

Evaluation of *Iyengariastellata* for its hypolipidemic and hepatoprotective activity

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Abstract: Cholesterol is believed to be the major regulator involved in the formation and progression of atheroma plaque. Seaweeds are known to possess enormous biological activities. They contain variety of active constituents, which have pharmacological significance. The objective of this study is to explore hypolipidemic and hepatoprotective activities of the brown seaweed *Iyengariastellata*. Ethanolic extract of seaweed was suspended in distilled water and administered orally at 10mg/200g body weight to rabbits for 30 days and total lipid level, cholesterol, triglycerides, HDL, LDL, VLDL, alkaline phosphatase, SGPT, SGOT, Gamma GT were assessed. The results showed overall decrease in lipid profile whereas *Iyengariastellata* increased liver enzymes except SGPT which was decreased highly significantly, since SGPT is more specific indicator of liver injury, decreased value of SGPT indicates that *Iyengariastellata* toxicity is less severe and reversible with marked hypolipidemic effect, but during the course of ingestion of the seaweed the liver enzymes must be carefully monitored to ensure liver safety.

Keywords: Hypolipidemic, *Iyengariastellata*, hepatoprotective, cholesterol, SGPT.

INTRODUCTION

Around the globe, the most prevalent cause of death is cardiovascular disease. Elevation in the plasma lipids behave as contributing factor to the progression of atherosclerosis and related cardiac abnormalities, gained focus of researchers on the necessity for the synthesis of newer and better hypolipidemic agents which maintain plasma lipid level within normal range (De Bono and Boon, 1992; Sliskovic and White, 1991). Kaneda *et al* (1963) screened several marine algae for their hypocholesterolemic effects in diet induced hyperlipidemic rats. It has been suggested that the addition of seaweeds including fish oils, seaweeds to the daily diet may turn out fruitful as the prophylactic management of coronary atherosclerosis (Chunacova, 1967; Velichko and Shevchenko, 1998). Red and brown algae are rich in polysaccharides content, which have hypolipidemic effects (Vazquez-Freire *et al.*, 1996).

There are reports that significant reduction in the plasma lipids occur after the ingestion of ethanolic extract of *Iyengariastellata* at the dose of 10mg/200g (Ara 2002). The possible mechanisms for these effects are that the algae not only induce the hepatic enzymes that are responsible for the oxidation of fatty acids (Murata *et al*, 1999), but according to another report the cholesterol lowering effect of seaweed might be because of its effect on cholesterol (Vazquez-Freire *et al*, 1996).

Khan in 2000 carried out phytochemical study on *Iyengariastellata* and isolated saringosterol, loliolide,

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propyl-4-hydroxy benzoate and methyl-4-hydroxy benzoate. Earlier researches on this alga have indicated the presence of aminoacids, carbohydrates and vitamins (Mehta and Parekh, 1978, Qasim and Rashida, 1991, Qasim, *et al*, 1985), polysaccharides (Hussein, 1975), proteins, amino acids, lipids and mannitol (Hussein, *et al*, 1983), fatty acid methyl esters as methyl-n-pentadecanoate, methyl hexadecanoate, methyl-n-heptadecanoate, methyl octadecanoate, methyl 9, hexadecenoate and methyl 9, octadecenoate (Usmanghani *et al.*, 1987). According to another investigation cholesterol with another new metabolitestellatol was detected from the extract of *Iyengariastellata* (Ali *et al.*, 1999). Evaluation of elemental composition of *Iyengariastellata* was done by Afzal (Rizvi *et al*, 2001).

The constituents those might play role in the lowering of lipid content are supposed to be saringosterol, polysaccharides and fatty acid methyl esters.

Saringosterol belongs to phytosterol and is found as free sterol in nature. Plant sterols are accountable for large number of biological activities in living system predominantly it show promise in reducing cholesterol level (Ostlund, 2007). The prime mechanism for free and esterified phytosterol that produces hypocholesterolemic effect inhibits cholesterol absorption from intestine. This inhibition might occur from different sites of intestinal tract. Several other mechanisms also believed to contribute to lower down cholesterol by the plant sterols, like they compete with cholesterol for solubilisation in dietary mixed micelles, other means of lowering cholesterol are that they co-crystallize with cholesterol to

form mixed crystals of sterols and cholesterol which are insoluble in nature, and last but not the least the impedance with the process of hydrolysis by lipases and cholesterol esterases are reported to help in reducing the levels of serum cholesterol by plant sterols. It has also been suggested that transport-mediated processes of cholesterol uptake has been hindered by phytosterols. Whatever be the underlying mechanism involved, the ultimate effect of the plant sterols is to decrease the cholesterol absorption and increase the excretion of cholesterol in faeces (Trautwein, *et al.*, 2003).

Polysaccharides are complex carbohydrates, which are not completely digestible but they may represent notable dietary components for humans. These soluble fibers interrupt the absorption of lipids by binding with bile acids and as bile acids aid in the digestion and absorption of fats, the formation of dietary fiber- bile acid complex lower down the plasma cholesterol levels (Anderson, *et al.*, 2009).

Methyl-n-pentadecanoate, methyl hexadecanoate, methyl-n-heptadecanoate, methyl octadecanoate are saturated fatty acids whereas methyl 9- hexadecenoate and methyl 9-octadecenoate are mono unsaturated fatty acids. Foods comprising of monounsaturated fats depress low-density lipoprotein (LDL) cholesterol, while probably raising high-density lipoprotein (HDL) cholesterol. They have dual benefits, similar to saturated fatty acids, they are highly impervious to oxidation and thus prevent the generation of carcinogenic free radicals and like poly unsaturated fats they have potential to reduce cholesterol.

MATERIAL AND METHOD

Prior to the initiation of the experimental work, collection of algae was done which was then identified by department of Botany, University of Karachi. Later drying followed by extraction was conducted to obtain the extract (Ali *et al.*, 1999).

Animals selection

Healthy albino rabbits of either sex weighing from 1500 to 2000grams were selected. All animals were equally divided into two groups, one group served as control while other received adequate doses according to their body weight for 30 days. Each group contained 10 animals. Before administration of drug, apparent health of these animals was monitored during the conditioning period under the laboratory environments for a week before administration of algal extract specifically noticing loss of hair, diarrhea, edema, ulceration and lack of activity. Diet and water was provided *ad libitum*. The animals were maintained under constant environmental conditions $23\pm 2^{\circ}\text{C}$. All animals were given standard diet prepared in the laboratory and water *ad libitum* for 30 days. They were housed individually in transparent cages, in a quiet room, under controlled condition of temperature

at least a week before the beginning of experiments, for acclimatization with the environment.

Dosing protocol

The dosing of *Iyengariastellata* was done daily. Ethanolic extract of seaweed was suspended in distilled water (dist. H_2O) and administered orally at 10mg/200g body weight daily for 30 days to the animals of the test group, while the same quantity of dist. H_2O was given orally to the animals of the control group. The doses were adjusted according to the body weight of individual animal (Ara, *et al.*, 2002). All animals received drugs orally. Body weight was monitored weekly.

Animals were handled as per specifications provided in Helsinki Resolution 1964 and study was approved by our Board of Advanced Studies and Research vide Resol. No, 1135 dated: 20-04-2011-22 & 27-04-2011.

Estimation of lipid profile

Serum was separated out by centrifuging clotted blood samples at 3000rpm for 15 minutes and following parameters were analyzed within 3 hours of sample collection on Humalyzer 3000, Semi-automatic chemistry analyzer. Model # 16700 (Human Germany), using standard kits supplied by Human.

Estimation of liver function test

After separating the serum, the liver enzymes (SGPT and SGOT) were measured by Humalyzer (Human Germany), using standard reagent kits of Human Germany.

STATISTICAL ANALYSIS

All values are compared with the controlled and standard drug by taking mean of all of them and the significance of difference between means is determined by student significance t-test. Values of $P < 0.05$ is considered as significant.

DISCUSSION

The effect of the current seaweed on the total lipid level has shown to be decreased significantly. Literature survey denotes that Polysaccharides from red and brown algae have also shown hypolipidemic activity (Bhakuni and Silva, 1974; Guven, *et al.*, 1979; Vazquez-Freire, *et al.*, 1996). Furthermore there are also reports that *Iyengariastellata* contain carbohydrates (Mehta and Parekh, 1978; Qasim and Rashida, 1991; Qasim, *et al.*, 1985), suggesting its role in lowering of total lipid content.

The present finding is important since the decrease in total lipids and cholesterol is associated with reduced incidence of various cardiovascular disorders and atherosclerosis.

Table 1: Effect of seaweed on total lipid levels

Effect of seaweed on total lipid levels after 30 days			
Parameters	Control	Treated	T-test
	Mean $\pm$ SD	Mean $\pm$ SD	P
Total lipid level	260.00 $\pm$ 6.70	208.00 $\pm$ 54.70	*0.015
Cholesterol	54.00 $\pm$ 1.49	45.00 $\pm$ 12.90	0.056
Triglycerides	47.20 $\pm$ 1.81	37.00 $\pm$ 13.80	*0.046
HDL	26.90 $\pm$ 1.79	20.60 $\pm$ 5.44	*0.006
LDL	45.00 $\pm$ 2.94	28.50 $\pm$ 11.40	**0.001
VLDL	9.00 $\pm$ 1.49	7.30 $\pm$ 2.45	0.082

Total number of animals (n=10).

Level of significance *p<0.05, **p<0.001, ***p<0.0001.

Table 2: Effect of seaweed on liver enzymes

Effect of seaweed on liver enzymes after 30 days			
Parameters	Control	Treated	T-test
	Mean $\pm$ SD	Mean $\pm$ SD	P
Alkaline phosphatase	9.00 $\pm$ 1.49	17.92 $\pm$ 6.74	*0.003
SGPT	100.00 $\pm$ 1.49	49.46 $\pm$ 7.24	***0.000
SGOT	43.00 $\pm$ 2.11	61.90 $\pm$ 24.70	*0.039
Gamma GT	5.00 $\pm$ 1.49	10.60 $\pm$ 4.14	*0.002

Total number of animals (n=10).

Level of significance *p< 0.05, **p<0.001, ***p<0.0001.

The seaweed has lowering effect on all types of lipids. There was significant reduction in cholesterol, triglycerides, HDL, LDL and VLDL following *Iyengariastellata* administration as shown in table 1. Cholesterol is involved in the early development and progression of atheroma plaque (Martin, *et al.*, 1986; Gordon, *et al.*, 1977). Aliya, *et al.* (1991) reported the presence of high amount of unsaturated fatty acid content as compare to saturated fatty acid, particularly clerosterol and many other sterols in seaweeds. Previous studies reports that diets consist of unsaturated fatty acids lower the plasma cholesterol level to moderate extent (Najam, 2003). According to Khan (2000), *Iyengariastellata* contain saringosterol, which is well known for its cholesterol lowering effect by reducing the intestinal absorption of cholesterol and by enhancing its excretion (Trautwein, *et al.*, 2003). Therefore the possible explanation of the lipid lowering effect of this seaweed is the presence of sterols in its composition.

There are reports that LDL and HDL levels in blood are more specific and sensitive biochemical markers of cardiovascular disease risk and furthermore HDL helps to protect against the development of atherosclerosis (Guyton and Hall, 2006). Various previous studies in the past few decades have revealed that significant increase in the serum levels of total cholesterol, LDL cholesterol and VLDL cholesterol is observed in acute myocardial infarction (Stampfer *et al.*, 1991). Thompson *et al.* (1996) reported that cholesterol is essential for VLDL synthesis therefore decreased cholesterol formation would lead to

lesser VLDL production and as VLDL is the precursor of LDL so reduced VLDL level will result in diminished LDL concentration.

Serum triglyceride may also be a risk factor (Carlson, *et al.*, 1979) especially in individuals with diabetes (West, *et al.*, 1983). In this study long term dosing of *Iyengariastellata* showed significant decrease in triglycerides.

Liver is the centre of biotransformation and metabolic disposition of large number of drugs and foreign agents thus drug induced liver injury is a potential complication of many medications (Lee, 1995). Serious underlying diseases can be evidenced by elevation of hepatic enzymes such as alanine transaminase (ALT) and aspartate transaminase (AST) (Giboney *et al.*, 2005). These two transaminases are most emergent risk markers of hepatocellular injury or necrosis because they are quite sensitive to liver damage.

The result showed a highly significant decrease in the concentration of ALT in the plasma (table 2), suggesting that the ethanolic extract from *Iyengariastellata* has the potential to resuscitate the hepatocytes. The probable explanation of this finding is the presence of vitamins in this seaweed (Mehta and Parekh, 1978; Qasim and Rashida, 1991; Qasim, *et al.*, 1985) as vitamins protects the liver from oxidative stress and inhibits the excessive free radicals accumulation thus exerting hepatoprotective effect. The other constituent of this seaweed which might

be responsible for its cell protective effect is loliolide (Khan, 2000).which shows antioxidant activity against various free radicals (Yang, *et al*, 2011). However there was a significant increase in the level of AST, which indicate that there may be some other factors contributing, not only liver damage.

In the current study the ethanolic extract of seaweed increased the Gamma-glutamyltransferase (γ GT) as in table 2. Generally the level of ALT and AST are slightly lower than the level of Gamma-glutamyltransferase (γ GT), might be because of the more generation of γ GT by the body cell. According to Ruttman, *et al* (2005), elevated level is also associated with pathogenesis of cardiovascular disease. Bidel *et al*. (2008) and Strasak *et al*. (2008), claimed that the liver enzyme gamma-glutamyltransferase (γ -GT) is a risk indicator of many common diseases such as diabetes and cancer.

Liver profile showed an increased Alkaline Phosphatase level (table 2). Alkaline Phosphatase is located in the biliary duct of the liver (Nyblom *et al.*, 2006) and also indicates liver toxicity.

Since the level of SGPT is more specific biochemical marker for hepatotoxicity, its decreased level indicates that *Iyengariastellata* toxicity is less severe and reversible but during the course of ingestion of the seaweed the liver enzymes must be carefully monitored to ensure liver safety.

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