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Anti-Hypercholesterolemic Activity of *Ulva fasciata* , Collected from Rameshwaram, Southeast Coast of India

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Abstract

Seaweeds such as *Ulva lactuca*, *Ulva fasciata*, *Caulerpa racemosa*, *Caulerpa lentillifera*, *Gracilaria corticata* and *Gracilaria dura* were analysed for its *in vitro* anti-cholesterol activity. After investigation, *Ulva fasciata* showed highest *in vitro* anti-cholesterol activity of 97.63 %, so *Ulva fasciata* were further analysed for *in vivo* anti-hypercholesterolemic activity by experimenting on Wistar albino rats. The serum total lipid profiles of treated rats were analyzed. The concentration of HDL is comparatively high in all groups. The concentration of LDL and Triglyceride is lowering subsequently in all the groups except the hypercholesterolemic group. The optimum lowering of LDL, TC and increase of HDL were observed for the group-IV of Wistar albino rats which were treated with 600 mg *Ulva fasciata*. The histopathological analysis of tissues of heart, liver, kidney and brain showed major inflammation and necrosis of tissues of the group-II rats which was treated with high fat died and seaweed treated rats showed no evidence of tissue inflammation and necrosis.

Keywords: *Ulva fasciata*; Anticholesterol activity; *In vivo* anti cholesterol activity; Hypercholesterolemia; Negotiation

Introduction

Due to the endemic burden of cardiovascular diseases throughout the world and the excessive burden of side effects of market available chemical based medicines, there is a need of cost effective bio based medicine with zero side effects. The market available statins groups of medicine have severe side effect of muscle pain, digestive problems, mental fuzziness and longtime medication cause liver damage. So, leading research is focusing to find out the plant based medicine or therapeutics. In my study, I selected *Ulva fasciata*, green seaweed on basis of its medicinal and nutritional utilities. *Ulva fasciata* Delile, 1813: A green seaweed (Chlorophyceae) is known as sea lettuce, edible as vegetables, raw in salads; cooked in soups and food source for human in India, Scandinavia; Great Britain; Ireland; China and Japan due to its high nutritive compositions with protein, soluble dietary fibre, variety of vitamins and minerals especially iron. It has various medicinal utilities also. The studied species is abundant and easily cultivated along the coastal areas of India. The histopathology analysis of liver, brain, heart and kidney all groups were analyzed for tissues conformational changes. It's showed significant results. From, this study, I may conclude that *Ulva fasciata* may use as therapeutics for hypercholesterolemia after further more investigation with human clinical trial.

Materials and Methods

Studied species and collection area

Two stations at Rameshwaram, Tamilnadu, Southeast Coast of India, had been selected for this study. Rameshwaram is a small island, in the Gulf of Mannar, 570 km away from south of Chennai. This famous pilgrimage centre, geographically located at 09°18'.390''N and 079°20'.076''E with the area coverage of 51.8 sq. Km. The Pamban Bridge connects the mainland with Rameshwaram Island. The first station, the Olaikuda is located at 09°18'.853''N and 079°20'.141''E near the Rameshwaram temple. This is influenced by tourism pressure throughout the year. The second station is Vadakkadu located at 09°19'.700''N and 79°19'.072''E, 8 km away from Rameshwaram temple (Figure 1). The six seaweeds such as *Ulva lactuca*, *Ulva fasciata*, *Caulerpa racemosa*, *Caulerpa lentillifera*, *Gracilaria corticata* and *Gracilaria dura* were collected from the mentioned area as listed (Table 1).

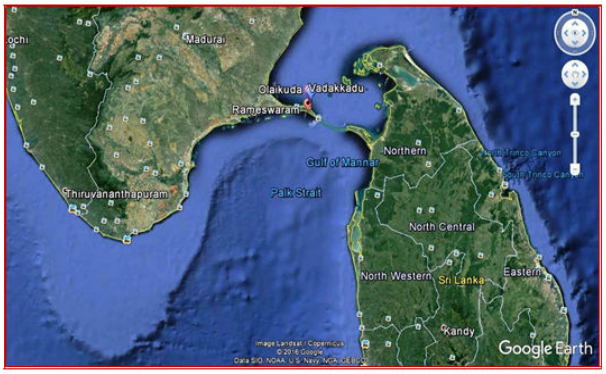


Figure 1: Map showing study area of Olaikuda and Vadakkadu coast, Rameshwaram, southeast coast of India.

Table 1: Taxonomic position, division, class, order and family.

Taxonomic position: Division: Chlorophyta; Class: Chlorophyceae; Order: Ulvales; Family: Ulvaceae	
	
Ulva lactuca	*Ulva fasciata*
Taxonomic position: Division: Chlorophyta; Class: Chlorophyceae; Order: Bryopsidales; Caulerpaceae	
	
Caulerpa racemosa	*Caulerpa lentillifera*
Taxonomic position: Division: Rhodophyta class: Rhodophyceae; Order: Gracilariales; Family: Gracilariaceae	

	
Gracilaria corticata	*Gracilaria dura*

Assay of *in vitro* anti-cholesterol activity

Each species of autoclaved seaweed of 250 mg was macerated and dissolved in 100 ml 50:1 chloroform: methanol solution. The adequate amount of this extract was used to test anti-cholesterol activity.

Blank: It was prepared with 20 µl distilled water and 2000 µl of Radox reagent kept in micro-titre.

Sample: The 10 µl seaweed extract was pipetted into microtitre plate and 2000 µl Randox and 10 µl cholesterol solutions were added to it.

Negative control: It was prepared with 20 µl of cholesterol solution and 2000 µl Randox reagent.

Standard: It was prepared with 20 µl Simvastatin and 2000 µl Randox reagent. All set up were incubated at room temperature in 3 minutes. The absorbances of all samples were measured at 500 nm in UV-VIS spectrophotometer [1]. Anticholesterol activity was measured by using below calculating equation:

$$\text{Inhibition (\%)} = \frac{\text{Negative control} - \text{Sample} \times 100}{\text{Negative Control}}$$

Acute toxicity test was done following standard protocol as mentioned previous literatures [2]. The most potent seaweed *Ulva fasciata* have been subjected to acute toxicity and sub-acute toxicity test to select the minimum lethal dose and to rule out the e ect of any toxins.

The acute toxicity test was carried out based on the Organization for Economic Co-operation and Development (OECD) guideline for testing of chemicals (410.423-2011).

Sample preparation

Pre-weighted quantity of 1500 mg and 2000 mg *Ulva fasciata* was well macerated in a mortar and pestle, and dissolved with 10 ml distilled water. The seaweed syrup like solution was made to use as sample for animal treatment for acute toxicity test. Seaweed syrup like solution was prepared everyday fresh prior to every day oral gavages to the experimental animals by feeding needle.

Animals collection and maintenance

I presented the proposal of laboratory animal experiment to members of Institutional animal ethics committee at Rajah Muthiah medical college, Annamalai University, Annamalai Nagar and got the ethical clearance for laboratory animal experiment, the proposal No. is AU-IAEC/1196/1/18 for 48 Wistar albino rats.

Total 48 Male Wistar albino rats weighing 150-250 g were obtained from the animal house of Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), (CPCSEA registration No.-190/GO/ReBiBt-S/ReRcBiBt- L/2000/CPCSEA and Serial No. 145) Chennai-600051, Tamil Nadu, India was used for the study. A ter collection, Wistar albino rats were transported keeping them within plastic rat cages in groups of 6 rats per cages, within AC car to the central animal house at Rajah Muthiah medical college, Annamalai University, Annamalai Nagar. All rats were housed in poly propylene rat cages in groups of 6 rats per cage in a room with temperature of 25 ± 2°C, relative humidity: 55 ± 65% 12 hours natural light and 12 hours darkness with free access to tap water and dry pellet feed provided from Central animal house itself. All rats were allowed to acclimatize for three days prior to the experiment. Among 48 animals, only 6 rats were utilized for acute toxicity test.

Animal experiments

The six groups of hypercholesterolemic rats which were fed with normal feed *i.e.,* control, normal feed; high fat diet group and seaweeds with different concentration (400, 600, 800 and 1000 mg) and one group treated with market available drug Simvastatin.

After completion of acute toxicity test of *Ulva fasciata*, I conducted the *in vivo* animal experiment in which the seven groups of wistar albino rats were treated as mentioned (Table 2).

Table 2: The seven groups of Wistar albino rats and their treatment.

Groups	Experimental set up
Group-I	Rats received standard solid diet and tap water ad libitum for 12 weeks.
Group-II	Rats received a high fat diet containing 1.5% cholesterol/day for 12 weeks.
Group-III	Rats received a high fat diet containing 1.5% cholesterol for 12 weeks+from 9th week's seaweed 400 mg/10 ml DW/kg body/day.
Group-IV	Rats received a high fat diet containing 1.5% cholesterol for 12 weeks+from 9th week's seaweed 600 mg/10 ml DW/kg body/day.
Group-V	Rats received a high fat diet containing 1.5% cholesterol for 12 weeks+from 9th week's seaweed 800 mg/10 ml DW/kg body/day.
Group-VI	Rats received a high fat diet containing 1.5% cholesterol for 12 weeks+from 9th week's drug Simvastatin/Atorvastatin 40 mg dissolved in polyethylene glycol/kg body/day.
Group-VII	Rats received a high fat diet containing 1.5% cholesterol for 12 weeks+from 9th week's seaweed 1000 mg/10 ml DW/kg body/day.

Steps of blood samples collection and analysis

Blood samples were withdrawn by retro-orbital puncture using capillary tubes. The blood samples were collected in Eppendorf tube containing EDTA. The samples were centrifuged at 3000 rpm for 15 minutes and clear supernatant sera were quickly removed and the sera samples were stored in -80°C till used. The blood samples were mixed with standard and reagent of chemicals of different kits such as total cholesterol kit, high density lipoprotein cholesterol kit and low-density lipoprotein cholesterol kit as well as triglycerides kit according to standard protocol. All the samples were incubated for 15 minutes in room temperature. Lipid profile reading was taken with Semi-biochemistry auto analyzer AGD 2020.

Steps of histopathological analysis different tissues of rats

At the end of the experiment mercy scarify of animals by ketamine injection and liver, brain, heart and kidney were collected and immediately cleaned with ice-cold saline (0.9% Sodium chloride). The histopathology of liver, brain, heart and kidney were examined by following the standard method [3]. The liver, kidneys, heart and brain were harvested and fixed with 10% formalin saline for 48 hrs and processed for paraffin wax embedding with an automatic tissue processor by dehydrating through 70%, 90%, 95% and two changes of absolute ethanol for 90 minute each. Sections were cut at 5 µm with rotary microtome. The sections were stained by haematoxylin and eosin (H and E) method, examined and photographed using a light microscope.

Results

In vitro anti-cholesterol activity

It was tested according to standard protocol and the results were added below with table and graphical form (Table 3 and Figure 2).

Table 3: Percentage of inhibition.

Sample ID	% of inhibition
Simvasatin	96.3
Ulva lactuca	78.63
Ulva fasciata	97.63
Caulerpa racemosa	85.89
Caulerpa lentillifera	89.63

Gracilaria corticata	65.32
Gracilaria dura	64.34

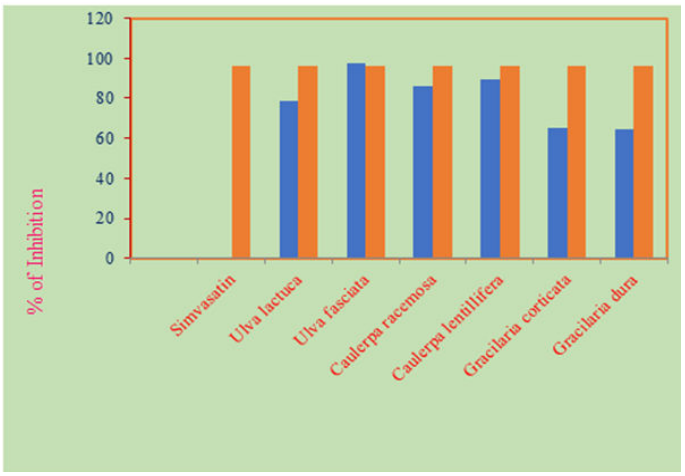


Figure 2: *In vitro* Anticholesterol activity of seaweeds and Simvastatin.

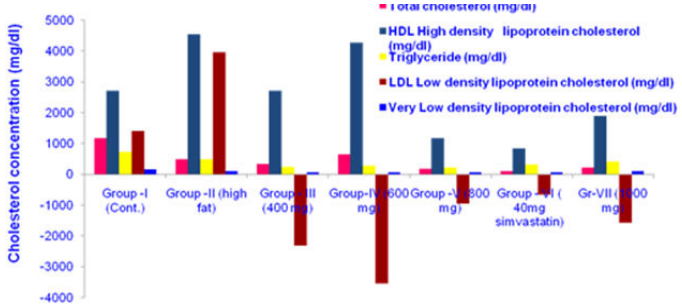
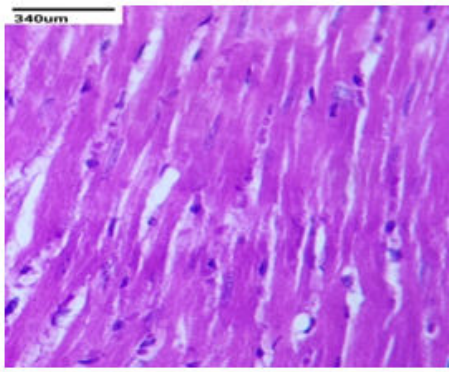


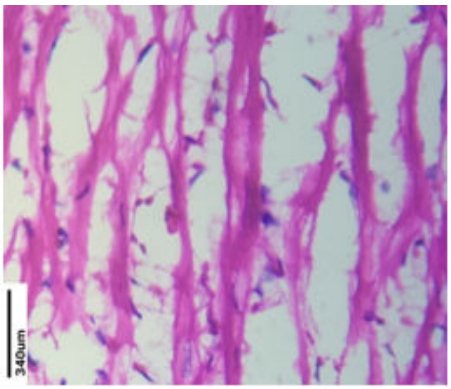
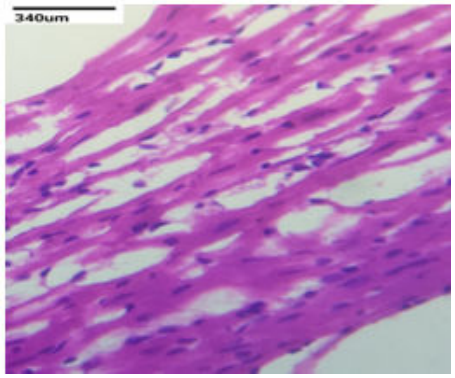
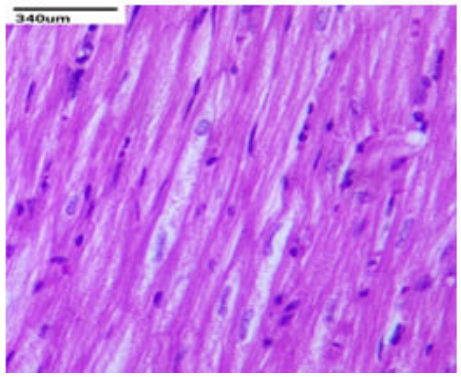
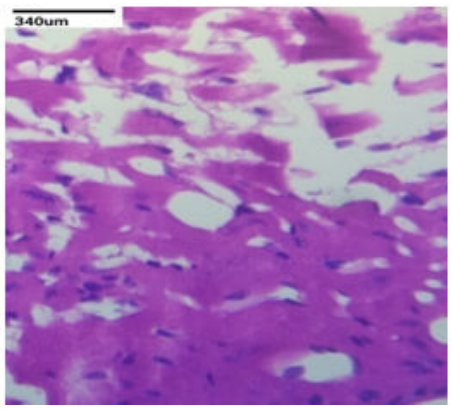
Figure 3: Representing the lipid pro ile of wistar albino rats treated with *Ulva fasciata* and market available medicine.

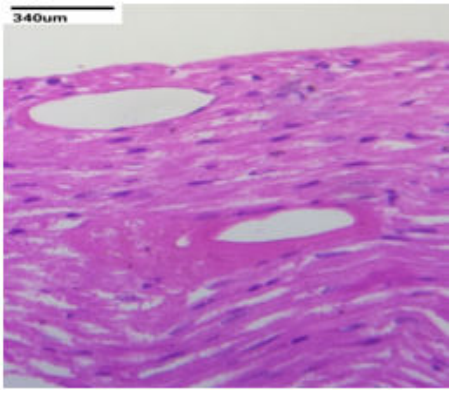
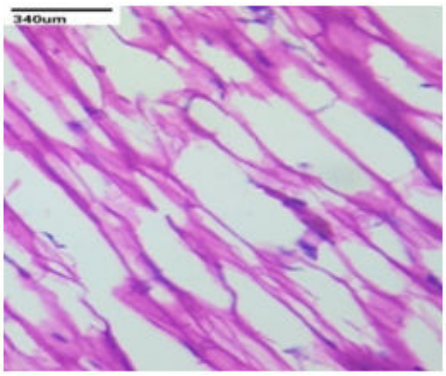
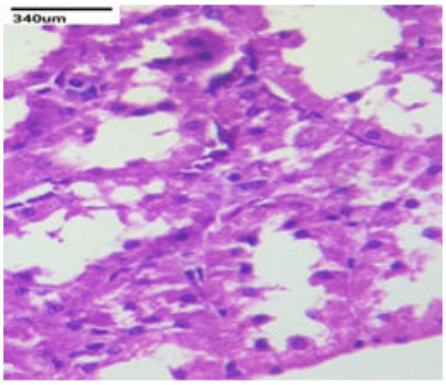
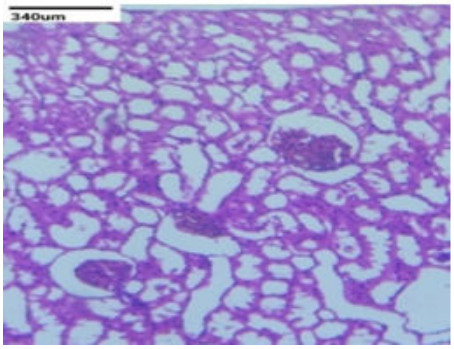
In vivo animal experiments

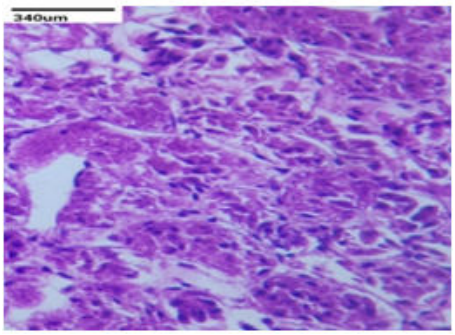
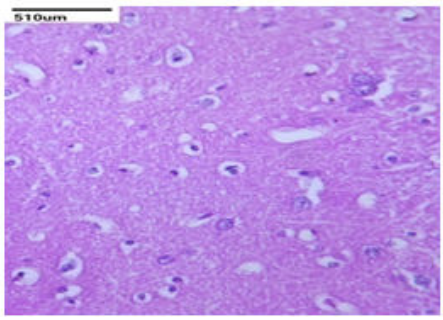
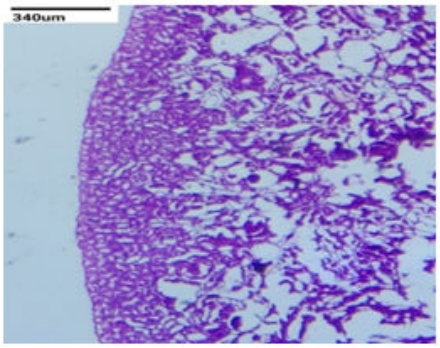
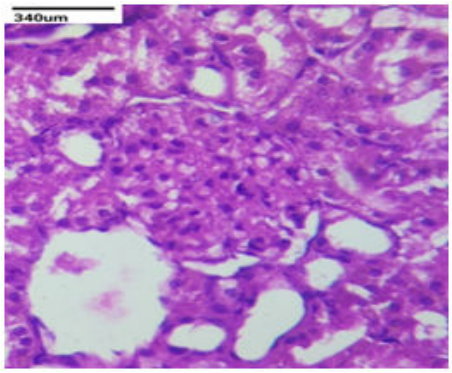
The *in vivo* animal experiments were conducted according to standard protocol and the results are elaborated in Figure 3 (Table 4).

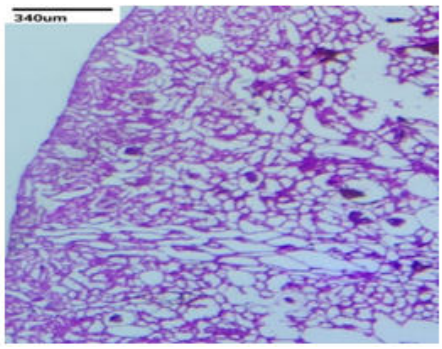
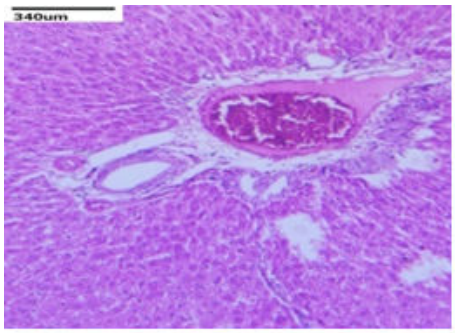
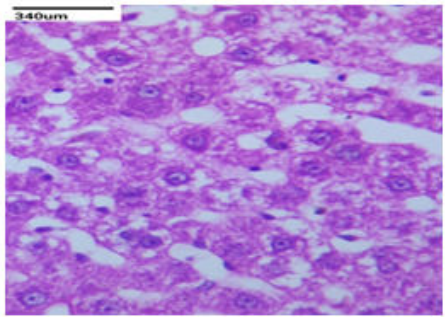
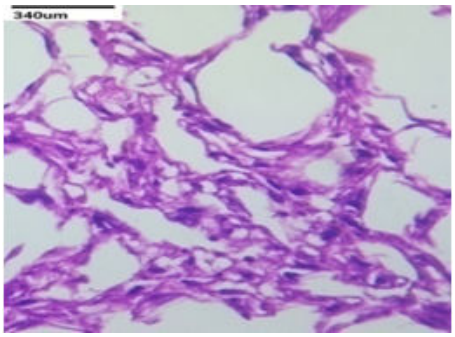
Table 4: Histopathological investigations of heart, kidney, liver and brain tissues.

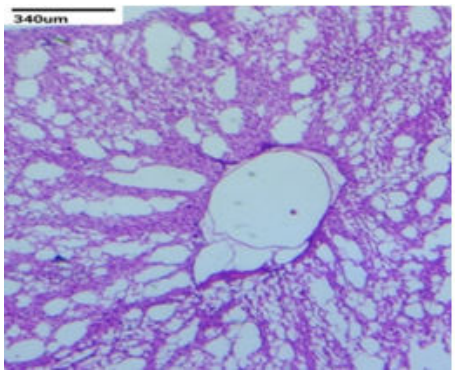
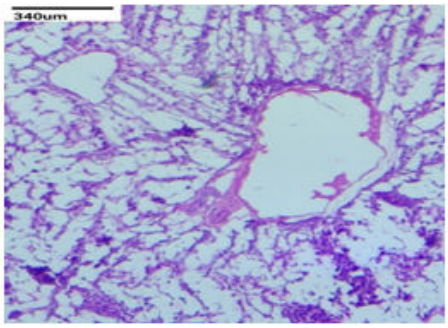
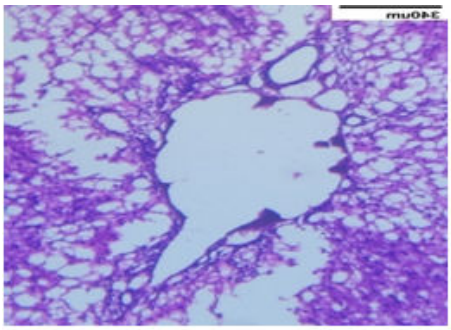
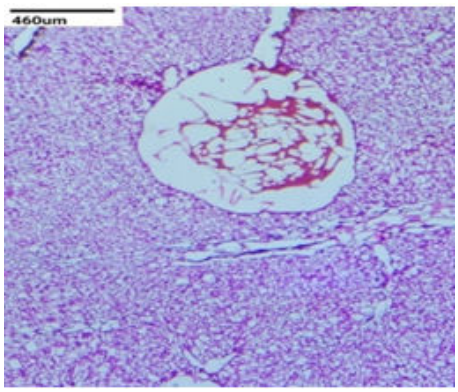
Histopathological investigations of heart tissues: Micrographs of heart tissues		
Groups	**Micrographs**	**Interpretations**
Gr- I (Control)		Normal cardiac myocytes.

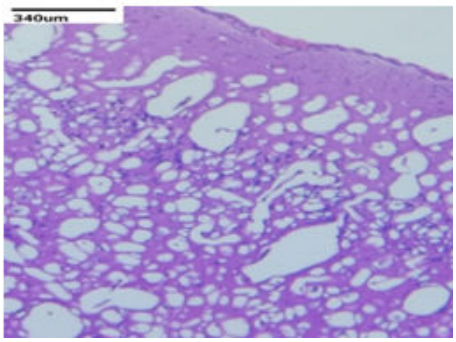
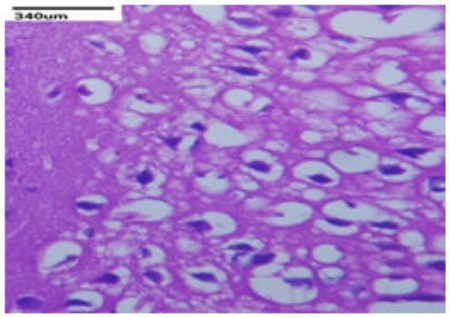
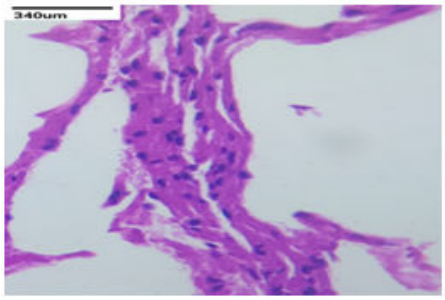
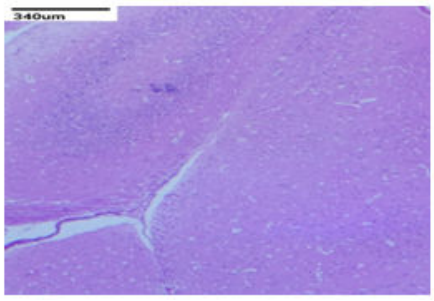
Gr-II – (High fat diet treated rat)		Cardiac myocytes with focal waviness of fibres. Evidence of active inflammation. The waviness of fibres in focal areas may indicate early ischemic changes.
Gr-III (High fat diet+400 mg seaweed treated rat)		Light evidence of active inflammation than group-II. Cardiac myocytes with focal waviness of fibres.
Gr-IV (High fat diet+600 mg seaweed treated rat)		Histological image showed the
Gr-V (High fat diet+800 mg seaweed treated rat)		No evidence of active inflammation.

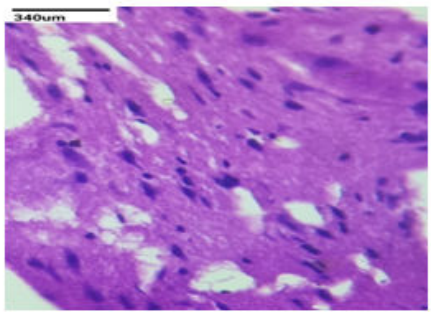
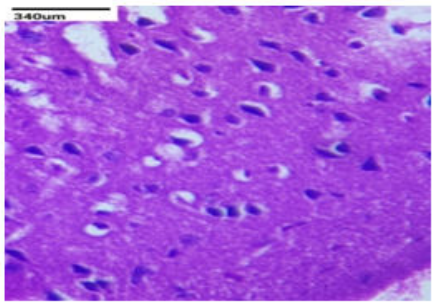
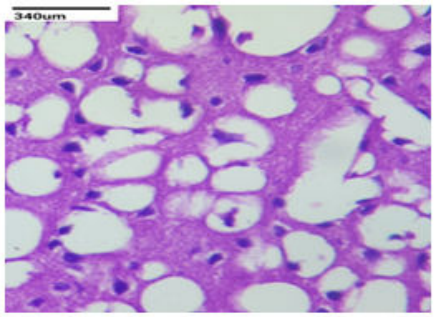
Gr-VI (High fat diet+40 mg Simvastatin)		No evidence of active inflammation.
Gr-VII (High fat diet+1000 mg seaweed treatment)		
Histopathological investigations of kidney tissues: Micrographs of kidney tissues		
Gr-I (Control)		Kidney parenchyma and glomerulus appear normal. There is no evidence of ATN (Acute Tubular Necrosis) or interstitial inflammation.
Gr-II–(High fat diet treated rat)		Kidney glomerulus appear accumulated, tubules appear fatty, evidence of interstitial inflammation/fibrosis.

Gr-III (High fat diet+400 mg seaweed treated rat)		There is no evidence of ATN (Acute Tubular Necrosis) or interstitial inflammation.
Gr-IV (High fat diet+600 mg seaweed treated rat)		There is no evidence of ATN (Acute Tubular Necrosis) or interstitial inflammation.
Gr-V (High fat diet+800 mg seaweed treated rat)		There is no evidence of ATN (Acute Tubular Necrosis) or interstitial inflammation.
Gr-VI (High fat diet+40 mg Simvastatin)		There is no evidence of ATN (Acute Tubular Necrosis) or interstitial inflammation.

Gr-VII (High fat diet+1000 mg seaweed treatment)		There is no evidence of ATN (Acute Tubular Necrosis) or interstitial inflammation.
Histopathological investigations of liver tissues: Micrographs of liver tissues		
Gr-I (Control)		Liver preserved architecture. No evidence of fatty change or inflammation. No peri-portal inflammation, interface hepatitis or lobular inflammation.
Gr-II–(High fat diet treated rat)		Liver extensive arti-factual changes. There is evidence of fatty change. evidence of fibrosis, inflammation or necrosis.
Gr-III (High fat diet+400 mg seaweed treated rat)		Medium evidence of fibrosis,inflammation or necrosis.

Gr-IV (High fat diet+600 mg seaweed treated rat)		No evidence of fibrosis, inflammation or necrosis.
Gr-V (High fat diet+800 mg seaweed treated rat)		No evidence of fibrosis, inflammation or necrosis.
Gr-VI (High fat diet+40 mg Simvastatin)		No evidence of fibrosis, inflammation or necrosis.
Gr-VII (High fat diet+1000 mg seaweed treatment)		No evidence of fibrosis, inflammation or necrosis.
Histopathological investigations of brain tissues: Micrographs of brain tissues		

Gr-I (Control)		K-Brain normal brain tissue. No evidence of active inflammation, gliosis or necrosis.
Gr-II – (High fat diet treated rat)		Extensive artifactual changes. May be evidence of active inflammation, gliosis or necrosis.
Gr-III (High fat diet+400 mg seaweed treated rat)		Brain appear normal with artifactual changes.
Gr-IV (High fat diet+600 mg seaweed treated rat)		K-Brain normal brain tissue. No evidence of active inflammation, gliosis or necrosis.

Gr-V (High fat diet+800 mg seaweed treated rat)		K-Brain normal brain tissue. No evidence of active inflammation, gliosis or necrosis.
Gr-VI (High fat diet+40 mg Simvastatin)		K-Brain normal brain tissue. No evidence of active inflammation, gliosis or necrosis.
Gr-VII (High fat diet+1000 mg seaweed treatment)		K-Brain normal brain tissue. No evidence of active inflammation, gliosis or necrosis.

Discussion

Hypercholesterolemia is considered as one of the leading causes of death in the world. It is characterized by elevated levels of lipids circulating in the blood is linked to the development of cardiovascular and metabolic syndrome. But majority of previous literatures studied anti-hypercholesterolemic activity with different extract of various source but yet didn't have significant satisfactory results, so there is still gap to work on *in vivo* and *in vitro* Anticholesterol activity. So, I studied *in vitro* and *in vivo* anti-cholesterol activity of some selected seaweeds depending their bioactivity. Among six seaweed *Ulva fasciata* extract showed highest percentage of cholesterol inhibition. In *in vitro* anticholesterol activity *Ulva fasciata* extract showed comparatively high cholesterol inhibition than market available anti-cholesterol drug Simvastatin. It showed 97.63% of inhibition. *Gracilaria cort cata* and *Gracilaria dura* have minimum cholesterol inhibition activity of 65.32 % and 64.34%.

The concentration of HDL is comparatively high in all groups. The concentration of LDL is lowering in all the groups except the hypercholesterolemic rats. Triglyceride's concentration is noted comparatively low in all the groups.

LDL cholesterol is considered the "bad" cholesterol, because it contributes to fatty build ups in arteries (atherosclerosis). This condition narrows the arteries and increases the risk for heart attack, stroke and peripheral artery disease, or PAD. HDL cholesterol is considered the "good" cholesterol for health as it carries excess cholesterol back to the liver for excretion. The liver then eliminates cholesterol through bile. Triglycerides are another type of fat in the blood. They are not a type of cholesterol but have a strong association with heart disease

Present study reveals that *Ulva fasciata* water solution oral administration to hypercholesterolemic rats significantly increase HDL cholesterol level and significantly decrease LDL cholesterol similarly triglycerides level. *Ulva fasciata* polysaccharides and it has been reported at hypolipidemic and anti-atherogenic [4,5]. *Ulva sp.* has been reported to induce a marked decrease in systolic blood pressure by 29 mm/Hg [6]. *Ulva linza* also decrease blood pressure [7]. *Ulva fasciata* ethyl acetate extract may be used as add-on therapy for controlling type 2 diabetes mellitus [8].

Conclusion

Drug development process is time consuming and laborious similarly expensive. But if food items use as a medicine, means single food item multi work target; its consumption fulfill nutrition and medication. *Ulva fasciata* is already use as food item and also useful for negotiating multi risk factor of cardiovascular disease. So, in future after human clinical trial with *Ulva fasciata*, it may be used as bio-medicine for negotiating risk factors of cardiovascular disease.

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Conflict of Interest

Author has no conflicts of interest to be declared.

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