside, Leucocyanidin-3-O-β-D-glucopyranoside, Subulin (New auroine glycoside) [14] and 1-8, Cineole, α-terpinyl Acetate [15]. The present study is aimed to evaluate the effects of *A. subulatum* on antioxidant status and lipid profile in hypercholesterolaemic rabbits.

2. Materials and methods

2.1 Collection of Plant Material

*A. subulatum* belongs to Family- Zingiberaceae and commonly known as Greater cardamom or Doda. Authentic
seeds of cardamom were obtained from the National Institute of Ayurveda, Jaipur.

2.2 Extraction of Plant Material
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 called “crude extract.” The extract was fractioned with petroleum ether and benzene.

2.3 Fractionation through Column Chromatography
The crude extract was washed with petroleum ether, benzene and chromatographed over a silica gel column (60-120 mash). Elution was carried out with various mixtures of chloroform and methanol of increasing polarity. Various fractions were collected and dried. Out of them fraction (25:75; CHCl₃:CH₃OH) was used for evaluation of its activity.

2.4 Animal Model
2.5 New Zealand white male rabbits weighing 1.50-2.0 kg were used. Animals were acclimatized to the laboratory conditions at room temperature prior to the experiment. Rabbits were housed in animal cages at constant temperature and also maintained on a standard pellet diet (Ashirwad Industrial Ltd., Punjab), green leafy vegetables and water ad libitum.

2.6 Experimental Design
The rabbits were divided into the following groups -

- **Group I (G1):** Control- Placebo treated for 120 days.
- **Group II (G2):** Cholesterol feeding for 120 days (atherogenic diet + 500mg chol./kg.b.wt./rabbit/day in 5ml coconut oil).
- **Group III (G3):** Cholesterol feeding for 60 days (atherogenic diet + 500 mg chol./kg.b.wt./rabbit/day in 5ml coconut oil) then atherodiet withdrawn and treated with fraction of *A. subulatum* (25:75; CHCl₃:CH₃OH) (100mg/kg.b.wt/day) for next 60 days i.e. from day 61-120.
- **Group IV (G4):** Cholesterol feeding (atherogenic diet + 500 mg chol./kg.b.wt./rabbit/day in 5ml. coconut oil) + fraction of *A. subulatum* (25:75; CHCl₃:CH₃OH) (100mg/kg.b.wt/day) from day 1-120 (Concurrent treatment).

2.7 Parameters studied
Following biochemical parameters have been estimated in serum and liver i.e. total cholesterol [16], HDL- cholesterol [17], LDL and Very Low Density Lipoprotein (VLDL) [18], triglyceride [19], phospholipids [20], Lipid per oxidation (LPO) [21], catalase [22] and Glutathione (GSH) [23].

2.8 Blood 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. During last week of experiments total faecal matter of control, hyperlipidaemic and the treated rabbits was collected daily and dried at 40°C. Collected faeces were homogenized,
Figure 1-3: Enzyme activity of *A. subulatum* fraction (25:75; CHCl₃:CH₃OH) treated rabbits in liver

Table 1: Serum and faecal biochemistry of *A. subulatum* fraction (25:75; CHCl₃:CH₃OH) treated rabbit

<table>
<thead>
<tr>
<th>Identification</th>
<th>Group</th>
<th>Triglyceride</th>
<th>LDL Chol.</th>
<th>VLDL Chol.</th>
<th>HDL Ratio</th>
<th>Total Cholesterol Serum mg/dl</th>
<th>Excreta mg/gm</th>
<th>Total phospholipids Serum mg/dl</th>
<th>Excreta mg/gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Placebo treated) from day 1-120</td>
<td>G1</td>
<td>69.75 ± 8.24</td>
<td>60.31 ± 2.28</td>
<td>13.95 ± 2.40</td>
<td>53.75</td>
<td>114.18 ± 9.07</td>
<td>51.02 ± 1.52</td>
<td>118.37 ± 10.14</td>
<td>24.10 ± 0.85</td>
</tr>
<tr>
<td>Atherodiet + Chol. feeding* from day 1-120</td>
<td>G2</td>
<td>+565.59</td>
<td>+1105.52</td>
<td>+565.59</td>
<td>-54.95</td>
<td>24.21 ± 21.39</td>
<td>98.97 ± 2.40</td>
<td>648.52 ± 15.28</td>
<td>34.16 ± 1.02</td>
</tr>
<tr>
<td>% Deviation (I)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atherodiet + Chol. feeding* from day 1-60 + <em>A. subulatum</em> fraction** for next 60 days</td>
<td>G3</td>
<td>194.54 ± 11.03</td>
<td>157.13 ± 29.5</td>
<td>38.90 ± 3.45</td>
<td>40.37</td>
<td>275.18 ± 10.72</td>
<td>129.50 ± 9.18</td>
<td>298.93 ± 14.06</td>
<td>40.12 ± 1.00</td>
</tr>
<tr>
<td>% Deviation (II)</td>
<td></td>
<td>-27.58</td>
<td>-63.34</td>
<td>-27.58</td>
<td>+31.28</td>
<td>-56.36</td>
<td></td>
<td>-53.90</td>
<td></td>
</tr>
<tr>
<td>Atherodiet + Chol. feeding* + <em>A. subulatum</em> fraction** from day 1-120 (concurrent feeding)</td>
<td>G4</td>
<td>218.15 ± 14.06</td>
<td>192.05 ± 22.14</td>
<td>43.63 ± 2.50</td>
<td>44.42</td>
<td>340.37 ± 30.12</td>
<td>125.30 ± 7.28</td>
<td>336.18 ± 20.00</td>
<td>39.74 ± 1.02</td>
</tr>
<tr>
<td>% Deviation (II)</td>
<td></td>
<td>-53.01</td>
<td>-73.58</td>
<td>-53.01</td>
<td>+83.47</td>
<td>-66.57</td>
<td></td>
<td>-48.16</td>
<td></td>
</tr>
</tbody>
</table>

VALUES ± 6 Determination

* Cholesterol feeding – 500 mg/kg b.wt/day in 5 ml coconut oil /day
** *A. subulatum* fraction – 100 mg/kg b.wt/day

- a - P ≤ 0.001 Highly Significant when G 2 compared with G 1
- b - P ≤ 0.01 Significant when G 3 & 4 compared with G 2
- c - P ≤ 0.001 Highly Significant when G 3 & 4 compared with G 2
extracted (chloroform: methanol), freeze-dried and stored at -20°C. Faecal cholesterol [16] and phospholipids [20] were estimated.

2.9 Statistical Analysis
Values are expressed 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.

3. Results
3.1 Serum biochemistry (Table-1)
Cholesterol feeding (120 days) to rabbits caused a significant increase (P ≤ 0.001) in serum total cholesterol, triglyceride and phospholipids levels as compared to control group. LDL and VLDL cholesterol levels showed significant elevation (P ≤ 0.001) in cholesterol fed group of rabbits for 120 days which decreased significantly (P ≤ 0.001) after administration of A. subulatum fraction (25:75; CHCl≤ CH₂OH) at 100mg/kg.b.wt./rabbit/day in treatment and concurrent group both.

3.2 Faecal Biochemistry (Table-1)
The hyperlipidaemic rabbits of Group 2 (120 days) showed a significant increase in faecal cholesterol and phospholipid levels in comparison to control animals. A. subulatum fraction (25:75; CHCl≤ CH₂OH) caused a significant increase in excretion of cholesterol and phospholipids in faeces as compared to atherodiet fed hyperlipidaemic rabbits.

3.3 Antioxidant parameters (Fig. 1-3)
Cholesterol feeding (120 days) to rabbits resulted in a significant elevation (P ≤ 0.001) in LPO whereas a significant decline (P ≤ 0.001) in the activity of GSH and catalase in liver as compared to control group. Oral administration of A. subulatum fraction (25:75; CHCl≤ CH₂OH) caused significant reduction (P ≤ 0.01) in the TBARS activity of liver whereas elevation (P ≤ 0.01) was observed in GSH content and catalase activity of the liver in group 3 and 4.

4. Discussion
In the present study, a significant elevation in serum cholesterol level after cholesterol feeding in rabbits has been observed which may be 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 [24, 25].

Administration of A. subulatum fraction (25:75; CHCl₃ : CH₂OH) to the hypercholesterolaemic rabbits significantly reduced total cholesterol in the serum. It may reduce the atherogenic diet and cholesterol feeding significantly increased triglyceride level in the serum of hypercholesterolaemic rabbits in 120 days because dietary fat is composed principally of triacylglycerol which after digestion and absorption stimulates the production of chylomicrons [26, 27]. Serum triglyceride levels were reduced after treatment with A. subulatum fraction. It has been shown that phospholipids level can be increased by cholesterol feeding [28] which reduced after treatment. An alteration in phospholipids level is the result of any change in the metabolism of chylomicrones and VLDL [29].

The HDL cholesterol ratio was decreased in cholesterol fed rabbits. A. subulatum fraction treatment showed the elevation in the serum HDL cholesterol ratio. HDL cholesterol particles prevent atherosclerosis by extracting cholesterol from the artery walls and disposing of them through the liver and increasing HDL plasma levels normalize impaired endothelial function in hypercholesterolaemic rabbits [30].

Cholesterol fed rabbits showed lower faecal excretion of cholesterol and phospholipids whereas treated rabbits with A. subulatum fraction excreted more faecal cholesterol and phospholipids contents in faeces. This increase in excreta might be due to intestinal cholesterol absorption through plant products [31]. The main mechanism believed to be responsible for cholesterol reduction by plant sterols was thought to involve competitive inhibition of cholesterol incorporation into the micelles leading to about 50% decreased absorption and, consequently, increased excretion in feces [32].

Cholesterol feeding to rabbits for 120 days caused a significant reduction in the activity of catalase. Catalase catalyses the decomposition of H₂O₂ into water and oxygen and thus, protect the cell from oxidative damage by H₂O₂ and OH [33, 34]. Administration of A. subulatum fraction caused a significant increase in catalase content in liver of rabbits. The reduction in GSH activity might be due to enhanced oxidative stress in the liver after cholesterol feeding [35], while the administration of A. subulatum fraction increased the GSH in the liver of the rabbits. Administration of cholesterol caused significant increase in TBARS activity of liver. Elevated TBARS in cholesterol fed group suggests the enhanced oxidative stress in hyperlipidaemic state as represented by earlier studies in liver [35, 36]. Treatment with A. subulatum fraction reduced LPO in liver may be due to its antioxidant property [37]. These results reveal that A. subulatum is a protective antioxidant action on living cells suffering from oxidative stress and hyperlipidaemia.

5. Conclusions
In conclusion, administration of A. subulatum fraction could be more beneficial in preventing hyperlipidaemia and oxidative stress. Treatment with A. subulatum fraction (25:75; CHCl₃ : CH₂OH) to the cholesterol fed or hypercholesterolaemic rabbits significantly reduced total cholesterol, triglyceride, phospholipid, LDL and VLDL in the...
serum whereas HDL ratio was increased significantly. It has the ability to activate antioxidant enzymes that reduces oxidative stress in liver. The protective activity of *A. subulatum* extract may be due to its antioxidant defense system and that reduction of LDL oxidation may provide a protective effect against the detrimental action of oxidized LDL.

References


Source of support: Nil; Conflict of interest: None declared