Evaluation of Anti-Hyperlipidemic Activity of *Ammomum subulatum* Seeds Extracts

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ABSTRACT

*Ammomum subulatum* is a perennial plant cultivated in swampy places in Bengal, Sikkim, Assam and Tamil Nadu. Plant bears fruit having numerous seeds which are traditionally used in spice. Fruits are used as stimulant, aromatic, stomachic, aphrodisiac, in infection of teeth and gums. The present study is an attempt to explore the Anti-Hyperlipidemic effect of acetone and methanolic extract of seeds of *Ammomum subulatum*, using experimental model: High cholesterol diet induced atherosclerosis in rats. Dose of 200 mg/ml, 400 mg/ml and 800 mg/ml of acetone and methanolic extract were evaluated for their Anti-Hyperlipidemic activity against high cholesterol diet induced atherosclerosis in rat. Both the extracts were able to show Anti-Hyperlipidemic activity in dose dependant manner. The data were found statistically significant by using one way ANOVA (P< 0.001).our data suggest that *Ammomum subulatum* significantly reduced Serum total cholesterol, triglyceride, LDL and increase HDL, VLDL in the high cholesterol diet induced atherosclerosis in rats treated with drugs.

Keywords: Ammomum subulatum; Zingiberaceae; anti-hyperlipidemia; atherosclerosis, cholesterol, triglyceride, HDL, VLDL.

INTRODUCTION

*Ammomum subulatum* Roxb. (Family: Zingiberaceae) commonly known as Large or Greater Cardamom, Moti elaichi. Large cardamom is a tall perennial herb found in Eastern Himalayas and sub-Himalayan region of West Bengal, Assam and Sikkim. The seeds are aromatic pungent, stimulant, stomachic, alexipharmic and astringent. Traditionally, it is used to treat stomach pain, flatulence, belching, indigestion vomiting, malarial disorders, and drunkenness from alcohol Consumption. In Ayurvedic and Unani medicines large cardamoms are used as a preventive as well as a curative for throat troubles, congestion of lungs, inflammation of eyelids, digestive disorders and in the treatment of pulmonary tuberculosis. A. subulatum contains 1, 8-Cineole, α-pinene and β-pinene and geraniol, subulin, chalcone, cardamonin and a flavanone, alpinetin.

MATERIAL AND METHODS

Plant Material and Extraction

The fruits of *Ammomum subulatum* Roxb
were collected from local market of Modasa and authenticated by Dr. H. B. Singh Scientist and Head of Raw Materials Herbarium & Museum Dept of National Institute of Science and Communication and Information Resources, New Delhi (NISCAIR) and preserved the herbarium in Smt. R. B. Patel Mahila Pharmacy College, Atkot, Rajkot, Gujarat. The seeds were dried at room temperature and mechanically powdered to obtain a coarse powder; defatted with petroleum ether (60-80°C) and Soxhlet extracted with acetone and methanol. Solvent removal under reduced pressure was afforded by acetone and methanolic extract. The dry methanolic extract was stored in cool and dry place which was further used for evaluation of the hypercholesterolemic activity.

**Animals**

Adult male Wistar rats weighing around 180-200g were obtained from zydus healthcare, Ahmedabad, India. The animals were kept in polypropylene cages (three in each cage) at an ambient temperature of 25±20C and 55-65% relative humidity 12±1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow and had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines. The study protocol was approved by institutional animal ethical commit, RBPMPC, Atkot, India. Animal described as fasted were deprived of food for 16 h but free access to water.

**Experimental design**

Adult wistar rat with an initial body weight of 180 to 200g were taken, and divided into eight groups each containing six animals.

- **Group I: Control (CON):** They were administered with vehicle (water) for 7 days. They were fed with standard laboratory diet and water *ad libitum.*
- **Group II: High cholesterol diet fed group (HCD):** They were administered with vehicle (water) for 7 days along with high cholesterol diet (2% cholesterol, 1% sodium cholate and 2% coconut oil).
- **Group III: Acetone extract 200 mg/kg + HCD (ACE 200 + HCD):** Acetone extract (200 mg/kg/day, p.o) was administered for 7 days along with high cholesterol diet.
- **Group IV: Acetone extract 400 mg/kg + HCD (ACE 400 + HCD):** Acetone extract (400 mg/kg/day, p.o) was administered for 7 days along with high cholesterol diet.
- **Group V: Acetone extracts 800 mg/kg + HCD (ACE 800 + HCD):** Acetone extract (800 mg/kg/day, p.o) was administered for 7 days along with high cholesterol diet.
- **Group VI: Methanolic extract 200 mg/kg + HCD (ME 200 + HCD):** Methanolic extract (200 mg/kg/day, p.o) was administered for 7 days along with high cholesterol diet.
- **Group VII: Methanolic extract 400 mg/kg + HCD (ME 400 + HCD):** Methanolic extract (400 mg/kg/day, p.o) was administered for 7 days along with high cholesterol diet.
- **Group VIII: Methanolic extract 800 mg/kg + HCD (ME 800 + HCD):** Methanolic extract (800 mg/kg/day, p.o) was administered for 7 days along with high cholesterol diet.
After the duration of treatment period collection of blood was done for the listed biochemical parameters. 

**Biochemical parameter:**

**Collection of serum:**
The blood samples were withdrawn from retro-orbital plexus under light ether anesthesia without any anticoagulant and allowed to clot for 10 minutes at room temperature. It was centrifuged at 2500 rpm for 20 minutes. The serum obtained was kept at 4°C until used.

**Estimation of parameters:**
The serum so collected was used for estimation of serum cholesterol, serum triglyceride, serum HDL, serum LDL. Triglycerides were estimated by GPO Method\(^7\), Cholesterol and high density lipoprotein (HDL) by CHO /POD Phosphotungstate Method\(^8,9\), and low density lipoprotein (LDL) and very low density lipoprotein concentrations were determined by Friedewald formula\(^8,10\).

**Statistical analysis:**
All the values are expressed as mean S.E.M. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using computer based fitting program (Prism, Graphpad.). Differences were considered to be statistically significant when \(p < 0.05\).

**Result and Discussion**

Graph 1 Effect of Acetone and methanol extracts of Amomum subulatum (200, 400 &800 mg/kg) on Serum total cholesterol and triglycerides in normal rats and HCD induced atherosclerosis in rats (* \(p<0.05\), **\(p<0.01\), ***\(p<0.001\))
Graph 2: Effect of Acetone and methanol extracts of Amomum subulatum (200, 400 & 800 mg/kg) on Serum HDL cholesterol and LDL in normal rats and HCD induced atherosclerosis in rats (* p<0.05, **p<0.01, ***p<0.001)

Graph 3: Effect of Acetone and methanol extracts of Amomum subulatum (200, 400 & 800 mg/kg) on VLDL cholesterol and Atherogenic index in normal rats and HCD induced atherosclerosis in rats (* p<0.05, **p<0.01, ***p<0.001)
Total cholesterol, triglycerides were significantly (p<0.001) increased in High cholesterol diet group when compared to normal group. Treatment with seed extracts (Acetone and Methanolic) reduced the elevated lipid profile in serum significantly when compared to the High cholesterol diet (HCD) group.

In HCD group there was a significant (p<0.001 in all the cases) rise in LDL and VLDL levels. The treatment with seed extracts (Acetone and Methanolic) reduced the LDL and VLDL levels significantly as compared to HCD group (p<0.001). HDL levels were found to increase.

HCD significantly increased atherogenic index (p<0.001) as compared to control group. However, treatment with seed extracts (Acetone and Methanolic) significantly reduced the atherogenic index (p<0.001).

Conclusion:
Lipid powering is the balance between reducing the risks of events (including death) related to coronary artery disease and the risk of drug treatment. Quite apart from the risk of drug toxicity, lowering lipid concentrations might in itself, however achieved, increase the risk in other ways. Lowering cholesterol concentrations may alter cellular function and behaviour and this warrants further investigations. One view would be that when the benefit of therapy is enhanced, as it is in those at very high risk in secondary prevention, the possible increase in risk of non-coronary artery disease can be accepted.

Methanol and acetone extracts of A. subulatum Roxb. seeds (200, 400, 800 mg/kg/day, p.o.) administered in rats fed with the high cholesterol diet for 7 days. During the experimental studies, the food intake was periodically assessed. At the end of the experiment, blood was collected. Histological study indicates Serum total cholesterol, triglycerides, LDL, VLDL, Atherogenic Index were significantly reduced in high cholesterol diet rats treated with drugs for 7 days. HDL was increased.

References
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