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RESEARCH ARTICLE

Hypolipidemic Activity of *Spinacia Oleracea* L. in Atherogenic Diet Induced Hyperlipidemic Rats.

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ABSTRACT

Spinacia oleracea (spinach) of family Amaranthaceae is an important plant used traditionally for medicinal purposes. Hyperlipidemia was induced by treated orally with atherogenic diet. In atherogenic diet induced hyperlipidemic model, the rats receiving *Spinacia oleracea* powder showed significant reduction in total cholesterol, triglycerides, total protein and elevation of high density lipoprotein cholesterol. *Spinacia oleracea* was found to possess significant hypolipidemic activity. The results also suggest that *Spinacia oleracea* powder at 200mg and 400 mg/kg b.wt. concentrations are an excellent lipid-lowering agent.

KEY WORDS: Cholesterol, *Spinacia oleracea*, Hyperlipidemic, Triglyceride.

INTRODUCTION:

Spinacia oleracea L. (Spinach; Amaranthaceae) is an edible flowering plant. It is native to central and south western Asia. Spinach has a high nutritional value and is extremely rich in antioxidants, especially when fresh, steamed, or quickly boiled. It is a rich source of vitamin A (especially high in lutein), vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, betaine, iron, vitamin B2, calcium, potassium, vitamin B6, folic acid, copper, protein, phosphorus, zinc, niacin, selenium and omega-3 fatty acids. Recently, opioid peptides called rubiscolins have also been found in spinach. It is a source of folic acid (Vitamin B9), and this vitamin was first purified from spinach[1]. The leaves of spinach are demulcent or soothing agents, diuretic, and laxative[2, 3, 4]. However, there is no systematic work has been under taken on analyzing the effect of *S.oleracea*. Based on the above information's the present research was focused on the hypolipidemic effect of *S.oleracea* on normal and atherogenic diet induced hyperlipidemia in rats.

MATERIALS AND METHODS:

CHEMICALS:

S.oleracea were collected from the local market of salipur, cutack, odisha, and authenticated by botanist of Revenshaw University, Cuttack. The plants were air dried and powdered for the use. The standard drug Gemfibrozil procured from Sun pharmaceuticals, India. Diagnostic kits for estimation of cholesterol (Merck), Triglyceride (Merck) and High density lipoprotein (CDR Diagnostic) were purchased from Modern Scientific Company, Coimbatore, India. Atherogenic diet was purchased from local market.

DRUG PREPARATION:

S.oleracea powder (200mg/kg, 400mg/kg) was dissolved in 9 ml saline. The suspension was given orally with the help of baby feeding tube to albino rats.

ANIMALS:

Male healthy adult Wistar rats (150-170 g, age 90 days) were obtained from the animal house of Institute of Pharmacy and Technology Salipur, Cuttack, Odisha. India. They were housed in plastic cages with filter tops under controlled conditions of a 12 h light/ 12 h dark cycle, 50% humidity and 28°C. The animals were fed standard rats chow (Lipton Lever Ltd., India) and water ad libitum. The animal experiments were approved by animal ethical committee of Institute of Pharmacy and Technology Salipur, Cuttack, Odisha, India, with registration number 1053/ac/07/CPCSEA. All the experiments were performed as per the CPCSEA guidelines. The composition of atherogenic diet used during the study was given in Tab. 1

INDUCTION OF HYPERLIPIDEMIA:

In order to induce hyperlipidemia, the method reported by Bopanna et al.[5] was followed. The animals were divided into 5 groups of 6 rats each and they received the following with or without treatment for 14 days orally.

EXPERIMENTAL DESIGN:

Animals were divided into different groups with six animals in each group. Group I served as normal control and received standard diet throughout experimental period. Group II, III IV and V received atherogenic diet throughout the treatment period. Group III and IV received S. oleraceae powder (200 mg/kg and 400mg/kg body weight). Group V received standard drug Gemfibrozil 50mg/kg body weight. Treatment periods for all these

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groups were 14 days. At the end of treatment period to all these groups, the animals were used for various biochemical parameters. The animals were deprived of food overnight, anesthetized using light ether and sacrificed by cervical decapitation. Blood was collected and centrifuged by using table top centrifuge at 2000 rpm for 30 minute so as to get serum.

MEASUREMENT OF SERUM LIPID PROFILE:

Total cholesterol (TC), total triglycerides (TG), total protein (TP) were estimated by the method of CHOD-PAP using standard kits and total high density lipoprotein (HDL) were estimated by the method of GPO-PAP using standard kits. The atherogenic index was calculated by using the following formula.

Atherogenic Index = $\frac{\text{Total Cholesterol} - \text{HDL}}{\text{HDL}}$

Statistical analysis:

Statistical analysis was carried out by Student's t-test [6].

RESULTS:

The results reveal that feeding of atherogenic diet increased serum total cholesterol, triglyceride and total protein and decreased serum HDL-cholesterol levels when compared to normal group at over a period of 14 days. Administration of 200, 400 mg/kg per day of S.oleracea powder and 50mg/kg per day of Gemfibrozil showed statistically significant decreased in total cholesterol ($p<0.05$), triglyceride ($p<0.001$) and total protein ($p<0.001$) level as compared to hyperlipidemic animals (Table 1, Figure-1a & 1b). At this time an increase of HDL-cholesterol level was also observed. Both 200 and 400 mg/kg body wt. *S.oleracea* treated animals and 50 mg/kg body wt of Gemfibrozil treated animals showed decrease in the atherogenic index and increased percentage of protection (Table 2, Figure-2).

Composition	Normal diet (%)	Atherogenic diet (%)
Protein (Milk powder)	12	10
Carbohydrates (Wheat flour)	71	61
Sugar	05	05
Fat (Butter)	05	16
Salts	04	04
Vitamins	01	02
Fibers	02	01
Cholesterol	--	01
Total Weight	100g	100 g

Table No. 1: Composition of Normal and Atherogenic Diet

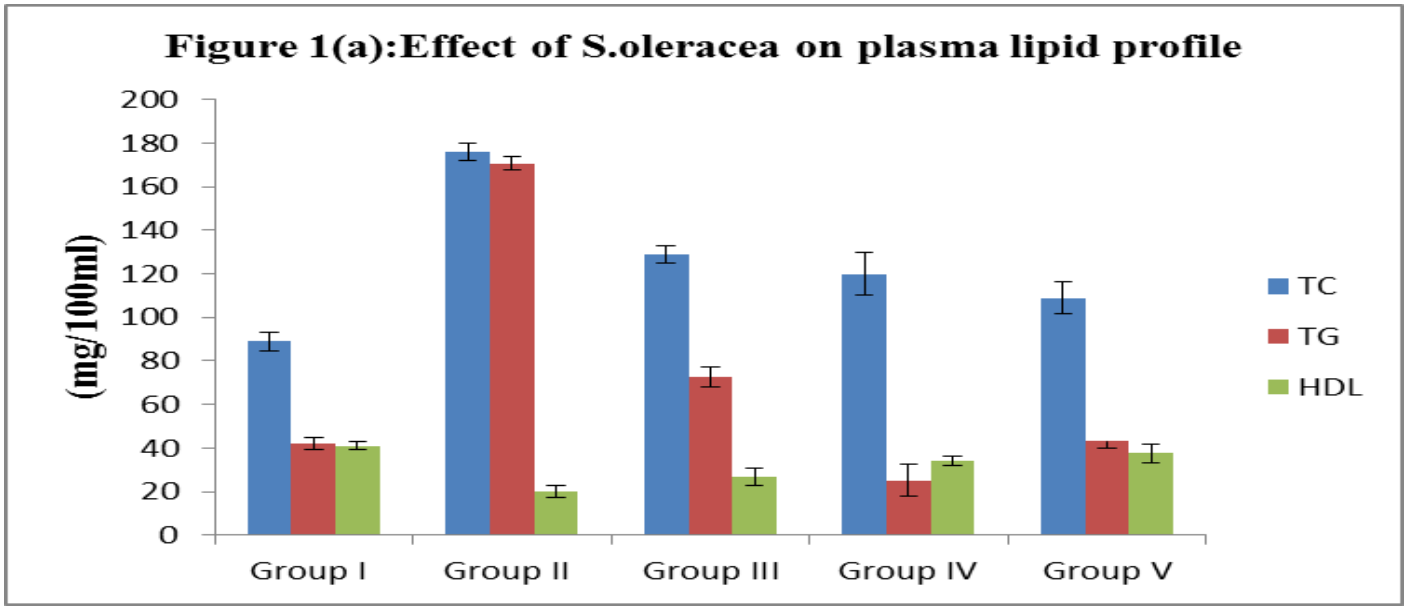
Groups	Total Cholesterol (mg/100ml)	Total Triglycerides (mg/100ml)	Total Protein (mg/100ml)	Total HDL (mg/100ml)
Group I (Normal)	89.0±4.2[a]	42.12±2.9[b]	4.13±0.7[b]	41.13±1.58[b]
Group II (Control) (Atherogenic diet only)	176.0±3.9	170.73±3.2	11.13±0.5	20.11±2.6
Group III (Atherogenic diet + S.oleracea 200mg/kg)	128.9±4.1[a]	72.8±4.7[b]	4.36±0.6[b]	26.87±3.8[b]
Group IV (Atherogenic diet + S.oleracea 400mg/kg)	119.8±9.8[a]	25.23±7.4[b]	4.23±0.8[b]	33.9±2.2[b]
Group V (Atherogenic diet+ Gemfibrozil 50mg/kg)	108.8±7.4[a]	43.24±3.4[b]	6.43±0.7[b]	37.53±4.3[b]

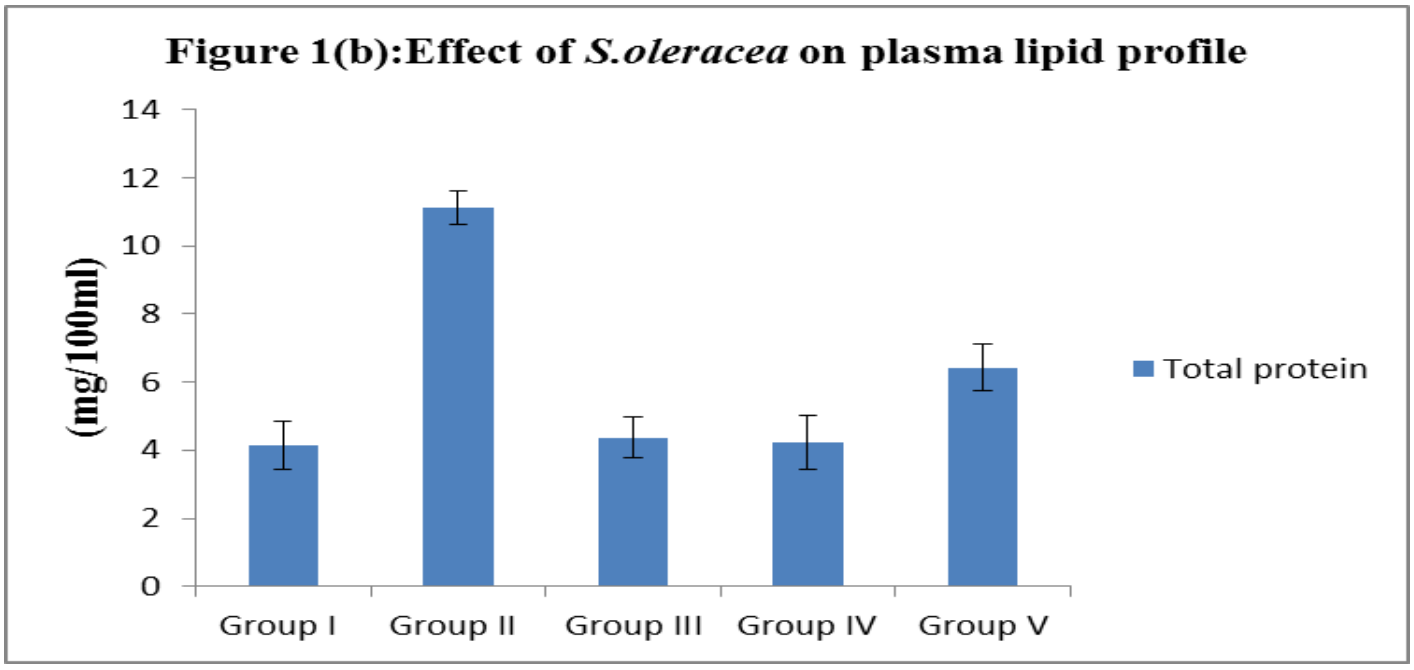
Table No. 2: Effect of S.oleracea on Plasma Lipid Profile of Normal and Atherogenic Diet Induced by Hyperlipidemic Rats
Statistical significant in comparison to group III, IV with group II, p values a<0.05, b<0.001

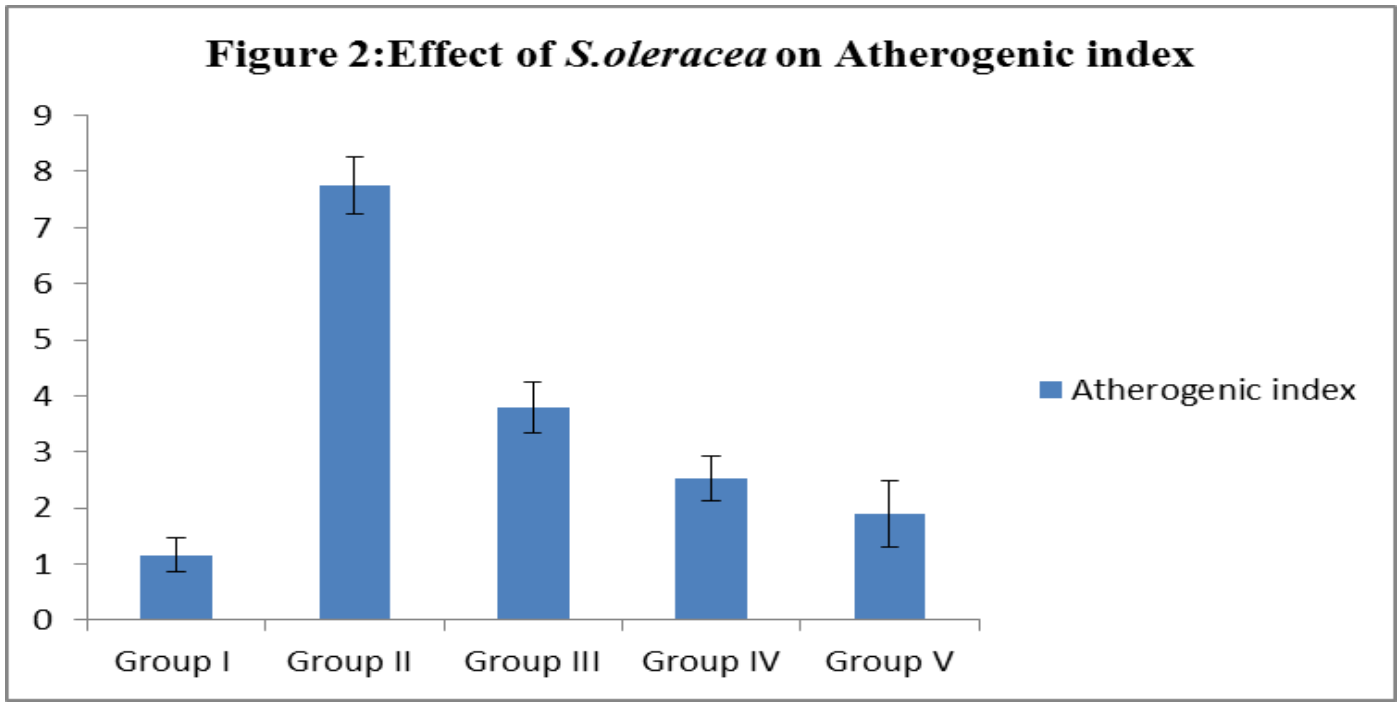
Groups	Atherogenic Index	Protection* (%)
Group I (Normal)	1.16±0.3	-
Group II (Control) (Atherogenic diet only)	7.75±0.5	-
Group III (Atherogenic diet + S.oleracea 200mg/kg)	3.79±0.45	51.09
Group IV (Atherogenic diet + S.oleracea 400mg/kg)	2.53±0.4	67.35
Group V (Atherogenic diet+Gemfibrozil 50mg/kg)	1.89±0.6	75.61

Table No. 3: Atherogenic Index in Various Groups

$$\text{*Protection (\%)} = \frac{\text{Atherogenic index of control} - \text{Atherogenic index treated group}}{\text{Atherogenic index of control}} \times 100$$







DISCUSSION:

Treatment with *S.oleracea* produced a significant decrease in the serum level of lipids in atherogenic diet induced hyperlipidemia in rats. Atherogenic diet induced hyperlipidemic model has been successfully employed for the evaluation of Hypocholesterolemic effect of protein [7,8] and S-allyl cystein sulphoxide of Allium sativum [9] in albino rats. As per literature S.oleracea is a rich source of vitamins such as vitamin C, vitamin A and vitamin E and minerals like magnesium, manganese, iron, calcium and folic acid. Spinach is also a good source of chlorophyll, which is known to aid in digestion. Spinach is also rich in the carotenoids beta-carotene and lutein. It is a good source of the bioflavonoid quercetin with many other flavonoids which exhibits anti-oxidant, antiproliferative, antiinfammatory, antihistaminic, CNS depressant, protection against gamma radiation, hepatoprotective properties in addition to its many other benefits[10]. Bok SH et al reported that Quercetin dihydrate and gallate supplements lower plasma and hepatic lipids[11]. So from the present study it may be concluded that the lipd lowering activity of the plant *S.oleracea* may be due to the bioflavonoid quercetin.

Faulty diet is a very common cause of heart disease. Particularly, with an increase in inclination towards fast foods, which are rich in saturated fats. An increase in coronary heart disorder (CHD) is being observed in the developing countries since past few decades [12]. A 1% decrease in HDLcholesterol is associated with a 3-4% increase in the risk of heart disease. For male and female, concentration of HDL-cholesterol below 1.0 and 1.2 mmol/L (39, 46 mg/dl) and especially below 0.8 and 1.0 and 1.2 mmol/L (31,39 mg/dl) confer an increased risk of CHD, whereas concentration exceeding 1.5 and 1.7 mmol/L (58, 66 mg/dl) diminishes the influence of other risk factors [13].

In the present study an increase in plasma HDL-cholesterol with a concomitant percentage decrease from other lipid was observed (Table 2 and 3). It can be concluded from the present data that the levels of total serum cholesterol, triglyceride and total protein which are actually raised in atherogenic diet, can be lowered significantly with *S.oleracea* (spinach).

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