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An Experimental Evaluation of Anti Atherogenic Effects for Two Marine Algae

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ABSTRACT

The study was undertaken to evaluate the marine algaeof Sargassum *polycystum* and *Gracilaria edulis* were dried undershade and then powdered, and extracted with 2-2.5 litters of 70% methanol by reflux.Preliminary phytochemical studies were carried out on methanolicextract of SP, andGE. Animals were feed with 5% of their body weight cholesterol rich diet to induceatherogenesis. Group 1 served as normal control while Group 2 was considered asatherogenic control. Group 3 was treated group I and was receiving *Sargassumpolycystum* (500mg/kg). Group 4 was treated group II and was receiving *Gracillariaedulis* (500mg/kg).

During the study fasting blood was taken at 0, 6th and 12th week. At the end of study,animals in all groups were sacrificed, blood sample, aorta were collected. Biochemicalparameters such as total cholesterol, HDL cholesterol, TG, LDL cholesterol,histopathological studies of aorta were performed. Oral administration of MSP(500mg/kg) and MGE (500mg/kg) showed antiatherogenic activity in atherogenicanimals. This was evidenced by lowering of serum cholesterol and triglyceridelevels, LDL cholesterol and increase HDL cholesterol level, hypolipidemiceffect. In this investigation the work is conclude Significant antiatherogenic activity of MSP and MGE observed in the presentinvestigation could be the result of decreased cholesterol and LDL level and increasedHDL level.Methanolic extract of SP and GE also showed improvisation in lipidprofile and may haveprotective effect in atherogenesis related cardiovascularcomplications.

Keywords: Methanolic extract, Sargassum polycystum, Gracillaria edulis, cholesterol,Antiatherogenic, HDL, LDL, TG.

INTRODUCTION

Atherosclerosis is a common condition that develops when a sticky substance called plaque builds up inside our body arteries.

Atherosclerosis develops slowly as cholesterol, fat, blood cells and other substances in our blood form plaque. When the plaque builds up, it causes arteries to narrow. This reduces the supply of oxygen-rich blood to tissues of vital organs in the body.

Several well recognized risk factors that contribute to the development of CHD include hypertension, smoking, diabetes, hyperlipidaemia, current cigarette smoking,and family history of premature CHD.[1][2]

The understanding of physiology of the circulation and diseases affecting the heart and the arteries were not part of mainstream Indian medicine. Ayurvedic diagnostic methods of these diseases and treatment also did not progress as well as in conventional medicine. This tardiness could also be due to the fact that atherosclerosis and coronary heart diseases were rare in India up to the early twentieth century.[3]

Many herbal products which used to lower the high cholesterol level available in India, such as Allium sativum, CommiphoramukulBoswellia serrata, Emblica officinalis, Terminalia arjuna, Trigonellafoenum graecum, Ocimum sanctum, Withaniasomnifera and Zingiber officinale. Antioxidants are substances that delay the oxidation process, inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions.Herbal drugs which are used as cardioprotective are Craegus oxycantha, Terminalia Arjuna, Inularacemosa, Commiphoramukul, Astragalus membranacous, Petasites vulgaris and Ruscusaculeatus[4]

Marine organisms are rich source of structurally biologically active metabolites and studies suggested that some bioactive compounds isolated from marine organisms had shown to exhibit anti-cancer, anti-microbial, anti-fungal or anti-inflammatory and other pharmacological activities. Several algae have been found to have secondary

metabolites most of which are phenolic compound, which had medicinal potentials. Some species of Cystoseira had been studied for its hypoglycaemic efficacy. The present study was planned to evaluate the Sargassum *polycystum* and *Gracilaria edulis*algae for antiatherogenic activity.

MATERIALS AND METHODS

Plant Materials

The marine algae *Sargassum polycystum* and *Gracillaria edulis* was collected during August 2015, from the Mandapam coast (latitude 90 17' Longitude 790 22, E), Gulf of manner. The sample was identified by Scientist in charge, at the Centre for Marine and Fisheries Research Institution (CMFRI), Mandapam Tamil Nadu.

Preparation of extract

The algae of *Sargassum polycystum* and *Gracilaria edulis* were chopped into small pieces and dried under shade at room temperature forseven days. The dried algae were powdered and passed through the sieve(coarse10/40). The powder was used for the preparation of methanolic extract.

Extraction and isolation

Dried and powdered algae of *Sargassum polycystum* and *Gracilaria edulis* (each 1.0kg) were extracted with boiling 70% Methanol in a reflux condition. After filtration, the solution was concentrated under a vacuum[5].

Experimental Animals

Rabbits weighing between 500gms - 800gms were procured from Karnataka Veterinary Hospital, Hebbal, Bangalore, Karnataka. Animals were housed separately and maintained under controlled temperature at $25^0C \pm 2^0C$ with 12hrs. Light/dark cycle. All animals were provided with free access to food and water *ad libitum*. Ethical clearance for performing the experiments on animals was obtained from the Institutional Animal Ethics Committee (IAEC).

Acute oral toxicity study

The acute oral toxicity study was performed on healthy female rabbit. Initially limit test was performed on a maximum of 5 animals with a test dose of 2000mg/kg. And if the animal dies main test is performed. Starting with a dose of 5mg/kg for one animal. if animal survive then dose for next animal in increased by factor of 3.2 times the dose100.[6]

Groups used in the study

24 rabbits were divided into 4 groups of 6 rabbits in each. Group 1normal controlgroup, Rabbits fed with normal chow (Oxoid) diet for 12weeks. Group 2disease control group,rabbits fed with 1.3% cholesterol enriched diet for 12 weeks. Group 3treated group I (Sargassum polycystum) **,** rabbits fed with cholesterol enriched diet for 6 weeks and 6 weeks of treatment along with cholesterol enriched diet. Group 4treated group II (Gracilaria edulis), rabbits fed with cholesterol enriched diet for 6 weeks and 6 weeks of treatment along with cholesterol enriched diet.

Blood samples were collected at the start of the study, after 6 weeks of the study and then at the end of treatment course for measurement of Serum lipid profile [total cholesterol (TC), serum triglyceride (TG) and high-density lipoprotein (HDL)], highly sensitive C-reactive protein (hsCRP). At the end of the study the aorta was removed for measurement of aortic malonyl dehydrogenase (MDA), glutathione (GSH) and Aortic intima- media thickness and sectioning for Histopathology.

Preparation of samples

From each rabbit, about 3 ml of blood was collected from the central ear artery without use of heparin after an overnight fasting. The blood sampling was done firstly at the start of the study i.e. at zero time and after 6 weeks of the induction period, and then at the end of treatment course (12week), allowing the blood samples to clot at 37°C and centrifuged at 3000 rpm for 15 min. Sera was taken, and analysed for determination of serum total

cholesterol, triglycerides, HDL-C and hs Creative protein.

Tissue preparation for oxidative stress measurement

20% homogenates of tissues were prepared in phosphate buffer at pH 7.5 containing 1 mmol/l Na2EDTA. The homogenates were centrifuged at 20,000 X g at 4°C for 30 min and the supernatants were used for biochemical measurements of GSH & MDA level.

Biochemical parameters

The biochemical parameters like Glucose, Triglyceride, Total Cholesterol, HDL, LDL, VLDL were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit. (DELTA LABS kit, Bengaluru, India) using Semi Auto analyser.

Statistical analysis

Results have been reported as mean value ± SEM. The variation in a set of data has been estimated by performing one-way analysis of variance (ANOVA).Individual comparisons of group mean values were done using Dunnet's test (Graph pad prism 6.0).

Histopathological Studies

The portion of aorta of rabbit was collected from group 1, 2, 3and 4 (Normal control, Disease control, Cholesterol rich diet + *Sargassum polycystum*, Cholesterol rich diet + *Glacilaria edulis*), respectively and fixed in 10 per cent formalin (10 ml of 40% formaldehyde added to 90 ml of water). The tissue was fixed for 48 hr and washed for 1 hr in running tap water. Then dehydration of the tissue was performed with increasing concentrations of ethanol (70, 90 and 100 per cent; each for 1 hr). Then the tissues were cleared in xylene for 1 hr for two changes. Paraffin embedding was carried by keeping the tissues in melted paraffin at 56º C for three changes. Longitudinal and transverse sections (5µm) were prepared with semiautomatic microtome and placed on glass slide coated with Meyer's egg albumin. Tissue sections were dried by incubating them for 2 hr at 40º C. Rehydration of fixed sections was carried in decreasing grades of alcohol (100, 90, 70 and 50 per cent; each for 1 hr) and then water. The sections were stained with haematoxylin and eosin stain as per Bancroft and Stevens (1996) with some modifications. Then the sections were covered with DPX (SRL, India) mounting medium with cover glass and observed under light microscope (Nikon, Japan) to study the histopathological changes.

RESULTS

Effect of methanolic extract of *Sargassum polycystum and Gracilaria edulis* (500mg/kg.po/day/6weeks) on cholesterol feed induced rabbit on blood glucose,serum cholesterol, triglyceride, HDL and LDL after 6 weeks of treatment.Introduction of high cholesterol rich diet to rabbits showed significant increase ($p<0.001$) in the blood glucose,total cholesterol, serum triglyceride, LDL level and VLDL level on 6th week of treatment when compared with normal control. Administration of methanolic extract of SP (500mg/kg.po/day/6weeks) to the cholesterol feed rabbits showed significant decrease ($p<0.001$) in the blood glucose,total cholesterol, serum triglyceride, LDL level and VLDL levelon 6th week of treatment when compared with disease control. Administration of methanolic extract of GE (500mg/kg.po/day/6weeks) to the cholesterol feed rabbits showed significant decrease ($p<0.001$) in the blood glucose, total cholesterol, serum triglyceride, LDL level and VLDL level on 6th week of treatment when compared with disease control.

Effect on serum HDL cholesterol (serum HDL-C) level of high cholesterol rich diet to rabbits showed significant decrease ($p<0.001$) in the serum HDL level on 6th week of treatment when compared with normal control. Administration of methanolic extract of SP (500mg/kg.po/day/6week) to the cholesterol feed rabbits showed significant increase ($P<0.001$) in the serum HDL level on 6th week of treatment when compared with disease control.Administration of methanolic extract of GE (500mg/kg.po/day/6weeks) to

the cholesterol feed rabbits showed significant increase ($p<0.001$) in the serum HDL level on 6th week of treatment when compared with disease control. Data are presented in Table1 and Figure 1, 2, 3, 4.

Table No:1. Effect of methanolic extract of *Sargassum polycystum and Gracilaria edulis* on blood glucose, serum cholesterol, triglyceride, HDL, LDL and VLDLafter 6 weeks of treatment.

Sl.	Parameters	Groups			
		NC (mg/dl)	DC (mg/dl)	SP (mg/dl)	GE (mg/dl)
1.	Glucose	101±0.26	160±0.51***	115±0.31 ###	109±0.50 ###
2.	LDL	77.7±0.33	184±0.36***	79.8±0.40##	86.5±0.44###
3.	Cholesterol	110±0.25	308±0.37***	128±0.42###	129±0.32###
4.	Triglyceride	110±0.30	185±0.31***	140±0.31###	139±0.45###
5.	HsCRP	0.82±0.05	4.01±0.23***	1.68±0.17#	2.19±0.26###
6.	VLDL	21.4±0.33	34.6±0.20***	25.4±0.26###	26.7±0.45###
7.	HDL	40.6±0.34	29.9±0.30***	45.6±0.29###	58.8±0.3###

The data are expressed as Mean ± SEM (n=6). ***P<0.001 when compared to normal control, #P<0.001 when compared to disease control

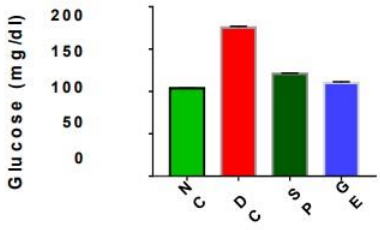


Fig:1: Effect of Methanolic extract of Sargassum polycystum and Gracilariaedulis on Glucose

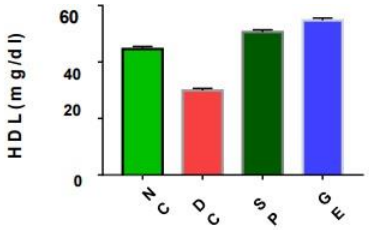


Fig:2: Effect of Methanolic extract of Sargassum polycystum and Gracilariaedulis on HDL

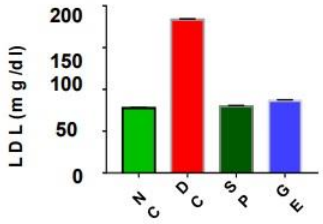


Fig:3: Effect of Methanolic extract of Sargassum polycystum and Gracilariaedulis on LDL

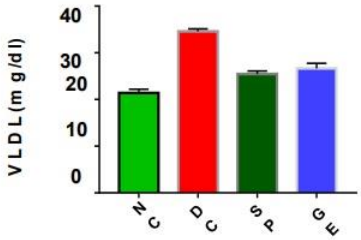
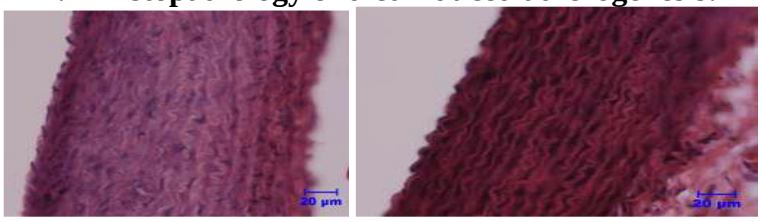


Fig:4: Effect of Methanolic extract of Sargassum polycystum and Gracilariaedulis on VLDL

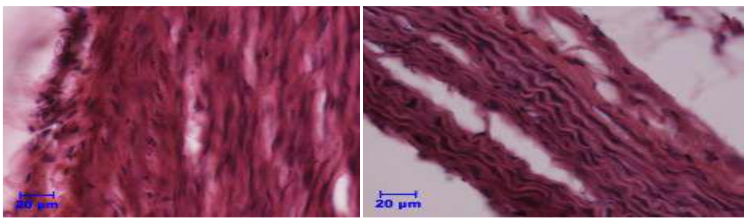
1.1 Histopathology of diet induced atherogenesis:



Thoracic Aorta Abdominal Aorta

Fig:5: Normalcontrol: Thoracic Aorta Abdominal Aorta

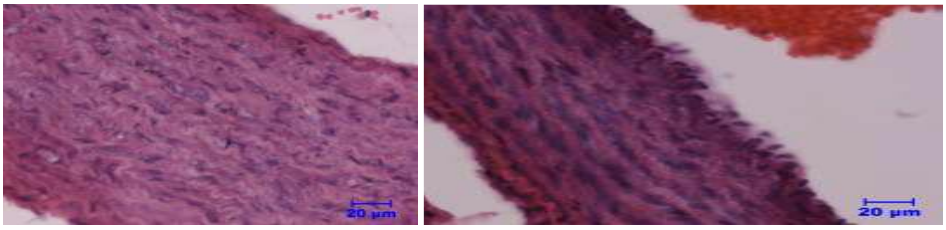
Fig 5 showed the thoracic and abdominal aorta of rabbits in the lining endothelial cells in tunica intima and smooth muscle fibres in tunica media with normal architecture of tunica.



Thoracic AortaAbdominal Aorta

Fig:6: Disease control:

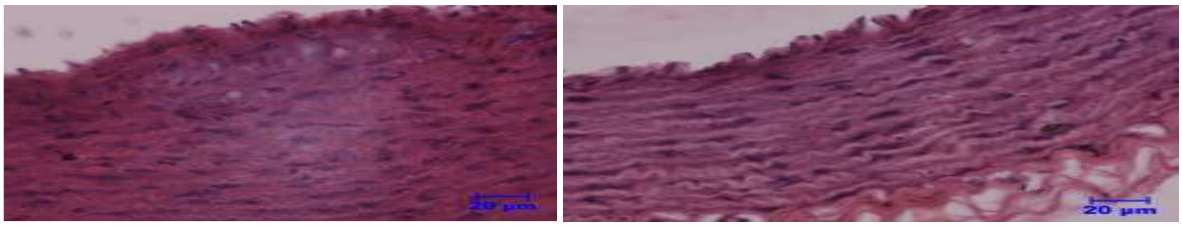
Fig 6 showed severe damage to endothelial layer which is evident by attachment of red blood cells and appearance of foam cells in between the smooth muscle fibres of tunica media.



Thoracic Aorta Abdominal Aorta

Fig:7: Sargassum polycystum + High Cholesterol diet:

Fig 7 revealed the presence of few foam cells in thoracic aorta but not is abdominal aorta and there was appearance of normal architecture of aorta.



Thoracic Aorta Abdominal Aorta

Fig:8: Gracilaria edulis + High Cholesterol diet.

Fig 8: The group 4 fed with cholesterol diet and *Glacilaria edulis* showed improvement in aorta architecture and appeared as of normal control aorta.

DISCUSSION

In the present study, Methanolic extract of SP, and GE were evaluated for their antiatherogenicactivity in cholesterol feed induced atherogenic rabbit.In present study we have observed that these algae *Sargassum polycystum and Gracilaria edulis* having anti atherogenic activity.Uncontrolled atherogenesis is associated with increase in total cholesterol,triglycerides and LDL cholesterol associated with decrease in HDL cholesterol.Atherogenesis is associated with lower rates of cholesterol synthesis and increasedabsorption of dietary cholesterol.

In present study, in atherogenic control group, there was marked increase in total cholesterol, LDL cholesterol and TG, while significant decrease in HDL cholesterol level, was found. Hyperlipidaemia is a known complication of atherogenesis and coexists with hyperglycaemia and is characterized by increased level of cholesterol, TG and LDL cholesterol, and all the lipid abnormalities associated with atherogenesis were significantly normalized by

treatment with methanolic extract of algae of SP and GE. In the present study histopathological picture of cholesterol feed aorta of rabbits showed appearance of foam cells in between the smooth muscle fibres of tunica media and there was separation of muscle fibres due to excessive deposition of fat between the muscle fibres, whereas methanolic extract of marine algae *Sargassum polycystum and Gracilaria edulis*, shows improvement in aorta architecture andappeared as of normal control aorta.

CONCLUSIONS

In conclusion, our data suggest methanolic extract of *Sargassum polycystum* and*Gracilaria edulis* possess potential antiatherogenic activity as it lowers serumcholesterol andtriglycerides levels, LDL cholesterol and increase HDL cholesterollevel significantly.Methanolic extract of SP and GE also possess significantantidiabetic activity as it lowers blood glucose level. Further studies are needed tocharacterize the antiatherogenic activity of the selected extract to find out the exactmechanism involved so that it can be formulated and may try clinically in future.

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