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Research Article

THE ANTI-HYPERCHOLESTEROLEMIC EFFECT OF ULVAN POLYSACCHARIDE EXTRACTED FROM THE GREEN ALGA *ULVAFASCIATA* ON AGED HYPERCHOLESTEROLEMIC RATS

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ABSTRACT

Objective: This study aims to evaluate the relation between hypercholesterolemia and aging, beside the role of *Ulva fasciata* polysaccharides (UFP) aqueous extracts in lowering cholesterol in aged hypercholesterolemia-induced rats was demonstrated.

Method: A total of 140 male Wister rats weighing 120±10 g, 6-9 months old were used. Hypercholesterolemia was induced in rats by feeding rats high-fat diet (cholesterol), cholesterol was orally administrated at a dose of (30 mg/0.3 ml olive oil/1 kg animal) 5 times a week for 12 consecutive weeks, lard fat was mixed with normal diet (1 kg of animal lard was added to 5 kg of normal diet), the occurrence of hypercholesterolemia was determined by measuring the lipid profile (TC, LDL-C, high-density lipoprotein-cholesterol [HDL-C], triglyceride [TG]), the old hypercholesterolemic (HC) rats were only used.

Results: The antihypercholesterolemic (HC) effects of ulvan, the sulfated polysaccharide extracted from the green alga *Ulvafasciata*, in aged rats, were studied. Algal treatment declared a significant reduction in serum total lipid level while, elevation of high-density lipoprotein-cholesterol level was noticed in HC rats. Moreover, the algal treatment significantly decreased serum liver and kidney functions biomarkers and improved the hepatic antioxidant levels in hyperlipidemic aged rats. In addition, ulvan administration significantly suppressed the expression of tumor necrosis factor-alpha, myeloperoxidase and cell vascular and intracellular adhesion molecules-1, while increased the anti-inflammatory cytokine level; interleukin-10. Furthermore, the histopathological examination of aorta, liver and kidney of HC-treated rats indicated that the *Ulva fasciata* polysaccharides (UFP), is a potent natural hypolipidemic nutraceutical for the amelioration of hyperlipidemia in aged rats.

Conclusion: It could be concluded that, in comparison with the standard anti-HC drug (fluvastatin) used in this study, both cold and hot UFP algal extracts of *U. fasciata* demonstrated appreciable anti-hypercholesterolemic property, in addition to their antioxidant activity even in the old HC stressed rats. Thus, it could be used as a natural lipid regulator.

Keywords: *Ulvafasciata,* Hypercholesterolemia, Polysaccharides, Rats, Sulfated polysaccharides, Aging, Hyper-cholesterolemia.

INTRODUCTION

Elevated levels of plasma cholesterol, particularly low-density lipoprotein-cholesterol (LDL-C), are known to be associated with an enhanced risk for atherosclerosis and coronary heart disease. Total cholesterol (TC) and LDL-C levels increase with age, as does the incidence of cardiovascular disease (CVD) [1,2].

Aging is characterized by the loss of homeostasis that leads to changes in the biochemical composition of tissues, reduced ability to respond adaptively to environmental stimuli and increased susceptibility and vulnerability to disease including coronary artery disease (CAD) [3]. A lot of events including deterioration in metabolism leading to acceleration of atherosclerosis are associated with aging [4].

Cholesterol homeostasis is maintained by a feedback regulatory system that senses the level of cholesterol in cell membranes and modulates both the transcription of genes encoding proteins involved in cholesterol biosynthesis and posttranscriptional events along with the uptake of cholesterol from plasma lipoproteins [5]. Maintenance of cholesterol homeostasis is regulated by both the receptor-mediated endocytosis of LDL by LDL receptors and *de novo* cholesterol synthesis *via* therate-limiting enzyme 3-hydroxy-3-methylglutaryl coenzymea reductase (HMGR) [6].

It was found that the metabolism of cholesterol is changed in aging [7] as TC and LDL-C levels were found to be elevated by 40% with aging [8,9]. In addition, during aging, hepatic lipid accumulation occur, elevation of both cholesterol level and hepatic cholesterol synthesis concomitant with HMGR enhancement f [10,11], due to increase in reactive oxygen species (ROS) related to aging [12-15].

Statins and fibrates are the most commonly used drugs for regulation of lipid. Although, the statins safety, some problems have been imitated related to the side effects, as they connected to the classes of drugs (rhabdomyolysis). In addition, statins do not suitable and powerful for all patients [16].

Recently, many sulfated polysaccharides (SPs) having antihyperlipidemic effect have been examined from different food sources as *Ulvapertusa* [17], *Ulvalactuca* [18]. It was found that natural polysaccharides are characterized by antioxidant and antihyperlipidemic, antitumor, anti-inflammatory, antiviral, activities [18,19], which can be used as apotent hypolipidemic nutraceutical.

Thus, this study aims to evaluate the relation between hypercholesterolemia and aging, beside the role of *Ulva fasciata* polysaccharides (UFP) aqueous extracts in lowering cholesterol in aged hypercholesterolemia-induced rats was demonstrated.

METHODS

Collection of the algal sample

U. fasciata, belongs to the family *Ulvaceae*, was collected from Abukir, Alexandria. The collected samples of alga were cleaned of epiphytes, barnacle, gastropod, and other contaminants at the site. After washing thoroughly in tap water, the samples were air dried at room

temperature in the shade, milled coarsely powdered, and stored in polyethylene plastic bags in a dry place. Herbarium specimens of the alga were identified by Dr. Shaalan S. A., Professor of Phycology, Faculty of Science, Alexandria University.

Preparation of UFP crude extracts

Chemical extraction

Air dried alga were soaked in 30% volume (w/v) of distilled water and kept overnight at 4-5°C. Then, the material was stirred well and allowed it to return to room temperature. The cold water extract was first filtered through muslin cloth and then with filter paper. The process was repeated till complete exhausted of polysaccharide (negative molish test). The extract was concentrated to 1/10 of its original volume under reduced pressure at 40°C using rotary evaporator with vacuum (BÜCHI Rota vapor R 200), and precipitated by the addition of 4-fold volume of 95% (v/v) ethanol, centrifuge at 3000 rpm for 10 minutes. The algal residue of cold water was soaked in sufficient distilled water and heated at 100°C for 3 hrs, and hot water extract was obtained following the same procedure used for the cold water extract. The precipitate was washed twice with absolute ethanol, the dried by freeze dryer to obtained a crude polysaccharide cold and hot then keep in refrigerator for chemical and biological investigation [20]. The yields of polysaccharides of *U. fasciata* were calculated on the basis of the dry weight of algal sample (w/w).

Chemicals

All chemicals and reagents were purchased from Biodiagnostic Company for diagnostic and research reagents, Egypt. Standard drug (Fluvastatin) was purchased from Novartis Pharmaceuticals. enzyme linked immunosorbent assay (ELISA) kits were provided by Uscn (USA.) for both C-reactive protein (CRP) and myeloperoxidase (MPO) Invitrogen (USA) for both tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-10, Eiaab (USA) for both vascular and intracellular adhesion molecules-1 (VCAM-1 and ICAM).

Induction of hypercholesterolemia

Hypercholesterolemia was induced in rats according to the method of Adaramoye *et al*. [21], by feeding rats high-fat diet (cholesterol), cholesterol was orally administrated at a dose of (30 mg/0.3 ml olive oil/1 kg animal) 5 times a week for 12 consecutive weeks, lard fat was mixed with normal diet (1 kg of animal lard was added to 5 kg of normal diet), the occurrence of hypercholesterolemia was determined by measuring the lipid profile (TC, LDL-C, high-density lipoprotein-cholesterol [HDL-C], triglyceride [TG]), the old hypercholesterolemic (HC) rats were only used.

Doses and routs of administration

- Negative cold extract administered to control old rats: Normal old rats given uphold extract at a dosage of 175 mg/kg body weight dissolved in distilled water for 4 weeks
- Negative hot extract aged control: Normal old rats given UFP hot extract at a dosage of 175 mg/kg body weight dissolved in distilled water for 4 weeks
- HC old rats received an oral dose of cold extract of 175 mg/kg body weight dissolved in distilled water daily for 4 weeks
- HC old rats received UFP hot extract at a dosage of 175 mg/kg body weight dissolved in distilled water daily for 4 weeks
- HC rats received an oral dose of 10 mg/kg body weight dissolved in distilled water of the anti-HC standard drug; fluvastatin, orally administered by gastric intubation for 4 weeks, this dose was calculated from the therapeutic dose of human [22].

Experimental design

Rats

A total of 140 male Wister rats weighing 120±10 g, 6-9 months old were used. The animals were 15-18 months old when purchased and were kept in-house until use. The rats were specifically raised for these experiments. Rats were kept under standardized conditions with free access to water, chow and lights cycle.

The animals were provided from the animal house of the National Research Center (NRC), and housed in a temperature-controlled environment (26-29°C), in steel mesh cages on wood-chip bedding, with a fixed light/dark cycle with free access to water and food *ad libitum.* This study was approved by the Ethical Committee of the NRC, Egypt, providing that the animals did not suffer at any stage of the experiment (Ethical approach number: 10 133).

Animals were randomly divided into seven equally sized groups of 20 old rats each (n = 20) as follows: Group 1: Normal aged control rats, administered normal diet and distilled water. Group 2: Negative cold extract control: Normal old rats administered cold UFP. Group 3: Negative hot extract control: Normal old rats administered hot UFP. Group 4: Aged HC-positive control rats. Group 5: Aged HC-rats treated with cold UFP extract. Group 6: Aged HC-rats treated with hot UFP extract. Group 7: Aged HC-rats treated with the anti-HC reference drug; fluvastatin.

Blood collection and tissue sampling

By the end of the 4th week, animals of different groups were sacrificed under diethyl ether anesthesia.

Blood samples were collected, left to coagulate at room temperature and centrifuged at 3000 rpm for 30 minutes. The clear, nonhemolyzed, and supernatant sera were quickly removed and kept at −80°C till used for biochemical investigations of lipid profile, liver function, kidney function parameters, and inflammatory markers.

Liver tissue was rapidly excised from each rat and accurately weighed; 0.5 g from each liver was homogenized in 5 ml bidistilled water using electrical homogenizer. The clear homogenate was used for estimation of nonenzymatic glutathione (GSH) antioxidant and lipid peroxide (LPO).

Biochemical examination

Cholesterol assay

Serum TC concentration was estimated according to the method of Allain *et al*. [23], serum total lipids (TLs) concentration was determined according to the method of Zollner *et al*. [24], serum TGs concentration was determined according to the method of Fassati and Prencipe [25], and serum HDL-C concentration was measured according to the method of Lopes-Virella *et al*. [26]. Serum LDL-C concentration was determined according to the method of Friedewald *et al*. [27] formula. Serum very LDL-C (VLDL-C) was determined according to the method of Norbert [28].

Liver and kidney functions assay

Both alanine aminotransferase (ALT) and aspartate aminotransferase (AST), activities were assayed according to the method of Reitman and Frankel [29]. Alkaline phosphatase (ALP) activity was determined according to the method described by Belfield and Goldberg [30]. Total bilirubin was assayed according to the method of Walters and Gerade [31]. Total protein (TP) was assayed in serum according to the method of Bradford [32]. Albumin (alb) was measured according to the method of Doumas *et al.* [33]. Glucose was determined in blood serum according to the method of Trinder [34]. The level of hepatic GSH was assayed in liver homogenate according to the method of Beutler *et al.* [35]. Liver malondialdehyde was estimated according to the method of Satoh [36].

Urea concentration was estimated according to the method of Fawcett and Scott [37], and serum creatinine concentration was measured according to the method of Schirmeister [38].

Estimation of serum cell adhesion molecules

Rat soluble ICAM-1 concentrations and rat soluble VCAM-1 were determined using ELISA.

Estimation of inflammatory markers TNF-α and CRP

Quantitative measurements of TNF-α, MPO, and CRP were performed by ELISA.

Estimation of antiatherogenic markers

Quantitative measurements of IL-10 were performed by ELISA.

Histopathological analysis

Parts of aorta, liver, and kidney were kept in neutral buffered neutral formalin (10%) for 8 hrs for fixation then processed in automatic processors and paraffin blocks were obtained. Sections of 3-6 µm thickness were stained using Hematoxylin and Eosin (H and E) stain to assess the cellular changes induced in the organsin all treatment modalities. The slides were examined and photographed under a light microscope at a magnification power of ×150.

Statistical analysis

Data were analyzed by comparing values for different treatment groups with the values for individual control. All data were expressed as mean±standard deviation of 15 rats in each group. Significant differences between the groups were statistically analyzed using one-way analysis of variance, co-stat software computer program. Differences were considered significant at p≤0.05.

RESULTS

Serumlipid profile

To explore the possible relationship between hypercholesterolemia andaging, we investigated 12-month-old rats that were experienced a HC induction of high-fat diet in comparison with untreated control animals. The plasma levels of TC and TL were 171.88%, 80.68% higher in the HC-induced rats as compared to old untreated control rats (Table 1). Negative control untreated old rats received both UFP extracts showed insignificant change as compared to normal control.

Analysis of plasma lipid profile revealed a significant increase of TG in HC-old rats (160.98%), LDL-C (371.92%) and VLDL-C (160.66%), while significant decrease in HDL-C (80.43%), was detected as compared to untreated old rats (Table 1).

UFP-treatment reversed the elevation of lipid profile level, while enhanced HDL-C level comparing with the diseased untreated rats as well as normal control old rats. Both groups of HC-rats responded positively to the algal treatments with a marked decrease of TC (62.49% and 64.57%), TG (51.51% and 53.39%), LDL-C (73.50% and 81.04%), and TL (42.86% and 43.75%) for both cold and hot UFPs, respectively. While, fluvastatin-treated HC rats showed mild ameliorative effects. In contrast, HDL-C was enhanced by 240.61%, 570.91% and 280.40%, respectively, for cold, hot UFPs and fluvastatin, in comparison to normal control old rats as well as untreated HC rats.

Liver enzymes activity and kidney functions

In this study, serum enzymes; ALT, AST, and ALP activities were significantly high in high-cholesterol fed diet as compared to normal old untreated rats by 38.48%, 33.56% and 110.87% (Table 2). In addition, This results revealed significant decrease in serum TP content and alb level by 14.17% and 10.42%, respectively. In contrast, glucose level showed significant increase (58%) in old-rats as compared to normal control old rats. On the other hand, negative control old rats received both UFP extracts showed insignificant change as compared to normal control (Table 3).

Moreover, high-fat diet intake caused significantly elevated serum urea and creatinine levels as compared to normal control by 102.06% and 98.7%, respectively (Table 4).

Rats treated with both cold and hot algal extracts showed decrease in enzyme activities: AST (23.34% and 22.22%), ALT (23.08% and 23.58%), and ALP (45.36% and 52.16%). While, TP content and alb level were increased by 14.68% and 17.21%, respectively, for cold UFP and 13.70% and 10.42%, respectively, for hot UFP extract. Treatment with fluvastatin, exhibited extensive reduction in AST, ALT and ALP enzyme activities (15.94%, 14.87%, and 47.94%) and lesser elevation in TP content and alb level (11.62%, 2.33%) as compared to normal control old rats as well as HC-untreated rats. HC-rats treated with cold and hot UFP aqueous extract showed a marked decrease in blood glucose level at the end of treatment by 29.32, 35.34, respectively, in comparison with fluvastatin standard drug (42.79%).

High-fat diet intake caused a significantly elevated serum levels of total urea and creatinine of HC-old rats as compared to normal controls (102.06% and 98.7%) (Table 4). Treatment of HC-rats with cold, hot UFP extracts as well as fluvastatin, declared marked reduction in total urea and creatinine levels by 54.88% and 36.60%, respectively, for cold extract, 55.37% and 36.60%, for hot extract. While treatment of HC rats with fluvastatin showed ameliorative percentages in total urea and creatinine levels reached to 45.36% and 36.60%, as compared to normal control and HC-untreated rats.

Table 1: Effects of UFP and fluvastatin supplementations on lipid profile TC, TG, LDL-C, VLDL-C, TL and HDL-C in HC-rats and different therapeutic groups

Groups	Parameters					
	TC (µg/dl)	TG (µg/dl)	LDL-C (µg/dl)	HDL-C (mg/dl)	VLDL-C	TL (mg/dl)
Negative control						
Mean±SD	63.63±10.85[a]	30.50±2.31[a]	32.23±1.25[a]	25.30±1.12[a]	6.10±0.65[a]	1087.5±33.90[a]
Negative cold extract						
Mean±SD	64.30±4.00[a]	29.40±1.75[a]	37.09±2.44[a]	21.33±1.33[c]	5.88±0.78[a]	1052.7±45.96[a]
Negative hot extract						
Mean±SD	66.73±4.00[a]	33.90±1.77[a]	31.7±2.76[a]	28.30±1.54[a]	6.80±0.99[a]	1087.7±34,88[a]
HC-rats						
Mean±SD	173.00±3.89[b]	79.60±3.86[b]	152.1±6.90[b]	4.95±0.33[b]	15.90±1.00[b]	1964.90±23.89[b]
% Change to control	171.88↑↑	160.98↑↑	371.92↑↑	−80.43↓↓	160.66↑↑	80.68↑↑
HC-cold extract						
Mean±SD	64.90±4.10[a]	38.60±1.75[a]	40.31±5.76**	16.86±0.65[c]	7.70±0.45[a]	1122.8±66.89[a]
% Change to HC	−62.49↓	−51.51↓	−73.50↓	240.61↑	−51.57↓	−42.86↓
HC-hot extract						
Mean±SD	61.30±4.23[a]	37.10±2.00[a]	20.67±1.20[c]	33.21±1.22[a]	7.42±0.55[a]	1105.3±11.23[a]
% Change to HC	−64.57↓	−53.39↓	−81.04↓	570.91↑	−53.46↓	−43.75↓
HC-fluvastatin						
Mean±SD	84.69±6.00[d]	38.20±2.12[a]	58.23±7.00[d]	18.83±0.98[c]	7.60±0.76[a]	1421.1±34.45[c]
% Change to HC	−51.05↓	−52.01↓	−61.72↓	280.40↑	−52.20↓	−27.68↓

TL: Total lipids, TG: Triglycerides, TC: Total cholesterol, LDL-C: Low density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, VLDL-C: Very low density lipoprotein cholesterol, AI: Atherogenic index, HC: Hypercholesterolemic, SD: Standard deviation. Data presented as mean±SD, n=15 for each treatment group, [a]significant at p≤0.05 to control normal rats; [b]significant to HC positive control, [c]highly significant at p≤0.001,

Table 2: Effects of UFP and fluvastatin supplementation on serum AST, ALT and ALP enzyme activities in HC-rats and different therapeutic groups

Groups	Parameters		
	AST (U/ml)	ALT (U/ml)	ALP (IU/L)
Negative control			
Mean±SD	43.32±2.56[a]	64.86±3.78[a]	46.00±2.87[a]
Negative cold extract			
Mean±SD	43.98±1.90[a]	69.75±273[a]	48.5±2.22[a]
Negative hot extract			
Mean±SD	44.43±2.87[a]	67.08±3.44[a]	42.5±1.87[a]
HC-rats			
Mean±SD	59.98±3.40[b]	86.63±2.90[b]	97.00±4.90[b]
% Change to control	38.48↑	33.56↑	110.87↑
HC-cold extract			
Mean±SD	45.98±1.23[a]	66.64±3.00[a]	53.00±5.67[a]
% Change to HC	−23.34↓	−23.08↓	−45.36↓
HC-hot extract			
Mean±SD	46.65±1.30[a]	66.20±2.45[a]	46.40±3.21[a]
% Change to HC	−22.22↓	−23.58↓	−52.16↓
HC-fluvastatin			
Mean±SD	50.42±2.45[a]	73.75±2.67[a]	50.5±2.20[a]
% Change to HC	−15.94↓	−14.87↓	−47.94↓

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, HC: Hypercholesterolemic, UFP: *Ulva fasciata* polysaccharides, SD: Standard deviation. Data presented as mean±SD, n=15 for each treatment group, [b]significant at p≤0.05 to control normal rats, [a]significant to HC positive control,

Table 3: Effects of UFP and fluvastatin supplementation on serum TP content, ALB and glucose levels of HC-rats and different therapeutic groups

Groups	Parameters		
	TP (mg)	Albumin (mg/dl)	Glucose (mg/dl)
Negative control			
Mean±SD	38.1±1.89[a]	4.80±0.25[a]	106.20±3.87[a]
Negative cold extract			
Mean±SD	38.00±1.67[a]	4.50±0.65[a]	99.40±2.99[a]
Negative hot extract			
Mean±SD	38.1±2.00[a]	5.20±0.21[a]	110.80±3.23[a]
HC-rats			
Mean±SD	32.70±1.88[b]	4.30±0.80[a]	167.80±3.88[b]
% Change to control	−14.17↓	−10.42↓	58.00↑
HC-cold extract			
Mean±SD	38.00±2.10[a]	5.04±0.23[a]	118.60±1.76[a]
% Change to HC	14.68↑	17.21↑	−29.32↓
HC-hot extract			
Mean±SD	37.5±2.22[a]	4.80±0.34[a]	108.50±2.9[a]
% Change to HC	13.70↑	10.42↑	−35.34↓
HC-fluvastatin			
Mean±SD	36.5±2.90[a]	4.40±0.22[a]	96.00±2.00[a]
% Change to HC	11.62↑	2.33↑	−42.79↓

TP: Total proteins, ALB: Albumin, SD: Standard deviation,
HC: Hypercholesterolemic, UFP: *Ulva fasciata* polysaccharides. Data presented as mean±SD, n=15 for each treatment group, [b]significant at p≤0.05 to control normal rats, [a]significant to HC positive control,

Table 4: Effect of UFP and fluvastatin supplementation, on total urea and creatinine levels in HC-rats and different therapeutic groups

Groups	Parameters	
	Urea (mg/dl)	Creatinine (mg/dl)
Negative control		
Mean±SD	34.00±1.60[a]	0.77±0.02[a]
Negative cold extract		
Mean±SD	30.00±1.00[a]	0.80±0.03[a]
Negative hot extract		
Mean±SD	31.66±1.22[a]	0.82±0.04[a]
HC-rats		
Mean±SD	68.70±2.87[a]	1.53±0.05[b]
% Change to control	102.06↑	98.7↑
HC-cold extract		
Mean±SD	31.00±2.10[a]	0.97±0.04[a]
% Change to HC	−54.88↓	−36.60↓
HC-hot extract		
Mean±SD	30.66±1.75[a]	0.97±0.03[a]
% Change to HC	−55.37↓	−36.60↓
HC-fluvastatin		
Mean±SD	37.33±2.0 0[a]	0.97±0.0 5[a]
% Change to HC	−45.66↓	−36.60↓

SD: Standard deviation, HC: Hypercholesterolemic, UFP: *Ulva fasciata* polysaccharides. Data presented as mean±SD, n=15 for each treatment group, [a]significant at p≤0.05 to control normal rats, [b]significant to HC positive control

Table 5: The antioxidant effect of UFP extracts and fluvastatin supplementation on hepatic MDA and GSH levels of HC-rats and different therapeutic groups

Groups	Parameters	
	GSH (mg/g tissue)	LPO (MDA) (nmol/g tissue)
Negative control		
Mean±SD	0.62±0.03[c]	7.10±0.44[a]
Negative cold extract		
Mean±SD	0.51±0.02[a]	6.37±0.54[a]
Negative hot extract		
Mean±SD	0.55±0.04[a]	6.18±0.88[a]
HC-rats		
Mean±SD	0.29±0.02[b]	11.17±0.90[b]
% Change to control	−53.23↓	57.32↑
HC-cold extract		
Mean±SD	0.54±0.01[a]	8.71±0.99[c]
% Change to HC	86.21↑	−22.02↓
HC-hot extract		
Mean±SD	0.50±0.05[a]	8.87±1.10[c]
% Change to HC	72.41↑	−20.59↓
HC-fluvastatin		
Mean±SD	0.41±0.02[a]	9.08±0.98[c]
% Change to HC	41.38↑	−18.71↓

LPO: Lipid peroxide, GSH: Glutathione reduced, MDA: Malondialdehyde
HC: Hypercholesterolemic, UFP: *Ulva fasciata* polysaccharides, SD: Standard deviation. Data presented as mean±SD, n=15 for each treatment group, [b]significant at p≤0.05 to control normal rats, [a]significant to HC positive control, [c]highly significant at p≤0.001,

Nonenzymatic oxidative stress markers

This results elucidated that, the old HC-control rats exhibited significant increase in LPO level, while GSH demonstrated significant decrease by 57.32% and 53.23%, respectively, as compared to normal control (Table 5). Comparing to normal control and HC-rats, algal treatments showed significant increase in GSH concentration with enhanced percentage of 86.21%, 72.41% and 41.38%, respectively, for cold, hot SP extracts and fluvastatin. On the other hand, LPO achieved marked reduction by 22.02%, 20.59% and 18.71%, respectively, for cold, hot extracts as well as fluvastatin reference drug.

Inflammatory markers

Table 6 revealed that the old HC-control rats exhibited significant increase in a the rogenic markers; CRP (41.50%), TNF-α (50.78%), MPO (15.13%) while it showed a marked decrease in the anti-inflammatory marker; IL-10 (36.43%) as compared to normal ones. On the other hand, HC-rats treated with both cold UFP, hot UFP and fluvastatin

Table 6: The anti-inflammatory effect of UFP (hot and cold extracts) and fluvastatin supplementation on serum MPO, IL-10, TNF-a and CRP levels in HC-rats and different therapeutic groups

Groups	Parameters			
	TNF-α (ρg/ml)	CRP (ηg/ml)	MPO (ρg/ml)	IL-10 (ρg/ml)
Negative control				
Mean±SD	119.24±11.9[a]	6.12±0.33[a]	121.83±9.98[a]	64.39±7.98[a]
Negative cold extract				
Mean±SD	113.80±0.20[a]	5.56±0.23[a]	123.853±8.90[a]	66.36±0.23[a]
Negative hot extract				
Mean±SD	117.27±0.19[a]	5.78±0.12[a]	125.64±5.80[a]	63.90±0.19[a]
HC-rats				
Mean±SD	179.79±14.00[b]	8.66±0.89[b]	140.26±10.54[b]	40.93±2.50[c]
% Change to control	50.78↑	41.50↑	15.13↑	−36.43↓
HC-cold extract				
Mean±SD	149.56±23.56[c]	7.12±0.67[a]	131.56±6.87[a]	51.46±5.01[c]
% Change to HC	−16.81↓	−17.78↓	−6.20↓	25.73↑
HC-hot extract				
Mean±SD	154.16±22.90[c]	7.22±0.4 3[a]	129.28±8.76[a]	51.09±7.67[c]
% Change to HC	−14.26↓	−27.21↓	−7.83↓	24.82↑
HC-fluvastatin				
Mean±SD	173.38±32.95[b]	6.92±0.01[a]	133.85±4.94[a]	49.53±2.33[b]
% Change to HC	−6.59↓	−16.32↓	−4.57↓	21.01↑

HC: Hypercholesterolemic, UFP: *Ulva fasciata* polysaccharides, SD: Standard deviation. Data presented as mean±SD, n=15 for each treatment group, [a]significant at p≤0.05 to control normal rats, [b]significant to HC positive control, [c]highly significant at p≤0.001,

Table 7: The antioxidant effect of UFP (hot and cold) extracts and fluvastatin supplementation on cell adhesion molecules in hypercholesterolemic rats and different therapeutic groups

Groups	Parameters	
	ICAM-1 (ηg/ml)	VCAM-1 (ηg/ml)
Negative control		
Mean±SD	245.97±6.90[a]	13029.62±134.90[a]
Negative cold extract		
Mean±SD	244.13±10.76[a]	12948.23±230.23[a]
Negative hot extract		
Mean±SD	247.99±16.98[a]	12832.28±155.87[a]
HC-rats		
Mean±SD	270.17±12.54[a]	15733.79±200.56[b]
% Change to control	9.83↑	20.75↑
HC-cold extract		
Mean±SD	261.69±21.93[a]	13290.95±201.78[a]
% Change to HC	−3.14↓	−15.53↓
HC-hot extract		
Mean±SD	260.72±23.76[a]	13370.78±167.89[a]
% Change to HC	−3.50↓	−15.02↓
HC-fluvastatin		
Mean±SD	267.19±11.88[a]	14668.72±222.19[b]
% Change to HC	−1.10↓	−6.77↓

VCAM-1: Vascular cellular adhesion molecule-1, ICAM-1: Intracellular adhesion molecule-1, HC: Hypercholesterolemic, UFP: *Ulva fasciata* polysaccharides, SD: Standard deviation. Data presented as mean±SD, n=15 for each treatment group, [a]significant at p≤0.05 to control normal rats, [b]significant to HC positive control, [c]highly significant at p≤0.001,

showed significant decrease in CRP (17.78%, 27.21%, and 16.32%), TNF-α (16.81%, 14.26% and 6.59%), and MPO (6.20%, 7.83% and 4.57%). On the contrary, UFP treatments elevated IL-10 in the HC-untreated old rats by 25.73%, 24.82% and 21.01 %, respectively, for cold, hot UFP and fluvastatin.

The antioxidant effect of UFP on cell adhesion molecules

Feeding of old rats with high-fat diet significantly increased both CAMs levels by 9.83% and 20.75%, respectively, for ICAM-1 and VCAM-1. By treatment, both CAMs levels were improved by 3.14%, 3.50%, and 1.10%, respectively, for ICAM-1 while VCAM-1 showed ameliorative percentages of 15.53%, 15.02%, and 6.77%, for cold, hot UFP and fluvastatin, respectively (Table 7).

Histopathological Examination of aorta,liver and kidney in different groups

Aorta aged normal control rats showed normal histological structure of the tunica intima, tunica media and tunica adventitia ,also, aorta of negative control aged rats treated with cold UFP extract of the green alga (*Ulva fasciata*) showing the normal histological structure of the tunica intima, tunica media and tunica adventitia . In addition , aorta of control aged rats treated with hot UFP extract of the green alga (*Ulva fasciata*) showing a normal histological structure (Figs. 1-3). Aorta of aged hypercholesterolemic (HC) rats showing significant atherosclerosis, vacuolation in the cells of the tunica media and marked HYPERLINK "http://en.wikipedia.org/wiki/Lumen_%28anatomy%29" \o "Lumen (anatomy)" luminal narrowing (Fig.4). Aorta of aged hypercholesterolemic rats treated with cold UFP extract of the green alga (*Ulva fasciata*) showing normal histological structure of the tunica intima, tunica media and tunica adventitia (Fig.5). Moreover, aorta of aged HC-rats treated with hot UFP extract of the green alga (*Ulva fasciata*) showing the normal histological structure of the tunica intima, tunica media and tunica adventitia (Fig.6). Furthermore, aorta of aged HC-rats treated with fluvastatin showing a perivascular hemorrhage and edema in the adventitia (Fig.7).

On the other hand , aged liver showing the architecture of a hepatic lobule. The central vein (CV) lies at the centre of the lobule surrounded by the hepatocytes (HC) with strongly eosinophilic granulated cytoplasm (CY), and distinct nuclei (N). Between the strands of hepatocytes the hepatic sinusoids are shown (HS), (Fig. 8). Aged rat liver treated with cold and hot UFP extract showing normal structure of the hepatic lobule (Figs. 9,10).Aged HC-rat liver showing fatty change of the hepatic lobule and hydropic degeneration (Fig.11). Aged HC-rat liver treated with cold UFP extract showing reduction of the fatty change as compared with the hypercholestermic one (Fig. 12). Aged HC-rat liver treated with hot UFP extract showing normal structure of the hepatic lobule (Fig.13). Finally rat liver treated with the reference drug (Fluvastatin) showing normal structure of the hepatic lobule (Fig.14).

In addition, aged kidney of control rat showing normal structure of the glomeruli and the renal tubules. Distal convoluted tubules (DCT) could be noticed and differentiated from the proximal convoluted tubules (PCT) as having larger and well defined lumina, less affinity to stain (Fig.15). Also, aged rat kidney treated with cold and hot UFP extract showing normal structure of the glomerulus and the renal tubules

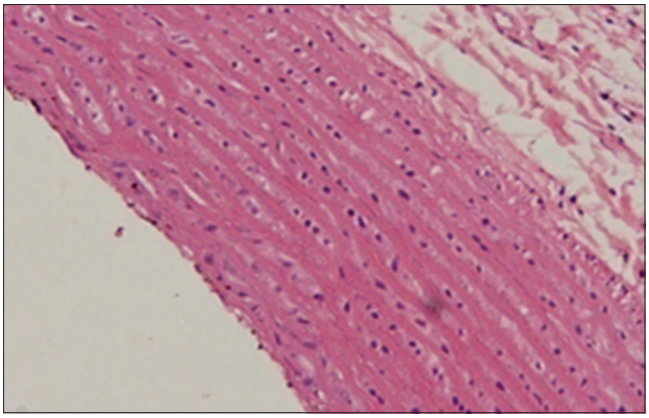

Fig. 1: Micrograph of transverse of aorta of aged normal control rats showing a normal histological structure of the tunica intima, tunica media and tunica adventitia (H and E, ×150)

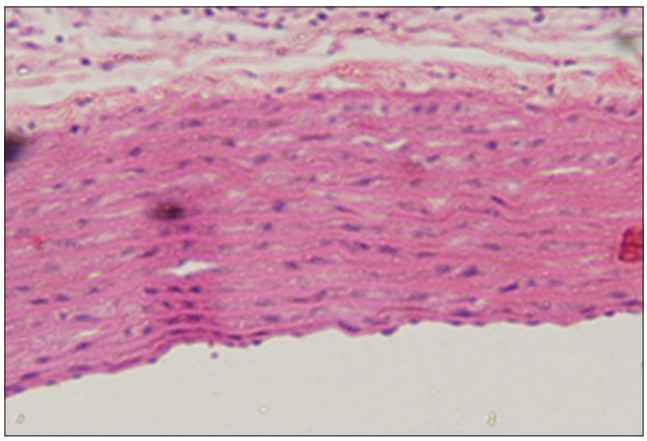

Fig. 2: Micrograph of transverse of aorta of negative control aged rats treated with cold *Ulvafasciata* polysaccharides extract of the green alga (*Ulvafasciata*) showing the normal histological structure of the tunica intima, tunica media and tunica adventitia (H and E, ×150)

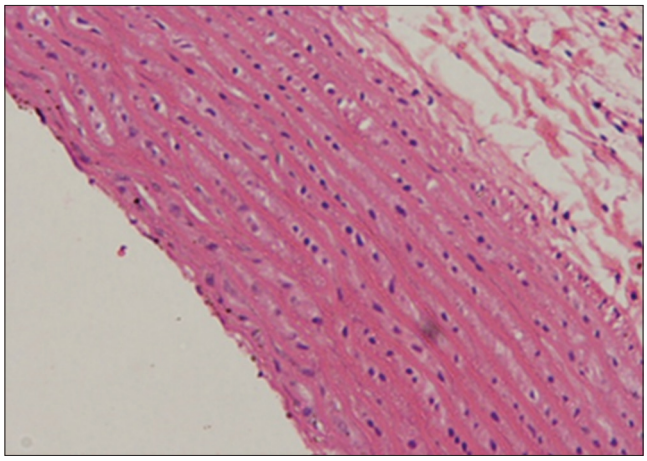

Fig. 3: Micrograph of transverse section of aorta of negative control aged rats treated with hot *Ulvafasciata* polysaccharides extract of the green alga (*Ulvafasciata*) showing a normal histological structure (H and E, ×150)

(Figs 16,17). While, aged HC-rat kidney showing hypotrophy of the glomerulus associated with wide urinary space. Note the epithelial

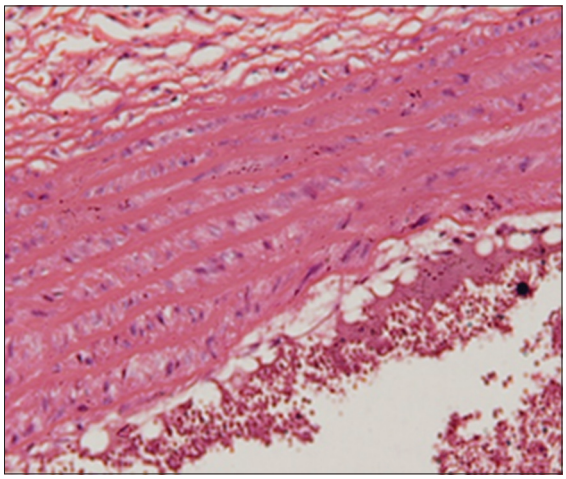

Fig. 4: Micrograph of transverse section of aorta of aged hypercholesterolemic rats showing significant atherosclerosis, vacuolation in the cells of the tunica media and marked luminal narrowing (H and E, ×150)

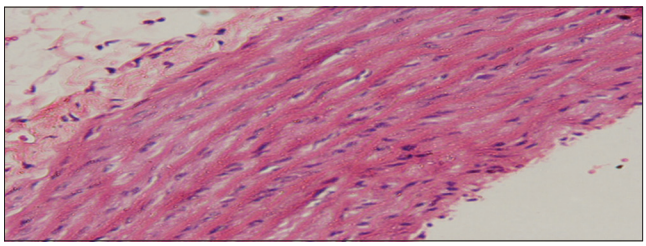

Fig. 5: Micrograph of transverse section of aorta of aged hypercholesterolemic rats treated with cold *Ulvafasciata* polysaccharides extract of the green alga (*Ulvafasciata*) showing normal histological structure of the tunica intima, tunica media and tunica adventitia (H and E, ×150)

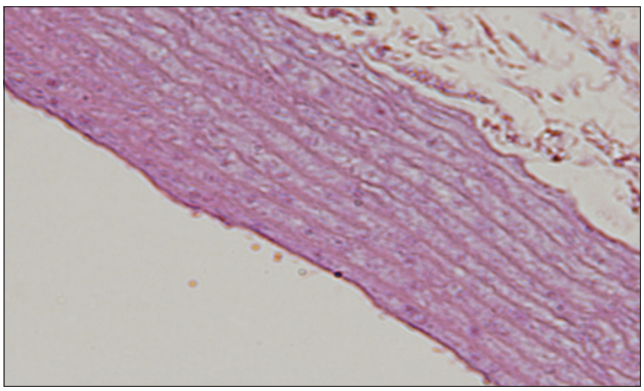

Fig. 6: Micrograph of transverse section of aorta of aged hypercholesterolemic-rats treated with hot *Ulvafasciata* polysaccharides extract of the green alga (*Ulvafasciata*) showing the normal histological structure of the tunica intima, tunica media and tunica adventitia (H and E, ×150)

detachment of the renal tubules and cellular debris in the lumen of the tubules (Fig.18). Aged HC-rat kidney treated with cold UFP extract showing normal structure of the glomerulus and the renal tubules (Fig. 19). Moreover, aged HC-rat kidney treated with hot UFP extract showing the normal structure of the glomerulus and the renal tubules (Fig.20). In addition , aged HC-rat kidney treated with the reference drug (fluvastatin) showing normal structure of the glomerulus and the renal tubules (Fig. 21).

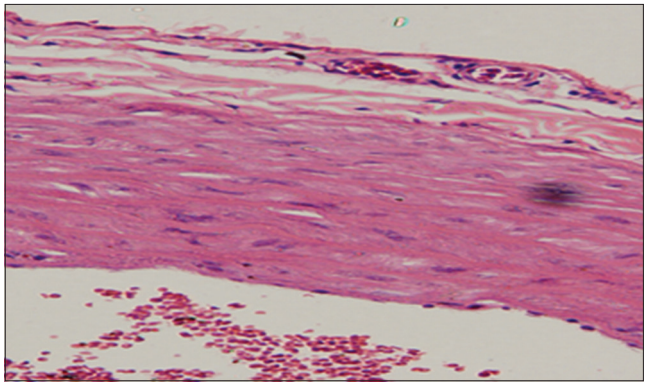

Fig. 7: Micrograph of transverse section of aorta of aged hypercholesterolemic-rats treated with fluvastatin showing a perivascular hemorrhage and edema in the adventitia (H and E, ×150)

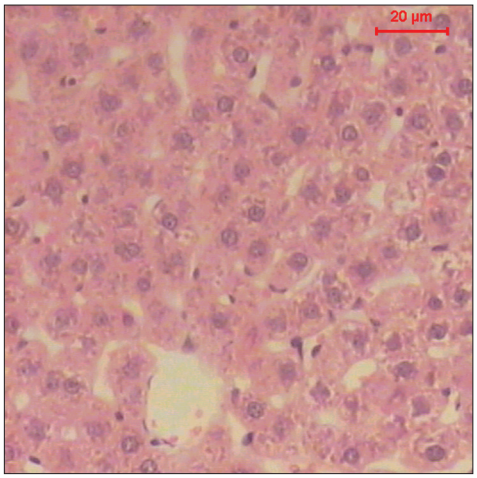

Fig. 8: Micrograph of liver of control of old rat shows normal structure of the hepatic lobule (H & E Stain).

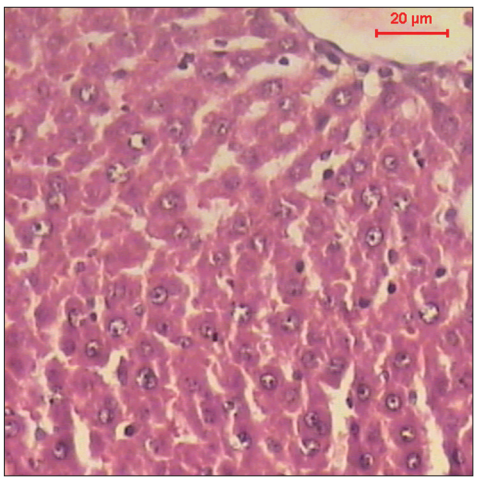

Fig. 9: Micrograph of liver of old rat treated rats with cold extract shows normal structure of the hepatic lobule (H & E Stain).

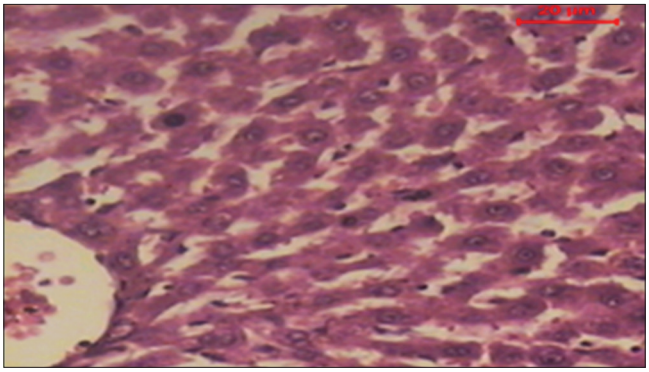

Fig. 10: Micrograph of aged rat liver treated with hot *Ulvafasciata* polysaccharides extract showing normal structure of the hepatic lobule (H and E, ×150)

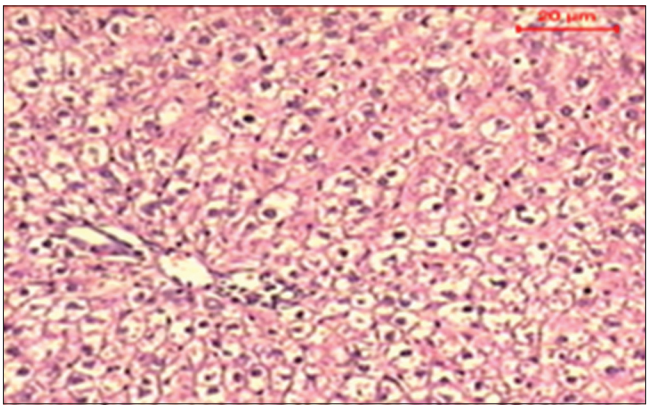

Fig. 11: Micrograph of aged hypercholesterolemic-rat liver showing fatty change of the hepatic lobule and hydropic degeneration (H and E, ×150)

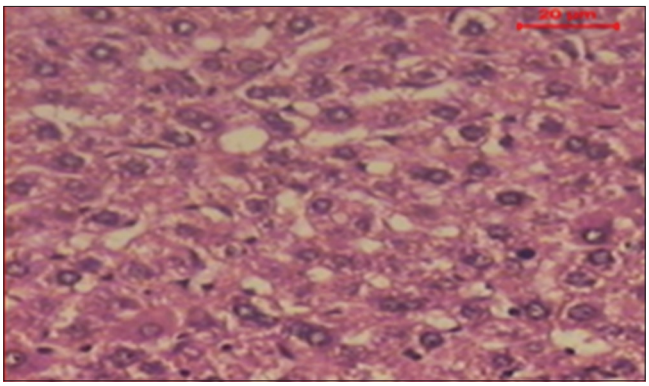

Fig. 12: Micrograph of aged hypercholesterolemic-rat liver treated with cold *Ulvafasciata* polysaccharides extract showing reduction of the fatty change as compared with the hypercholestermic one (H and E, ×150)

DISCUSSION

These results declared the biological relationship between aging and hypercholesterolemia. One of the critical aspects linked with aging is the elevation risk chronic artery disease (CAD) and, more generally, CVDs. Aging has been defined as the series of the deteriorative changes occurring during the adult period of life that underlie increased vulnerability to challenges and decreased survival [39]. This deterioration is responsible for both the commonly recognized sequential changes that accompany advancing age and the progressive increase in the chance of disease and death and is usually manifested as a progressive decrease in physiological functions.

In view of the obtained data, this study suggests that algal treatment of *U. fasciata* extract elicited anti-HC properties by significantly lowering blood cholesterol level in HC-old rats as compared to untreated ones. Algal SPs were reported to possess potent medicinal value, such as

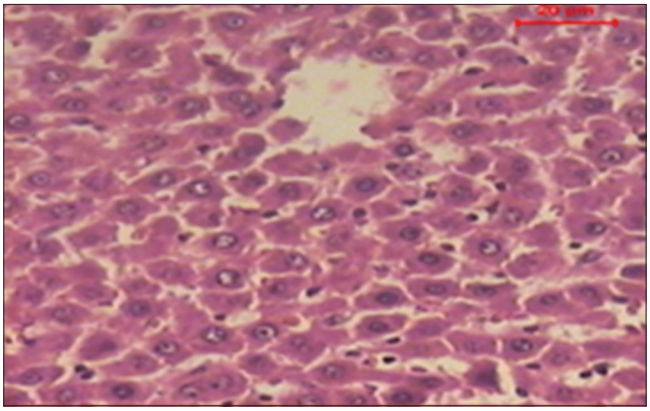

Fig. 13: Micrograph of aged hypercholesterolemic-rat liver treated with hot *Ulvafasciata* polysaccharides extract showing normal structure of the hepatic lobule (H and E, ×150)

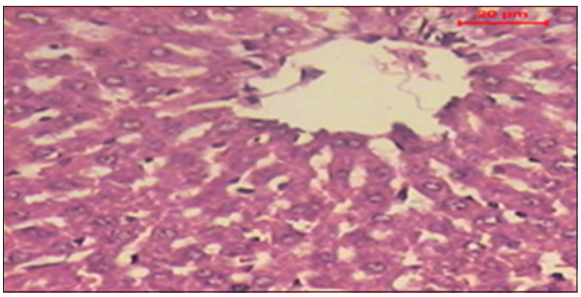

Fig. 14: Micrograph of aged hypercholesterolemic-rat liver treated with the reference drug (fluvastatin) showing normal structure of the hepatic lobule. Few fatty vacuoles are noticed (H and E, ×150)

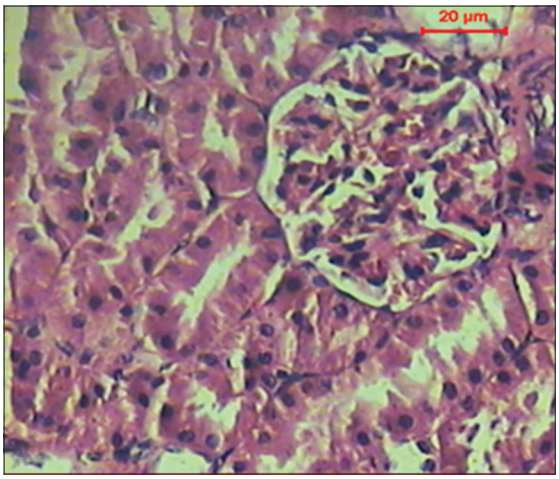

Fig. 15: Micrograph of aged kidney of control rat showing normal structure of the glomeruli and the renal tubules. Distal convoluted tubules could be noticed and differentiated from the proximal convoluted tubules as having larger and well defined lumina, less affinity to stain (H and E, ×150)

anticoagulant, antitumor, anti-inflammatory, antiviral, and antioxidant as well as antihyperlipidemic activities [17,40]. The finding is also consistent with Castro *et al.* [41] who proved that ulvan is a remarkable polysaccharide with different attractive properties that make it suitable for a wide range of applications.

The present research is investigated to demonstrate the anti-HC, antioxidative, and antiatherosclerotic activities of green alga *U. fasciata.*

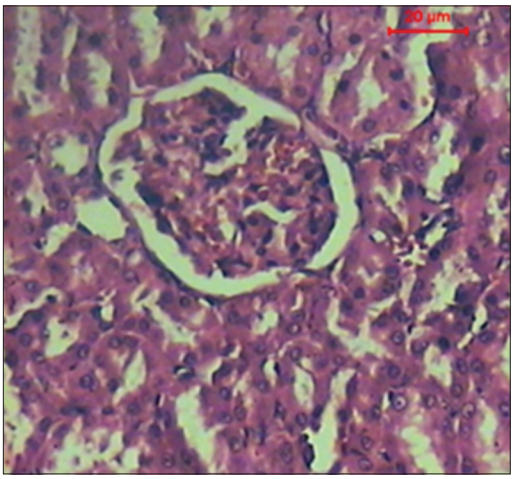

Fig. 16: Micrograph of aged rat kidney treated with cold *Ulvafasciata* polysaccharides extract showing normal structure of the glomerulus and the renal tubules (H and E, ×150)

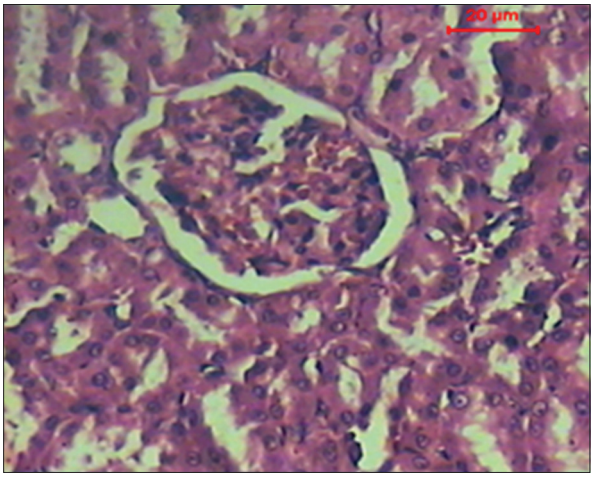

Fig. 17: Micrograph of aged rat kidney treated with hot *Ulvafasciata* polysaccharides extract showing the normal structure of the glomerulus and the renal tubules (H and E, × 150)

It was suggested aging is well-connected with growth hormone (GH), deficiency leading to aging-related hypercholesterolemia, due to GH has a powerful role in cholesterol metabolism [42].

It was found that total and LDL-C values were elevated dependant on aging, leading to an incidence of CVD. Of particular interest is the finding of during aging a gradual reduction in the LDL-C from the circulation was happed. In addition, the capacity for body cholesterol removeal though the conversion of cholesterol to bile acids is also progressively reduced with age, and a decrease in the activity of the bile acid biosynthesis, cholesterol 7α-hydroxylase enzyme activity, has been demonstrated in the aging rat [41-44].

Our results showed that after 12 weeks of feeding old rats with a high-fat diet, plasma lipid profiles showed significant increase in TC (171.88%), TLs (80.68%), TAG (160.98%), LDL-C (371.92%), VLDL-C (160.66%). On the contrary, HDL-C showed a significant decrease (80.43%), as compared to normal control rats.

Hypercholesterolemia plays a critical role in atherosclerosis and there is strong incidence that the production of ROS causes endothelial cell injury, which in turn trigger the first step in atherosclerosis [45]. Stalenhoef and DeGraaf declared that the high level of serum TGs is an insignificantly risk biomarker for coronary heart disease [46].

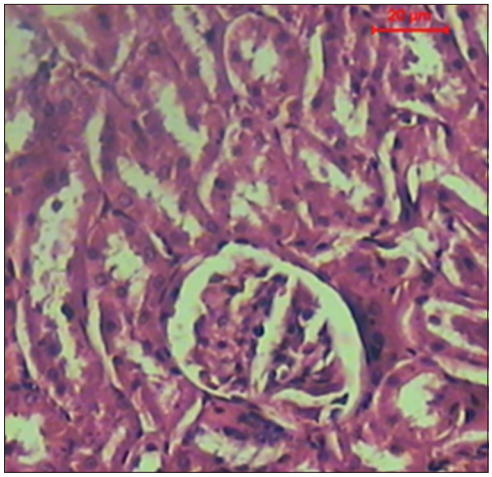

Fig. 18: Micrograph of aged hypercholesterolemic-rat kidney showing hypotrophy of the glomerulus associated with wide urinary space. Note the epithelial detachment of the renal tubules and cellular debris in the lumen of the tubules (H and E, ×150)

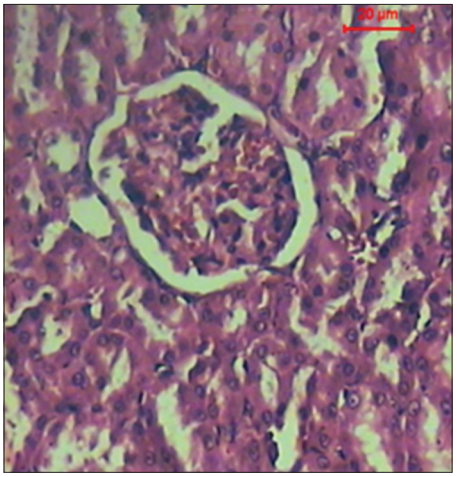

Fig. 20: Micrograph of aged hypercholesterolemic-rat kidney treated with hot Ulvafasciata polysaccharides extract showing the normal structure of the glomerulus and the renal tubules (H and E, ×150)

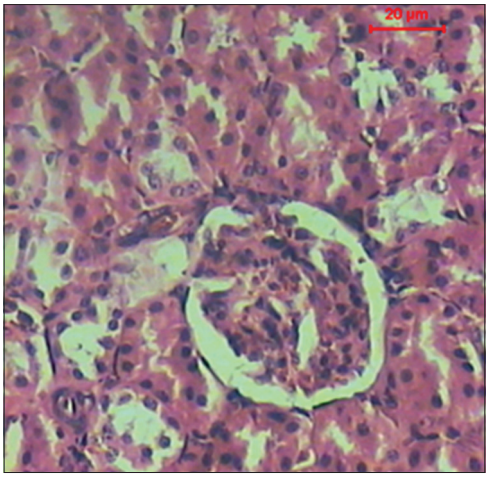

Fig. 19: Micrograph of aged hypercholesterolemic-rat kidney treated with cold *Ulvafasciata* polysaccharides extract showing normal structure of the glomerulus and the renal tubules (H and E, ×150)

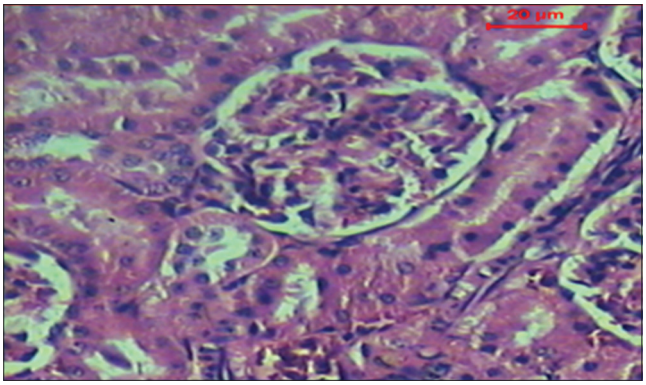

Fig. 21: Micrograph of aged hypercholesterolemic-rat kidney treated with the reference drug (fluvastatin) showing normal structure of the glomerulus and the renal tubules (H and E, ×150)

As compared to normal control old rats and positive control untreated ones, the algal supplementation to atherogenic old rats improved notably their lipid profile by induction of marked significant decrease of serum TLs, TC, triacylglycerols and LDL-C concentrations and elevated HDL-C level which may be due to the ability of the extract to hasten the decomposition of free radical species generated during cholesterol administration; this corroborates the report of a previous study by Godard *et al*. [47].

Qi *et al*. [17] reported that polysaccharides are considered as bile acid synthesis stimulator. Bile acids are reabsorbed in the small intestine and return to the liver so that the bile acid pool remains essentially constant. Bile acid interrupting the enterohepatic circulation and increasing the fecal excretion of bile acids so that fewer bile acids return to the liver.

Concerning the liver functionality, the present study revealed that serum ALT, AST, and ALP enzyme activities showed significant surge by 38.48%, 33.56%, 110.87%, respectively, in old HC-rats as compared to normal rats. Under pathological conditions of liver, there is a leak of these enzymes into the circulation, thus raising their activities [48]. The elevated enzyme activities in serum of HC- rats reflect the alterations in serum membrane integrity and/or permeability [49]. In consistent with the present results, Sudhahar *et al*. [50] and Kim *et al*. [51], found that; the activities of ALT and AST tend to increase according to the exogenous cholesterol contents from diet and that hypercholesterolemia state significantly stimulate ALT and AST enzyme activities in the serum.

The elevated liver function enzyme activities in aged HC rats were significantly reduced in response to treatment with both cold and hot algal SP extracts as compared to corresponding values in control old and HC rats. This state of declined enzyme activities secondary to drug and the algal treatments may be attributed to their ability to maintain membrane integrity thereby restricting the leakage of these enzymes and thus leading to decrease of enzyme activities in serum.

On the other hand, serum TP and alb showed significant decrease by 14.17 and 10.42%. These results run in parallel with Awada *et al*. [52], who observed that serum TP content and alb level were significantly decreased in HC-rats, and attributed these reductions to increase in protein catabolism and increase in alb excretion in urine. While, serum glucose showed a significant increase (58%) in old HC-control rats as compared to normal rats that prove the link existing between hyperglycemia and hypercholesterolemia in addition to hypertriglyceridemic.

By treatment of HC rats TP content and alb level were restored near to their normal values, while glucose showed a marked decrease at the

end of treatment; this glucose-lowering effect of UFP may be attributed to gluconeogenesis and the regulation of serum lipid levels [53].

High-fat diet elevated the excretion of renal dysfunction such as urea, creatinine in HC-rats comparing to normal control, the aqueous extracts of *U. fasciata* reduced their levels, this runs in agreement with Herreo and his colleagues [54]. This enhancement in kidney function implies that sulfated polysaccharides of UFP might interfere with creatinine metabolism and eventually excreted it from the blood.

Oxidative stress is considered as a critical mechanism of nonalcoholic fatty liver disease, [55]. Hypercholesterolemia-induced oxidative stress resulted in elevated hepatic LPO and decreased GSH level, the nonenzymatic antioxidant defense system.

Administration of algal polysaccharides was found to enhanced the activity of the nonenzymatic antioxidant defense system. Zhang *et al*. [56] declared administration of polysaccharide isolated from the alga *Porphyrahaitanesis* caused elevation of an antioxidant level in aged mice. These results postulated that the high activity of antioxidant is associated with the high sulfate content of ulvan. This runs in parallel with the finding of Qi *et al*. [57] who demonstrated the antioxidant activity of polysaccharides isolated from the green alga *Ulvapertusa in vitro* and they noticed that strong scavenging activity against hydroxyl radical, reducing power and chelating ability.

The present study reveals also that, old HC-rats, exhibited strong activation of TNF-α, MPO, and CRP, while suppression in IL-10 level, as they all implicated in pathophysiological alterations, which may become crucial mediators accelerating the progression of inflammatory disease during hyperlipidemia. In good agreement with the present finding Sun *et al*. [58], found that significant elevation in CRP level in hyperlipidemic rats and this elevation is tidily linked to concern of atherosclerosis.

As compared to atherogenic rats, treatment of the old HC-rats with both algal extracts showed significant decrease in atherogenic inflammatory markers CRP, MPO, and TNF-α and a marked increase in IL-10, that might be attributed to the direct inhibition of CRP expression, MPO and TNF-α, while exert activation of anti-inflammatory cytokine. This may be attributed to reduction in pro-inflammatory markers of endothelial function, suggesting an attenuation of endothelial activation and improvement in endothelial function. This may lead to a reduction in the progression of atherosclerosis and local production of the cytokines by inflammatory cells that have accumulated [59]. In addition, UFP extracts significantly lowered serum TNF-α level in HC rats independent of its metabolic actions, which may at least partly be due to direct inhibition of TNF-α expression and secretion of adipocytes [59].

Induced hypercholesterolemia in old rats led to over-induction of ICAM-1and VCAM-1. The high glucose, and oxidized LDL-C levels, are the contributor factors oxidative stress, and the ignition of ICAM-1 and VCAM-1. Both CAMs caused monocytes activation, and moving to sub-endothelial layer leading to promotion of vascular diseases [60].

It could be concluded that algal extracts of *U. fasciata* were noticed to suppress VCAM-1/ICAM-1 which is considered as a therapeutic agent against CVDs. The observed decrease in the number of adhesion molecules may be also attributed to anti-oxidative effects of UFPs that decrease the oxidation of LDL-C to ox-LDL-C.

Histopathological investigations of aorta revealed that normal amount of collagen fibers and connective tissues are exhibited in the tunica adventitia of the aortic tissue of normal rats. Most of the medial smooth muscle cells (SMCs) of the tunica media oriented horizontally to the aortic canal. The aorta of cholesterol-stressed old rats demonstrated, multifocal degeneration, necrosis, disorientation of SMC and irregularity of the wall of the aorta with increased of wall thickness, loss of normal corrugation and discontinuity of endothelium and desquamation in the lining endothelium and hemorrhage in perivascular tissue with vacuolation in the cells of the tunica media, and minor increase in the thickness of aorta wall, these findings are in concomitant with Kamesh and Sumathi [61].

In addition, hepatocytes of negative control rat liver were arranged with well-distinct cytoplasm and nuclei, in which sinusoids radiated from the central vein, this observation was agreed with Posuwan *et al.* [62]. In contrast, the present histopathological examination of liver sections of HC-rats showed cytoplasmic vacuolization and fatty changes of hepatocytes which run in parallel with Rezq and El-Khamisy [63]. On the other hand, hepatic cells of HC-rats treated with both algal extracts were improved with fewer endothelium injuries and less fat vacuoles, showed considerable reduction in the pathological changes and exhibited an almost normal figure as the control, and the hydropic degeneration of the hepatocyte disappeared. The circular fat droplets in the cytoplasm decreased significantly, and only minor inflammatory cell infiltration was observed in portal areas fluvastatin treatment showed liver recovery included decrease signs of fatty liver with fewer fatty vacuoles, with fewer endothelium injuries.

Moreover, histopathological observation of normal control kidney revealed, normal histology of the glomerulus, well-spaced tubules and normal orientation of nephrons with adequate glomeruli, similar to nephro-histological data presented by Jouyban *et al.* [64]. Light microscopy observations of old HC-rats demonstrated, mild glomerular injury with mild vascular and inflammatory changes, signs of moderate vascular congestion, mesangial hyperplasia and dilatation of vascular lumen with no evidence of fat deposits, this proves that hypercholesterolemia induces glomerular injury. Treatment of HC-rats with algal extracts and fluvastatin showed, milder tubular injury, no glomerular or tubular alterations, no basement membrane thickening and fibrosis were discerned. Furthermore, normal histology of the glomerulus with well-spaced tubules, no congestion and no inflammation were detected.

The current results proved that hot UFP exhibited stronger antihyperlipidemic and anti-oxidative activities than cold UFP. Cold and hot UFPs used had strong hypolipidemic, hypotriglyceridemic and HC effects in serum of old cholesterol-stressed rats, inducing a reduction in LDL-C levels and an increase in HDL-C levels. Lipid peroxidation, a degenerative pathway of the membrane components mediated through the free radicals produced in the cell, is a hallmark feature of oxidative stress. In this study, there is an upsurge in lipid peroxidation in liver due to dietary conditions and that was clearly demonstrated in the hepatic histopathology in addition to that of kidney, this state of oxidative stress further decreased with supplementation of algal UFP extracts, that proves their antioxidant activity. Thus, understanding age-related physiological deterioration and the mechanisms driving increased cellular and plasma cholesterol content during aging is essential in defining specific intervention points.

CONCLUSION

It could be concluded that, in comparison with the standard anti-HC drug (fluvastatin) used in this study, both cold and hot UFP algal extracts of *U. fasciata* demonstrated appreciable antihypercholesterolemic property, in addition to their antioxidant activity even in the old HC-stressed rats. Thus, it could be used as a natural lipid regulator. Further studies are therefore in progress to identify the mechanism of the hypolipidemic effects of *U. fasciata* and to be elucidated at the molecular and cellular levels, in particular, by further determining the effects of SP on HMG-CoA reductase.

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