

PROTECTIVE ROLE OF SEAWEEDS AGAINST HEART DISEASES

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ABSTRACT

Epidemiological studies have demonstrated a direct relationship of increased lipid profile, particularly low density lipoprotein cholesterol to the incidence of coronary heart diseases. Ethanol extract of test species of seaweed *Iyengaria stellata* (brown) and *Solieria robusta* (red) significantly decreased the serum total cholesterol, triglycerides and low density lipoprotein (LDL)-cholesterol levels in normal rats and triton-induced hyperlipidaemic rats. Feeding of the extracts @ 10 mg /200 g body weight daily for 12 days in animals maintained on high fat diet showed decrease in lipid and LDL-cholesterol with subsequent significant increase in high density lipoprotein (HDL)-cholesterol in rats treated with these seaweeds. *Solieria robusta* was found most effective in reducing the lipid profile particularly in high fat diet-induced hyperlipidaemic rats. Water extract of *I.stellata* and *S. robusta* showed significant hypolipidaemic potential in normal, triton and high fat-diet induced hyperlipidaemic rats. In triton-induced hyperlipidaemic rats both the seaweeds demonstrated more than 50% reduction in the level of total cholesterol, triglycerides and LDL-cholesterol. *Iyengaria stellata* showed better results in lowering LDL-cholesterol level as compared to *S. robusta* in high fat-diet induced hyperlipidaemic rats.

Keywords: Seaweeds, heart diseases, algal extract, biological activity, lipid, blood.

INTRODUCTION

Atherosclerosis-related diseases, particularly coronary artery disease is a major cause of death in developed countries. Coronary artery disease (CAD) accounts for more than 50% of all deaths in the United States. (Fuster *et al.*, 1992; Levy, 1981). Many factors are thought to be associated with the development of atherosclerosis particularly an increase in blood lipid profile is a contributing factor in the pathogenesis of atherosclerosis and associated cardiac disorders. Over the past several decades, tremendous efforts have been underway to develop effective therapeutic hypolipidaemic agents, which reduce very low density lipoprotein (VLDL) and low density lipoprotein (LDL)-cholesterol, increase high density lipoprotein (HDL)-cholesterol and reduce triglyceride levels without significant side effects. This had led to the discovery of hundreds of compounds that showed significant serum cholesterol and triglyceride lowering effects, such as bile acid sequestrants, nicotinic acid, fibric acids, 3-hydroxy-3-methylglutaryl (HMG) coenzyme A (CoA) reductase inhibitors, probucol, and many others (Marx, 1979). Several polysaccharides from red and brown algae have also shown hypocholesterolaemic activity (Bhakuni & Silva, 1974; Guven *et al.*, 1979; Vazquez-Freire *et al.*, 1996). Besterman (1970) reported hypolipidaemic effect of laminarian sulphate in rabbits. Similarly Michanek (1979) demonstrated the hypocholesterolaemic effect of carrageenan, agar and alginic acid. Cytotoxic (Ara *et al.*, 1999) and antibacterial (Ara *et al.*, 2002) activities of seaweeds from Karachi coast have been reported. The present reports describes the hypolipidaemic activity of ethanol and water extracts of two seaweeds *Iyengaria stellata* and *Solieria robusta*, collected from Karachi coast, in normal, triton and high fat diet- induced hyperlipidaemic rats.

MATERIALS AND METHODS

Algal material:

Seaweed were collected Buleji, a coastal beach of Karachi, under low tide and brought to the laboratory. Algal material was washed thoroughly under tap water and dried under shade and powdered in a miller and stored in polyethylene bags at room temperature until used.

Preparation of ethanol extract of seaweeds:

Dry powder of each seaweed (500 g) was extracted three times with ethanol (4 vol.) for 1 week. Extracts were pooled, filtered through cotton wool and concentrated to dryness on a rotary vacuum evaporator (Eyela NE).

Preparation of water extract of seaweeds:

Water extracts of a brown seaweed *Iyengaria stellata* and a red seaweed *Solieria robusta* were obtained after soaking dry powder in distilled water and homogenized using Ultrataurrex and then filtered. The filtrate was lyophilized on a Freeze dryer (Eyela) and stored in a freezer until used.

Animal:

Adult male albino rats were purchased from Aga Khan University, Karachi, Pakistan and were housed in uniform hygienic conditions and kept on a standard pellet diet and water *ad libitum*.

Normal rats model:

In the first set of experiment with normal rats, animals were divided in control and test groups. Both groups of rats were fed on normal diet throughout the course of study. Ethanol extracts of seaweeds @ 10 mg/200 g body weight (b.wt.) suspended in distilled water (D.H₂O) and water extract (100mg of freeze dried powder/ 200 g b.wt in D.H₂O) were administered orally for 12 days to the animals of test group while same quantity of D.H₂O was given orally to the rats of control group. At the end of experiment, rats were fasted overnight and blood samples were collected (Khanna *et al.*, 1994) and serum was obtained for the estimation of total cholesterol (Tc), triglycerides (TG), high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) levels.

Triton model of hyperlipidaemia:

The rats were divided into three groups: control, triton only and triton plus seaweed extract-treated groups. Each group contained six animals. Triton WR-1339 (Sigma Chemicals Company, St. Louis, Mo., USA) was administered @ 400 mg/kg by intra-peritoneal injection. Crude ethanol extract suspended in D.H₂O @ 10 mg and water extract @ 100 mg/200g b.wt. were fed orally simultaneously with triton. Control animals were given same volume of D.H₂O while control of triton group received triton only. Blood was drawn after 18 hours from all these groups. The serum was used for the estimation of lipid profile.

High fat diet-induced hyperlipidaemic rats model:

In another set of experiment, hyperlipidaemia was induced by high fat diet. The normal diet was supplemented with cholesterol (1%), cholic acid (0.5%), coconut oil (5%) and normal laboratory feed (93.5%) (Vazquez-Friere *et al.*, 1996). The rats were divided into two groups viz., high fat diet control group (Group I) and high fat diet-treated group (Group II). Rats of Group I were fed with high fat diet throughout the course of study. Group II were fed with high fat diet plus water extracts of seaweeds (@ 100 mg/200g b.wt.) and ethanol extract of seaweeds (@ 10 mg/200g b. wt.) once daily for 12 days. Equal volume of D.H₂O was given to control of high fat diet group. On day 12, animals were fasted overnight and blood samples were collected. Serum lipid profile was determined.

Estimation of blood lipid profile:

Serum total cholesterol (Tc) and HDL-cholesterol were estimated using kits (Merck, Germany) by CHOD-PAP method, while serum triglycerides (TG) level was determined by GPO-PAP method on Microlab-200 analyzer. Friedwald formula (Friedwald, 1972) was used to calculate the LDL-cholesterol.

RESULTS**Effect of ethanol extracts of seaweeds:**

Ethanol extracts of seaweeds *Iyengaria stellata* and *Solieria robusta* were given to normal Albino rats. Significant reduction in the serum cholesterol and triglyceride levels of rats treated with *I. stellata* was observed in comparison with control (Table-1). *Solieria robusta* showed no significant effect on the cholesterol level. Highest hypocholesterolaemic activity was displayed by *I. stellata* (20%). *Solieria robusta* exhibited activity against LDL-cholesterol (LDL-c) followed by an increase in HDL-cholesterol level (HDL-c) (Table 1).

These seaweed were also assayed in triton-induced and high fat diet-induced hyperlipaemic rats. Administration of triton in rats increased serum Tc, TG, HDL-c and LDL-c levels significantly (Table 2). Treatment with *I. stellata* and *S. robusta* partially lowered the level of these serum lipids in triton + extract treated animals. Both the species of seaweed showed significant decrease in the levels of cholesterol and triglycerides. Rats treated with triton plus seaweed extracts *I. stellata* and *S. robusta* reduced the levels of Tc (20.3-21.87%), TG (23.85-21.3%) and LDL-cholesterol (34.8-39.3%). Significant activity against LDL-c was recorded by *I. stellata* (39.3%) and *S. robusta* (34.8%) (Table 2).

Table 1. Effect of ethanol extracts of seaweeds @ 10 mg/200 g body weight on lipid profile in normal rats after 12 days treatment.

	Tc Mg %	TG mg %	HDL-c mg %	LDL-c mg %
Control	75.0±3.60 ^a	60.0±3.60 ^a	37.66±0.57 ^b	25.3±2.80 ^a
<i>Iyengaria stellata</i>	60±2.0 ^b	40±5.0 ^c	35±2.0 ^b	17.0±2.64 ^b
% Deviation	(-) 20%	(-) 33.3%	(-) 7.06%	(-) 32.8%
<i>Solieria robusta</i>	77.0±2.64 ^a	55.0±2.64 ^b	54.66±2.88 ^a	14.0±2.42 ^b
% Deviation	(+) 2.66%	(-) 8.3%	(+) 45.14%	(-) 44.66%
LSD _{0.05}	4.85	5.62	3.16	4.70

Tc, total cholesterol; TG, triglycerides; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol. % Deviation in comparison with control. Values are means ± SD with n=6.

Means followed by same superscript letters are not significantly different by Duncan's Multiple range test.

Table 2. Effect of ethanol extracts of seaweeds @ 10 mg/200 g body weight on lipid profile in Triton-induced hyperlipidaemic rats after 12 days treatment.

	Tc mg %	TG mg %	HDL-c mg %	LDL-c mg %
Control	76.33±1.53 ^c	59.33±1.53 ^c	38.0±3.0 ^d	26.46±4.20 ^c
Triton-induced Hyperlipidaemic rats	320±11.36 ^a	394±5.29 ^a	161±3.60 ^a	80.2±4.20 ^a
<i>Iyengaria stellata</i> + Triton	255±11.13 ^b	300±11.13 ^b	146.33±5.50 ^b	48.66±13.14 ^b
% Deviation	(-) 20.31%	(-) 23.85%	(-) 9.11%	(-) 39.33%
<i>Solieria robusta</i> + Triton	250±13.23 ^b	310±13.23 ^b	135.66±4.0 ^c	52.3±11.84 ^b
% Deviation	(-) 21.87%	(-) 21.3%	(-) 15.74%	(-) 34.78%
LSD _{0.05}	14.04	14.65	7.5	15.23

Tc, total cholesterol; TG, triglycerides; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol. % Deviation in comparison with control of Triton-induced hyperlipidaemic rats. Values are means ± SD with n=6.

Means followed by same superscript letters are not significantly different by Duncan's Multiple range test.

High fat diet treatment for 12 days significantly increased the serum lipid profile like total cholesterol, triglycerides and LDL-cholesterol levels in rats (Table-3). The reduction in lipid profile by ethanol extracts of *I. stellata* and *S. robusta* was in the range from 11.2-37.6% for cholesterol, 17.3- 28.6% for triglycerides and 51.88-86% for LDL-c level. *Solieria robusta* showed greater hypolipidaemic activity in high fat diet- induced hyperlipidaemic rats as compared to *I. stellata* (Table 3).

Effect of water exyracts of seaweeds:

In normal rats, lyophilized powder of *I. stellata* and *S. robusta* demonstrated significant influence on total cholesterol, triglycerides and LDL-c. The results also revealed a significant increase in HDL-c followed by reduction in LDL-c by the both test seaweeds (Table 4).

Table 3. Effect of ethanol extracts of seaweeds @ 10 mg/200 g b. w. on lipid profile in diet-induced hyperlipidaemic rats after 12 days treatments.

	Tc mg %	TG mg %	HDL-c mg %	LDL-c mg %
Control	75.0±3.0 ^c	57.66±2.51 ^c	35.66±2.08 ^c	27.8±1.70 ^c
High fat diet control	107.33±2.51 ^a	82.66±2.51 ^a	25±1.0 ^d	65.8±3.0 ^a
<i>Iyengaria stellata</i>	95.0±2.0 ^b	68.33±1.52 ^b	49.66±0.58 ^a	31.66±2.70 ^b
% Deviation	(-) 11.2%	(-) 17.33%	(+) 66.24%	(-) 51.88%
<i>Solieria robusta</i>	67±2.0 ^d	59.0±1.0 ^c	46.66±1.53 ^b	9.2±0.8 ^d
% Deviation	(-) 37.58%	(-) 28.62%	(+) 56.1%	(-) 86%
LSD_{0.05}	3.66	3.58	2.32	3.40

Tc, total cholesterol; TG, triglycerides; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol. % Deviation in comparison with control of high fat diet group. Values are means ± SD with n=6.

Means followed by same superscript letters are not significantly different by Duncan's Multiple range test.

Table 4. Effect of water extracts of seaweeds @ 100 mg/200 g body weight in normal rats after 12 days treatment.

	Tc mg %	TG mg %	HDL-c mg %	LDL-c mg %
Normal	79.33±1.53 ^a	100.33±3.05 ^a	38.0±3.0 ^b	21.26±1.89 ^a
<i>Iyengaria stellata</i>	69.0±1.0 ^c	79.0±2.0 ^b	41.66±1.15 ^a	11.53±1.30 ^c
% Deviation	(-) 13.02%	(-) 21.26%	(-) 9.63%	(-) 45.77%
<i>Solieria robusta</i>	71.6±1.53 ^b	81.6±3.05 ^b	40.33±1.53 ^a	15±2.8 ^b
% Deviation	(-) 9.66%	(-) 18.60%	(-) 6.13%	(-) 29.44%
LSD_{0.05}	2.30	5.87	3.64	3.54

Tc, total cholesterol; TG, triglycerides; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol. % Deviation in comparison with control. Values are means ± SD with n=6.

Means followed by same superscript letters are not significantly different by Duncan's Multiple range test.

Table 5. Effect of water extracts of seaweeds @ 100 mg/200 g body weight in Triton-induced hyperlipidaemic rats after 12 days treatment.

	Tc Mg %	TG mg %	HDL-c mg %	LDL-c Mg %
Normal	77.33±6.11 ^c	106.33±4.04 ^c	33±7.0 ^c	23.06±0.8 ^c
Triton-induced hyperlipidemic rats	263±14.42 ^a	312.67±11.68 ^a	128.67±7.77 ^a	71.67±8.0 ^a
<i>I. stellata</i>	122.67±6.10 ^b	128.67±7.09 ^b	64±4.36 ^b	32.9±1.68 ^b
% Deviation	(-) 53.35%	(-) 58.84%	(-) 50.26%	(-) 54.10%
<i>S. robusta</i>	112.33±2.89 ^b	130±3.0 ^b	67±3.46 ^b	19.33±0.84 ^c
% Deviation	(-) 57.28%	(-) 58.42%	(-) 47.93%	(-) 73.0%
LSD_{0.05}	14.47	17.03	9.87	7.08

Tc, total cholesterol; TG, triglycerides; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol. % Deviation in comparison with control of Triton-induced hyperlipidaemic rats. Values are means ± SD with n=6.

Means followed by same superscript letters are not significantly different by Duncan's Multiple range test.

Table 6. Effect of water extracts of seaweeds @ 100 mg/200 g body weight in diet-induced hyperlipidaemic rats after 12 days treatment.

	Tc mg %	TG mg %	HDL-c mg %	LDL-c mg %
Normal	79.33±1.53 ^c	100.33±3.05 ^b	38.0±3.0 ^a	21.26±1.89 ^c
High fat diet control	113.6±5.13 ^a	113±8.18 ^a	28.66±3.0 ^b	62.4±5.6 ^a
<i>I. stellata</i>	97±2.0 ^b	101.33±3.05 ^b	32±1.0 ^b	44.73±2.04 ^b
% Deviation	(-) 14.74%	(-) 10.33%	(+) 11.65%	(-) 28.31%
<i>S. robusta</i>	101±2.0 ^b	99.33±4.50 ^b	31.0±1.0 ^b	50.1±3.40 ^b
% Deviation	(-) 11.14%	(-) 12.1%	(+) 8.16%	(-) 19.87%
LSD _{0.05}	5.18	8.74	3.87	5.80

Tc, total cholesterol; TG, triglycerides; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol. % Deviation in comparison with control of high fat diet group. Values are means ± SD with n=6. Means followed by same superscript letters are not significantly different by Duncan's Multiple range test.

In triton induced hyperlipidaemic rats, lyophilized powder of test species viz. *I. stellata* and *S. robusta* exhibited significant reduction in the concentrations of Tc, TG, HDL-c and LDL-c as compared to control triton-induced hyperlipidaemic rats. Both the tested seaweeds demonstrated more than 50% reduction in the levels of Tc, TG and LDL-c (Table 5).

In diet-induced hyperlipidaemic rats, seaweeds *I. stellata* and *S. robusta* showed significant hypocholesterolaemic and hypotriglyceridaemic activities with significant decrement in LDL-c. Brown seaweed, *I. stellata* demonstrated greater activity in lowering cholesterol and LDL-c levels. (Table 6).

DISCUSSION

Natural products and biological substances with therapeutic effect are of increasing interest. Algae are rich and varied source of pharmacologically active natural products (Konig & Wright, 1993). In the present study, two seaweeds *Iyengaria stellata* and *Solieria robusta* were tested for hypolipidaemic activity in normal, triton- and high fat diet-induced hyperlipidaemic rats, demonstrated promising results in lowering the serum cholesterol, triglycerides and LDL-cholesterol level followed by an increase in HDL-cholesterol as have been observed with other natural hypo-cholesterolaemic agents (Singer, 1981; Tsi *et al.*, 1995). Epidemiological studies have demonstrated a direct relationship of low density lipoprotein cholesterol to the incidence of coronary heart diseases (Castelli *et al.*, 1986; Gordon *et al.*, 1985; Miller & Miller, 1975). The protective role of HDL-cholesterol in atherogenesis was shown by Frick *et al.*, (1987) emphasizing that an 11% increase in HDL-cholesterol concentration can reduce myocardial infarction by 34%. Triton- and diet-induced hyperlipidaemic rats showed significant decrease in serum cholesterol, triglycerides and LDL-cholesterol when treated with ethanol and water extracts of *I. stellata* and *S. robusta*. Triton acts as a surfactant to block the uptake of lipoproteins from the circulation by extra-hepatic tissues, resulting in an increase in the level of circulatory lipids (Schurr *et al.*, 1972). The hypolipidaemic effects of seaweeds observed in the present study are consistent with the finding of Murata *et al.*, (1999) that seaweeds increased the activity of hepatic enzymes responsible for fatty acid oxidation. Algae from Rhodophyta contain nontoxic sterols that have the ability to reduce blood cholesterol level and also reduced fat accumulation in liver and heart (Patterson, 1977). Similarly sterols from *Sargassum muticum* and *Fucus gardneri* significantly reduced plasma cholesterol level in experimental animals (Reiner *et al.*, 1962). Protein fractions of *Cystoseria corniculata* from Turkish coast of Mediterranean sea showed lipolytic and hypoglycaemic activity (Güven *et al.*, 1980). Cholesterol is the most commonly occurring sterol in red algae and has the ability to reduce blood cholesterol level (Bhakuni & Silva, 1974). The effectiveness of green algae and *Porphyra* on the lowering of the plasma cholesterol level in rats was studied by Abe & Kaneda, (1972) and betaines as well as an unidentified compound were detected in the active fraction of seaweed.

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