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Research Article

CHEMOPREVENTIVE POTENTIAL OF *CARALLUMA ADSCENDENS* (ROXB.) AGAINST 1,2 DIMETHYL HYDRAZINE INDUCED COLON CARCINOGENESIS

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ABSTRACT

Colorectal cancer is increasingly common nowadays in Asian countries and still remains the second leading cause of cancer death in the United States. The chemopreventive and hypolipidemic effect of *Caralluma adscendens* (Roxb.), an edible succulent, was studied in 1,2-dimethylhydrazine (DMH)-induced colon cancer. Rats were given a weekly subcutaneous injection of DMH (20 mg/kg body weight), a known colon carcinogen, in the groin for 16 weeks. *Caralluma adscendens* (Roxb.) (50 & 100 mg/kg body weight P.O.) was given at the initiation stage of carcinogenesis. The animals were sacrificed at the end of the experimental period of 30 weeks. The tissue lipid profile was evaluated using various biochemical estimations. The levels of cholesterol, HMG CoA reductase, free fatty acids, and triglycerides were significantly increased, whereas the levels of tissue phospholipids were decreased in DMH-treated rats as compared to control rats. On administering *caralluma adscendens* (Roxb.) at the initiation stage of colon carcinogenesis, the levels of tissue cholesterol, HMG CoA reductase, free fatty acids, triglycerides were significantly decreased, whereas the levels of phospholipids were increased as compared to unsupplemented DMH treated rats in dose dependent manner. Thus, C*aralluma adscendens* (Roxb.) supplementation was found to reduce the risk of colon cancer markedly by virtue of its hypolipidemic and antioxidative effects.

Keywords: colorectal cancer, 1,2 Dimethyl hydrazine, *Caralluma adscendens (Roxb.),* HMG CoA reductase

INTRODUCTION

Cancer is the second leading cause of death after cardiovascular diseases in India. Lung, breast, colon and stomach cancers are the four most common cancers worldwide. Medicinal plants are frequently used by traditional healers to treat a variety of ailments and symptoms including diabetes and cancer. According to the World Health Organization, over 80% of the world's populations rely upon such traditional plant-based systems of medicine to provide them with primary healthcare[1].

1, 2-Dimethylhydrazine (DMH), a potent procarcinogen with selectivity for colon, can also produce colon cancer in experimental rats. Experimental colon cancer induced by DMH in rats mimics human colon cancer and is therefore an ideal model for chemoprevention studies[2].

Tissue lipids play an important role in the maintenance of membrane integrity and regulation of cellular processes. Lipid profile alterations have been considered to be important aspect of malignant transformation and tumor development[3].

The plants belonging to the Asclepiadaceae comprises of about 180 genera and 2200 species which are distributed mainly in the tropical and subtropical regions of the world. *Caralluma,* a genus with about one hundred and seventy five species, is widely distributed in Spain, Africa, Arabia, Middle East Pakistan and India. The genus *Caralluma* was listed in the wealth of India printed in 1956. In dictionary of economic products of India, *Caralluma adscendens* Roxb. is described as the fleshy, nearly leafless herb, known by the local name cullimulayan (Tamil), is usually met within arid places in Deccan peninsula and this plant is often eaten by the natives in the form of pickles or is made into chutney. *Caralluma* plants elaborate pregnane type of molecules. Generally Asclepiadaeceae plants are abundant in esterified polyoxy pregnane glycosides, which promise a source of antitumour agents[4].

The aim of the study was to investigate the efficacy of orally administered ethanolic extract of *Caralluma adscendens (Roxb.)* as a chemopreventive agent against 1,2 dimethyl hydrazine induced colon cancer by using tissue lipid profile and HMG CoA reductase as biomarkers.

MATERIALS AND METHODS

Chemicals and Carcinogen

1,2-dimethyl hydrazine (DMH) was purchased from Sigma Chemical Company (St Louis,MO, USA). All other chemicals and reagents used were of analytical grade.

Collection Of Plant Material

The whole plant material was collected from the nearby places Coimbatore, and its botanical identity was confirmed by a botanist in the herbarium center of Botanical survey of India, Coimbatore.

Preparation Of Extract

The plant was cut into small pieces and shade dried for the experimental studies. Dried leaves were powdered and then the extract was prepared. The ethanolic extract was prepared by dissolving 10 of the plant powder in 100 ml of ethanol in a conical flask and kept in the orbital shaker for 48hr. Then it was filtered with the help of what man no.1 filter paper. The solvent was evaporated to dryness and a semisolid mass was obtained and stored in an eppendorf tubes.

Tumor Induction

DMH was dissolved in 1 mM EDTA just prior to use and the pH was adjusted to 6.5 with 1mM sodium bicarbonate to ensure the stability of the chemical. The rats were given a weekly subcutaneous injection of DMH in the groin at a dose of 20 mg/kg body weight for 16 weeks.

Experimental Animals

Female Wister rats of 100-150 g were obtained from the inbred population of the animal house of KMCH College of pharmacy and maintained at 27 ±2 ° C with a 12 hours light /

12 h dark cycle. A commercial pellet diet containing 4.2 % fat (Hindustan lever limited Mumbai, India) was powdered and mixed with 16% of peanut oil, making a total of 20% fat. This was then fed to all the rats throughout the experimental period of 30 weeks.

The animals were then housed in polypropylene cages with a gilled top and a hygienic bed of husk (regularly changed). Water was given ad libitium. The rats were randomly assigned to 5 groups of 6 animals each. Animals were maintained as per the principles and guidelines of the ethical committee for animal care of KMCH college of pharmacy in accordance with the Indian national law of animal care and use (Proposal no: KMCRET / PHD/ 5/2009).

Rats of group I animals were received distilled water alone. Group II animals received daily 200 mg / kg b.wt of (the highest dose) of plant extract suspended in distilled water, by oral route for 30 weeks and served as control. Group III animals were administrated with DMH weekly at a dose of 20 mg/kg body weight as subcutaneous injections (freshly prepared) for 16 weeks. Group IV animals were administrated with plant extract at a dose of 100 mg/ kg body weight daily by oral gavage along with weekly administration of DMH. Group V animals were administrated with plant extract at a dose of 200mg/kg body weight daily by oral gavage along with weekly administration of DMH for 30 weeks.

The experiment was terminated at the end of 30 weeks and all the animals were killed by cervical dislocation after an overnight fast. The colon was split open longitudinally and gross tumors were counted. Colonic tissues were then processed and used for various biochemical estimations. Tissue samples were immediately transferred to ice-cold containers weighed and homogenized using the appropriate buffer in a tissue homogenizer.

Biochemical Estimations

Extraction of lipids from colon tissue was done and total cholesterol, Phospholipids, Free Fatty acids were estimated[5-8] .The activity of HMG CoA reductase in liver and colon tissue were also estimated[9].

Statistical Analysis

The statistical significance of the data has been determined using one-way analysis of variance (ANOVA) and significant difference among treatment groups were evaluated by Duncan's multiple range test (DMRT). The results were considered statistically significant at P < 0.05. All statistical analyses were made using SPSS 17.0software package (SPSS, Tokyo, Japan).

Table I: Effect of *Caralluma adscendens (*Roxb.*)* on tissue total cholesterol, phospholipids, C/P ratio and free fatty acids (FFA) of control and experimental animals

Groups	Total cholesterol (mg/100 tissue)	Phospholipid (mg/100g tissue)	Total (C/P) ratio	FFA (mg/100g tissue)
Group I control	482.5 ± 30.1 ^{a}	798.5 ± 50.2 ^{a}	0.60 ± 0.045 ^{a}	615. 5 ± 58.1 ^{a}
Group II *Caralluma adscendens* (Roxb.) (200mg/kg b.w)	470.5 ± 22.9 ^{a}	783.5 ± 42.6 ^{a}	0.60 ± 0.03 ^{a}	635. 6 ± 51.2 ^{a}
Group III DMH	544.9 ± 66.7 ^{b}	474. 6 ± 40.2 ^{b}	1.14 ± 0.02 ^{b}	720. 3 ± 52.7 ^{b}
Group IV DMH+*Caralluma adscendens* (Roxb.) (100mg/kg b.w)	506.4 ±65.5 ^{c}	734.3 ± 20.2 ^{c}	0.69 ± 0.03 ^{d}	620.4 ± 40.5 ^{c}
Group V DMH+*Caralluma adscendens* (Roxb.) (200mg/kg b.w)	490.4 ± 25.2 ^{a}	755.4 ± 60.5 ^{a}	0.64 ± 0.02 ^{a}	610.6 ± 30.7 ^{a}

Values are mean ± S.D of six rats in each group. Values not sharing a common superscript (a-d) differ significantly.

Table II: Effect of *Caralluma adscendens* (Roxb.) on tissue HMG Co A reductase activity of control and experimental animals

Groups	HMG Co A reductase activity In liver	HMG Co A reductase activity In colon tissue
Group I control	5.5 ±0.45 ^{a}	2.0 ± 0.19 ^{a}
Group II *Caralluma adscendens* (Roxb.) (200mg/kg ,b.w)	5.3 ± 0.67 ^{a}	2.2 ± 0.22 ^{a}
Group III DMH	2.1 ± 0.20 ^{a}	0.95 ± 0.10 ^{b}
Group IV DMH+ *Caralluma adscendens* (Roxb.) (100mg/ kg b.w)	4.4 ± 0.60 ^{a}	1.5 ± 0.15 ^{c}
Group V DMH+ *Caralluma adscendens* (Roxb.) (200mg/ kg b.w)	5.1 ± 0.35 ^{a}	1.9 ± 0.10 ^{a}

Values are mean ± S.D of six rats in each group. Values not sharing a common superscript (a-d) differ significantly

RESULTS AND DISCUSSION

Effect of *Caralluma adscendens (Roxb.)* on Colon Tissue Lipid Profile of Different Experimental Group of Animals

Cell membrane is an integral part of a live cell and it plays an essential role in life processes. It makes the cell an isolated system and it determines its specific properties and its capability of moving. The most important properties of biological membrane are its electric charge and its potential drop between the membrane and surrounding solution. Electric properties of the membrane are determined by acid base and complex formation equilibria of membrane and solution components. Most membrane components namely proteins, phospholipids and fatty acids are involved in those equilibria[10].

Tissue membrane lipids play an important role in the maintenance of membrane integrity and regulation of cellular process. Lipid profile alterations have been considered to be an important aspect of malignant transformation and tumor development[3].Studies on tissue lipids are of paramount importance due to their role in maintenance of membrane integrity and regulation of cellular process.

In highly lipogenic tissues such as liver, adipose tissue, and the lactating breast, the lipid synthetic pathway functions normally to store excess energy intake, synthesize lipid from carbohydrate or protein if the diet is low in fat, or produce lipids for milk production. However, in cancer cells endogenously synthesized fatty acids are esterified predominantly to phospholipids for membrane lipid synthesis, which promotes cell replication, rather than used for triglyceride energy storage. In fact, pharmacological and small interference RNA-mediated inhibition of FAS decreases the synthesis of phospholipids, suggesting that the high level of lipogenesis in cancer cells is primarily for the synthesis of membranes[11].

The levels of colon tissue total cholesterol, phospholipids, Cholesterol/phospholipid (C/P) ratio and free fatty acids of control and experimental animals are given in Table I

Tissue phospholipid content was depleted in rats given DMH as compared to control rats whereas in rats treated with *Caralluma Adscendens* Roxb. along with DMH, the levels were significantly ($p < 0.05$) elevated as compared to DMH alone treated rats. Thus *Caralluma adscendens* Roxb.Supplementation increased the phospholipid levels in DMH-treated rats to near normal levels.

In DMH-treated rats (group 3), the cholesterol levels were significantly ($p < 0.05$) elevated, as compared to control (group 1). Administration of *Caralluma adscendens* Roxb. to DMH-treated rats (group 4) decreased the tissue cholesterol levels as compared to DMH alone treated rats (group 3). The cholesterol levels of control rats (group 1) did not differ significantly from control + *Caralluma adscendens* Roxb. rats (group 2). Thus *Caralluma adscendens* Roxb. supplementation reduced the cholesterol levels in DMH-treated rats to near normal levels.

In DMH alone treated rats, the cholesterol/ phospholipid ratio was increased significantly as compared to control rats. Administration of *Caralluma adscendens* (Roxb.) to DMH-treated rats significantly decreased the tissue cholesterol/phospholipid ratio. Thus *caralluma* supplementation reduced the cholesterol/phospholipid ratio levels in DMH-treated rats to near normal levels.

The tissue free fatty acid content were significantly increased in DMH administered animals as compared to control animals. On *Caralluma adscendens (Roxb.)* supplementation to DMH administered animals; the free fatty acid content was significantly decreased as compared to DMH alone treated rats. Thus *Caralluma adscendens (Roxb.)* supplementation reduced the free fatty acid levels in DMH-treated rats to near normal levels. Free fatty acid content did not differ significantly between the control and control + *Caralluma adscendens* Roxb. rats.

Many membrane proteins have been shown to be dependent on the lipid environment[12]. Any alteration in the phospholipid metabolism will therefore affect the properties and functions of these proteins, as phospholipids are vital components of biomembranes. The observed decrease in the phospholipid content may also be due to decreased synthesis of phospholipids. Moreover, a number of studies have demonstrated that tumor cells have reduced levels of phospholipids[13].

Based upon a strong relationship between arachidonic acid metabolites and colon carcinogenesis, several studies have focused on the importance of the rate limiting enzymes of arachidonic acid metabolism, commonly called as phospholipases. Colon carcinogen treatment increased the activities of colonic mucosal Phospholipase activity in rats fed a high corn oil diet and increased Phospholipase A_2 activity was found in human colorectal cancer. Increased phospholipase A and Phospholipase C activities in DMH treated animals may result in increased hydrolysis (degradation) of the substrate phospholipids resulting in decreased levels of colonic and intestinal phospholipid content[14].

Biological membranes are composed of phospholipids and proteins. The lipid bilayer is highly impermeable to most of the polar molecules and ions, but yet they are quite fluid in nature, because of which they act as a solvent system for membrane proteins. Addition of cholesterol causes a transition from the gel to crystalline phase, resulting in alteration in the nature and extent of lipid interaction and a decrease in amplitude of motion of the chain axes and reduced fluidity of the system. Studies on natural and artificial membranes have shown that cholesterol affects the central portion of the acyl chain, resulting in decreased motion or increased viscosity of the chain. The change in the molecular motion of the lipid bilayer of the cell membrane will greatly affect its properties and function[15].

Carcinogen treatment results in doubling of biological membrane cholesterol and gross distortion of cell shape, with changes in (1) lipid composition, (2) membrane fluidity, and (3) morphology. Thus, the increased cholesterol content in the colon of DMH-treated animals may be associated with one of the properties of malignant cells such as cellular pleomorphism[16].

The cholesterol/ phospholipid ratio is closely related to membrane fluidity, a lower ratio indicating membrane stability. DMH+ *Caralluma adscendens* Roxb. rats showed decreased levels of cholesterol/phospholipids ratio and thus increased membrane stability. Alterations in the lipid fluidity and composition of plasma membranes of colonocytes may play a role in the early stage(s) of the malignant transformation. Additional factor(s) would then be necessary for the development of carcinoma in these cells. The results on the membrane fluidity correlate with the lipid composition. The cholesterol/phospholipids ratio increased to an appreciable extent in the DMH-induced rats indicating less fluidity, as shown by others[17].

The observed changes in lipid profile in *Caralluma adscendens* Roxb.. treated groups may be due to the presence of pregnane glycosides. *Caralluma fimbriata*, also known as *Caralluma adscendens*, belongs to the family Asclepiadaceae. *Caralluma fimbriata*, a traditional Indian “famine food” with no history of adverse effects contains pregnane glycosides. Pregnane glycosides act directly on adipose tissue, by inhibiting adipocyte proliferation and differentiation. CFE has pronounced dose-dependent appetite suppressant and antiobesogenic effects in this model. These effects were reflected in the feed intake, body weight, liver weight, fat pad mass, and serum lipid profiles of the rats in our various treatment groups[18].

Effect of *Caralluma Adscendens* Roxb. on Colon Tissue HMG CoA Reductase Activity of Different Experimental Group of Animals

HMG CoA reductase is the rate-limiting enzyme for cholesterol synthesis which plays an important role in maintaining cholesterol homeostasis. The activity of HMG CoA reductase in the hepatic microsomal membranes is relatively high and to a certain extent is controlled by the cholesterol content[19-20]. Moreover catabolism of cholesterol produces bile acids in the liver, which on reaching the intestines can cause hyperproliferation of colonocytes eventually resulting in colon carcinoma[17].

Table II shows the HMG CoA reductase activity of control and experimental groups. The activity of HMG CoA reductase in DMH treated rats was significantly increased as compared to control rats, whereas administration of plant extract to DMH-treated animals showed significant reduction in the HMG CoA reductase activity as compared to DMH alone treated rats. These results reveal that *Caralluma adscendens* Roxb. inhibits the activity of HMG CoA reductase due to its high sterols, saponin glycosides and flavonoids content.

Treatment with *Caralluma adscendens* Roxb.results in the inhibition of cholesterol formation due to HMG CoA reductase inhibition and may alter the cell/mitochondrial membrane integrity thereby leads to the apoptosis favorably in tumor cells as compared to normal cells. Tumor cells are highly proliferating cells requiring high levels of cholesterol to maintain the cell/mitochondrial membrane integrity[21].

HMG-CoA reductase inhibitors (statins) block the rate limiting step of cholesterol biosynthesis. Cholesterol synthesis may be blocked by the luteolin (a well known flavanoid) through the inhibition of HMG-CoA reductase enzyme and the mechanism is similar to statin drugs such as atorvastatin[22].

Caralluma attenuate Wight. [Syn.: *C. fimbriata* Hook.] (Asclepiadaceae), is found to be a rich source of glycosides and chemical investigation of the plant have resulted in the isolation of luteolin-4%-O-neohesperidoside, i.e. luteolin-4%-O-[a-(L-rhamnopyranosyl- (1.2)-b-D-glucopyranoside)] as the major chemical constituent of this plant[23].

On administration of C*aralluma adscendens* Roxb. at the initiation stage of colon carcinogenesis, the levels of tissue cholesterol, HMG CoA reductase, free fatty acids, were significantly decreased, whereas the levels of phospholipids were increased as compared to unsupplemented DMH treated rats in dose dependent manner. Thus, C*aralluma adscendens* Roxb. supplementation was found to reduce the risk of colon cancer markedly by virtue of its hypolipidemic and antioxidative effects.

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