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Original article

Antihyperlipidemic and Antioxidant Effects of Ethanol Fraction of *Sargassum angustifolium* in Dexamethasone-Induced Dyslipidemic Rats

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Abstract

Background and objectives: Recent data propose the beneficial antihyperlipidemic effects of several marine brown alga belonging to the genus *Sargassum*. In the current study, the effects of ethanol fraction of *Sargassum angustifolium* were assessed on dexamethasone-induced dyslipidemia in rats. **Methods:** The fraction was prepared by maceration method and then using a reverse phase column chromatography. It was evaluated for total phenolic and salt contents. Seven groups of six male rats were used as the following: group 1 (normal control) received vehicle for 1 week; group 2 (*Sargassum* control) was treated only with 80 mg/kg *S. angustifolium* for one week; group 3 (dyslipidemic control) received dexamethasone (10 mg/kg/day, subcutaneously) for one week; groups 4-6 (test groups) received dexamethasone and were simultaneously treated orally with 20, 40 or 80 mg/kg *S. angustifolium* and group 7 (reference) received dexamethasone and atorvastatin (40 mg/kg, orally) for one week. At the end of experiment, fasting blood glucose, lipid markers and malondialdehyde levels were evaluated in serum specimens. Livers were weighed and processed for histopathological inspection. **Results:** The content of total phenolics was 87.21 ± 2.4 mg/g as gallic acid equivalent and salt as NaCl was 6.5 g/100 g. Treatment with *S. angustifolium* significantly decreased serum blood sugar, triglycerides, total cholesterol, low-density lipoprotein-cholesterol and malondialdehyde levels and also alleviated steatotic changes in liver tissues compared to the dexamethasone-induced dyslipidemic control group. **Conclusion:** Findings of the current study revealed anti-hyperglycemic, hypolipidemic and anti-lipid proxidative properties of *S. angustifolium* ethanol fraction in an animal model of dyslipidemia.

Keywords: dexamethasone; hyperlipidemias; lipid peroxidation; rats; *Sargassum*

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Introduction

Hyperlipidemia is an acquired or genetic medical condition, which is elucidated by an increase in one or more of the plasma levels of lipids [1]. Abnormal high levels of plasma lipids concomitant with low levels of high density lipoprotein (HDL)-cholesterol are the popular risk factors for the development of diseases such as atherosclerosis and cardiovascular disorders [2]. Moreover, hyperlipidemia is related with increased oxidative stress and decreased antioxidant status which may lead to the pathogenic progress of this disorder and its complications [3]. The major therapeutic interventions for hyperlipidemia include lipid lowering drugs, such as fibrates and statins but these synthetic drugs may be associated with

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severe adverse effects such as toxicity in the liver and muscles in long time usage [4]. Recently, alternative natural products have been considered for developing new hypolipidemic drugs with fewer or no side effect [4-6].

Marine habitat is an important source of biologically active metabolites. Marine compounds are different from terrestrial natural metabolites due to the unique physical and chemical conditions in the sea substrates [7]. Various potent metabolites with characteristic chemical structures have been identified in marine organisms such as sponges, fungi, corals, seaweeds and ascidians [8]. About 2400 natural bioactive components have been identified only in seaweeds from different parts of seas and oceans [9]. Algae are important sources of compounds with diverse structures and bioactivities. They are divided into three phyla including Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae) [10]. *Sargassum* is a genus with approximately 300 species in Sargassaceae family and is geographically widespread in all tropical and temperate oceans. Recent investigations have shown that some species of *Sargassum* have beneficial cardiovascular properties including lipid-lowering effects. These species have been able to reduce energy intake, improve insulin sensitivity and lipid profile and as a result reduce adipose tissue content in laboratory animals [11,12]. The antihyperlipidemic activities of seaweed extracts are related to their various active components [13,14]. Latest data have shown that there are more than 300 species of marine algae in the coastal areas of Iran [15]. However, only a few studies have been done for the assessment of hypolidimedic properties of the marine algae in this region especially *Sargassum* species. In this study, the effect of ethanol fraction of brown seaweed *Sargassum angustifolium* was evaluated in dexamethasone-induced dyslipidemia in rats.

Materials and Methods

Ethical considerations

The study was approved by the Institutional Research Ethics Committee of Isfahan University of Medical Sciences with ethic approval ID: IR.MUI.RESEARCH.REC.1399.367. The animal experimental practice was carried out in accordance with the international guidelines for laboratory animal use and care (European Directive 2010/63/EU) [16].

Chemicals

Dexamethasone was purchased from Darou Pakhsh Pharmaceutical Co. (Iran). Atorvastatin was obtained from Tehran Chemie Pharm Co. (Iran). The assay kits for evaluation of serum triglycerides, total cholesterol, high-density lipoprotein-cholesterol (HDL), very low-density lipoprotein-cholesterol (VLDL), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were prepared from Pars Azmoon Co. (Iran). The kit for malondialdehyde (MDA) assay was purchased from Hakiman Shargh Research Co. (Iran). All other chemicals including Folin-Ciocalteu reagent were obtained from Merck Co. (Germany).

Plant metarial

The seaweeds were collected in Oct 2019 from the Persian Gulf coasts of Iran close to Bushehr Province. They were identified as *Sargassum angustifolium* C.Agardh by Agricultural and Natural Resources Research Center of Bushehr and the voucher specimens was coded as 2662 and deposited at the Herbarium of the School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences (Isfahan, Iran).

Based on the reports for high total phenolics content [17] and also for the low salt content, the ethanol fraction was selected.

To prepare the ethanol fraction of *S. angustifolium*, the algae were entirely washed with running tap water and air-dried at room temperature in shade. Then powdered algae (2000 g) was exhaustively extracted with 70% ethanol (10 L) for 72 h at room temperature, using maceration method for three times. After that, the hydroalcoholic extract was rinsed with water followed by ethanol through a reverse phase column chromatography for separation of the salts as the highly polar compounds that dissolve in water from the total extract. The aqueous phase was discarded and the ethanol phase was collected and concentrated under vacuum by rotary evaporator [18]. The ethanol fraction was kept in the refrigerator for further tests. The fraction was dissolved in water for oral gavage in the animals.

Total phenolics assay

Total phenolics content was measured using

Folin-Ciocalteau method [17]. The reagent was diluted and mixed with samples of *S. angustifolium* ethanol fraction then left for 5 minutes. After that, a solution of 20% sodium carbonate was added to the mixture and again left for 2 hours at room temperature. Finally, the absorbance of mixture was measured at 765 nm by a UV-visible spectrophotometer (Bio-Tek, PowerWave XS, USA). The content of total phenolic components was determined by a standard curve obtained from various concentrations of gallic acid (0-5500 µg/mL) and stated as milligram of gallic acid equivalents (GAE) per gram of the dried *S. angustifolium* fraction.

Salt content assay

The content of salt as sodium chloride was evaluated by Mohr method based on the silver nitrate and potassium chromate titration of the chloride ion in samples of *S. angustifolium* ethanol fraction [19].

Animals

Wistar male rats, 200-220 g were supplied by animal house of the School of Pharmacy and Pharmaceutical Sciences (Isfahan, Iran). The animals were housed in standard polypropylene cages under regular laboratory settings of temperature and humidity, with a 12 h light-dark cycle. They were allowed to access a standard rat diet and water ad libitum. Rats were adapted to the laboratory condition one week before the tests.

Induction of dyslipidemia

Dexamethasone as a potent glucocorticoid was used for induction of dyslipidemia in rats. It was injected subcutaneously (s.c.) at a dose of 10 mg/kg/day for 7 days [20]. The animals' body weights were recorded regularly. Food intake was measured during the period of test based on the remaining chow after a 24 h period which was normalized to the total body weights from each cage.

At the end of the experimental period, the blood samples of overnight fasted rats were collected from orbital sinus plexus with heparinized capillary tubes under anesthesia by intraperitoneally injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The serum was taken for evaluation of biochemical and antioxidant markers. Finally, the animals were euthanized with exposure to carbon dioxide and the livers were excised and weighed. The livers were immersed in 10% formalin and examined histopathologically after processing.

Experimental groups

Randomization was done by using a random number table in which all animals had an equal and independent chance of being selected for the sample group. Forty-two rats that were comparable in age and weight were randomly divided to seven experimental groups of 6 rats each as follows:

Group 1: Normal control, received vehicle (normal saline) orally and s.c. for one week.

Group 2: *Sargassum* control, received *S. angustifolium* (80 mg/kg) by oral gavage daily for one week.

Group 3: Dexamethasone-induced dyslipidemia control, received dexamethasone (10 mg/kg, s.c.) daily for one week.

Groups 4, 5 & 6: Test groups received ethanol fraction of *S. angustifolium* (20, 40 and 80 mg/kg) orally and were simultaneously given dexamethasone for one week [21].

Group 7: Reference group, received atorvastatin (40 mg/kg, orally) and simultaneously dexamethasone for one week [22].

Biochemical assays

Serum lipid profile including total cholesterol, HDL, triglycerides, ALT and AST were assessed enzymatically by the standard respective kits according to the manufacturer's instructions.

The intra-assay coefficients of variation were 0.95%, 0.78%, 1.60%, 2.0% and 2.54% for total cholesterol, HDL, triglycerides, ALT and AST, respectively. The inter-assay coefficients of variation were 1.09%, 1.8%, 1.23%, 2.01% and 3.61% for total cholesterol, HDL, triglycerides, ALT and AST, respectively. The assay sensitivities of kits were 5 mg/dL, 1 mg/dL, 5 mg/dL, 4 IU/L, and 2 IU/L for total cholesterol, HDL, triglycerides, ALT and AST, respectively.

Low-density lipoprotein (LDL)-cholesterol was estimated by the formula LDL = (total cholesterol) - (HDL) - (triglycerides/5)

and VLDL-cholesterol was calculated through dividing the triglyceride value by 5. The blood glucose level was measured based on glucose oxidase method. The intra- and inter-assay coefficients of variation were 1.50% and 0.90% for glucose and the kit had assay sensitivity of 5 mg/dL.

Malondialdehyde (MDA) assay

Serum lipid peroxidation was evaluated through determination of MDA concentration. In this assay, a colorimetric product is produced from the reaction of MDA with thiobarbituric acid (TBA). Briefly, serum sample was vortex mixed with trichloroacetic acid (20%) and the precipitate was dispersed in H_2SO_4. Then TBA solution (0.67%) in Na_2SO_4 was added to the mixture and heated in a boiling water bath for 1 hour. After fast cooling, each sample was mixed with n-butanol and absorbance of the pink colored product was determined at 532 nm by a spectrophotometer and expressed as MDA equivalents in nmol/mL. Calibration curve was plotted using different concentrations of MDA tetrabutyl ammonium.

Histopathological examination

Histological examination was performed using routine methods. The right liver lobes were washed instantly with saline and then fixed in 10% buffered neutral formalin solution for 24 h. The samples were dehydrated with gradient series of ethanol and then embedded in paraffin blocks. The blocks were cut into 5 μm thickness sections and 3 serial sections were stained with hematoxylin and eosin (H&E). The sections were briefly deparaffinized, re-hydrated, stained in hematoxylin, counterstained in eosin solution, dehydrated and finally mounted with mounting medium. The slides were examined microscopically for histopathological alterations.

Statistical analysis

Data were presented as mean±standard error of mean (SEM) and analyzed using one-way analysis of variance (ANOVA) in the statistical package for the social sciences (SPSS software version 25.0). The normality of the distribution of the variables was checked using Kolmogorov-Smirnov test. Levene's test was used to verify homogeneity of variance components between experimental treatments. Tukey post-hoc test was done to compare the differences between the means at 5% probability. P value less than 0.05 reflected the significance level.

Results and Discussion

In this investigation, the yield of *S. angustifolium* ethanol fraction was 7.48 % (w/w). Folin-Ciocalteu assay showed total phenolics components as 87.21 ± 2.4 mg GAE in 1 g of dried ethanol fraction of *S. angustifolium* which proposes this alga as a rich source of antioxidants. In a recent study by Farvin et al, evaluation of aqueous, hydroalcoholic and ethanol extracts of various species of *Sargassum* revealed significant variation in total phenolics content and antioxidant properties. They reported lowest amount as 22.9 ± 5.0 and the highest value of total phenolics content as 138.9±12.6 mg GAE/g in water and ethanol extracts of *S. angustifolium*, respectively [17]. The amounts of phenolic compound in the extracts may be affected by many factors such as geographical places, harvesting time, process of preparation and extraction methods [23].

The amount of salt as NaCl was 6.5 g/100 g in dried ethanol fraction of *S. angustifolium*. It has been proposed that seaweeds could be used as the substitute for salt in diet due to the natural salty taste and containing high amount of healthy mineral salts such as potassium and magnesium, however low amounts of seaweed must be consumed as food for preventing unwarranted salt intake [24].

In the present study, dexamethasone-induced dyslipidemia was used as an animal model for estimation of potential effects of *S. angustifolium* ethanol fraction on glucose and lipid profile. After one week exposure of rats to dexamethasone (10 mg/kg, s.c.), significant increase in serum fasting blood glucose (p=0.043), triglyceride (p=0.000), total cholesterol (p=0.014), LDL (p=0.000) and VLDL (p=0.035) levels and a notable decrease in HDL (p=0.006) level was observed compared to the normal control group (Figures 1-6).

Excessive and long-term administration of glucocorticoids is known to affect synthesis and clearance of plasma lipoproteins and also apolipoprotein genes and lipoprotein receptor genes [25]. Glucocorticoid excess can evoke plasma lipids and glucose elevation and lead to metabolic syndrome through inhibition of intravascular lipolysis of triglycerides, promotion of adipose tissue lipolysis, enhancing hepatic fatty acid synthesis, inducing hyperinsulinemia and insulin antagonism in peripheral tissues. Fatty liver happens because of marked rises in the uptake and synthesis of free fatty acids and increased activity of hepatic lipogenesis enzymes, and inadequate synthesis of triacylglycerol [20,26]. Increased VLDL secretion from the liver. Decreased lipoprotein catabolism due to the declining in lecithin cholesterol acetyl transferase

(LCAT) activity and down regulation of LDL receptor are also contributed in elevated plasma LDL and total cholesterol levels during corticosteroid therapy [27].

Figures 1 to 6 show the effect of treatment with *S. angustifolium* (20, 40 and 80 mg/kg) and atorvastatin (40 mg/kg) on biochemical markers in dexamethasone-induced dyslipidemic rats. Atorvastatin as a reference hypolipidemic drug resulted in a notable decrease in serum triglycerides (47.2%, p=0.000), total cholesterol (31.2%, p=0.037) and LDL level (61%, p=0.000) without any change in VLDL (p=0.079) and fasting blood glucose levels (p=0.981). It also increased the HDL level (51.7%, p=0.001) compared to the dyslipidemic control rats. Treatment with *S. angustifolium* significantly reduced fasting blood sugar and atherogenic lipid markers. There was a reduction of 30.7% in blood glucose (p=0.000), 30.9% in triglycerides (p=0.027), 50.2% in total cholesterol (p=0.000) and 59.5% in LDL level (p=0.000) after one week administration of *S. angustifolium* extract at the dose of 80 mg/kg in rats. However, no significant effect was observed on HDL (p=0.166) and VLDL (p=0.096) levels.

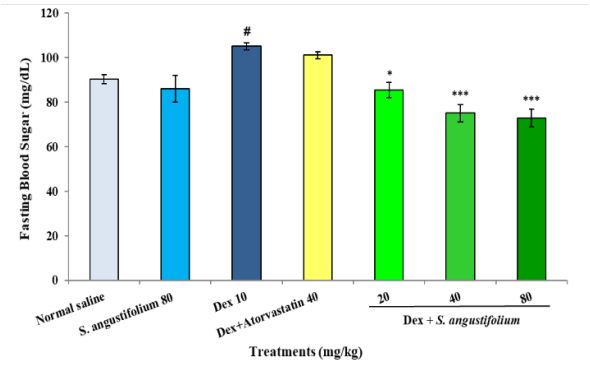

Figure 1. Effects of *Sargassum angustifolium* extract (20, 40 and 80 mg/kg) and atorvastatin (40 mg/kg) on fasting blood sugar in dexamethasone (Dex)-induced dyslipidemia. Values are means ± SEM (n=6). $^{\#}$p<0.05 versus normal control, and *p<0.05, **p<0.01 and ***p<0.001 versus Dex

Recent investigations have shown that some species of the *Sargassum* genus have beneficial cardiovascular properties, including lipid-lowering effects. In the study of Ahmed et al., administration of methanolic extract of *S. subrepandum* in atherogenic diet-induced hyperlipidemic rats has been associated with antihyperlipidemic activity through reduction of plasma cholesterol (38.2%), triglycerides (21.7%), LDL (35.4%), and increase in HDL (50.9%) level.

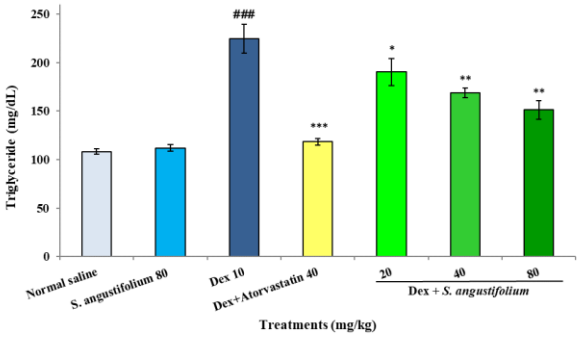

Figure 2. Effects of *Sargassum angustifolium* extract (20, 40 and 80 mg/kg) and atorvastatin (40 mg/kg) on serum triglycerides level in dexamethasone (Dex)-induced dyslipidemia. Values are means ± SEM (n=6). ###p<0.001 versus normal control, and *p<0.05, **p<0.01 and ***p<0.001 versus Dex

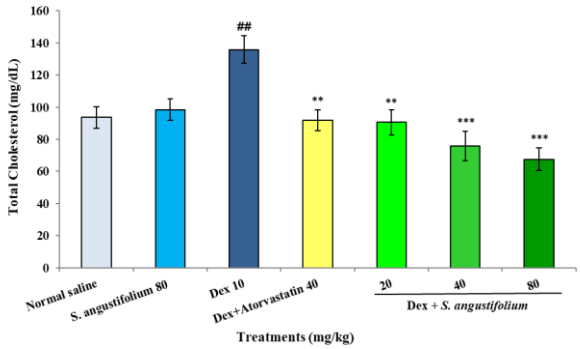

Figure 3. Effects of *Sargassum angustifolium* extract (20, 40 and 80 mg/kg) and atorvastatin (40 mg/kg) on serum total cholesterol level in dexamethasone (Dex)-induced dyslipidemia. Values are means±SEM (n=6). $^{\#\#}$p<0.01 versus normal control, and **p<0.01 and ***p<0.001 versus Dex

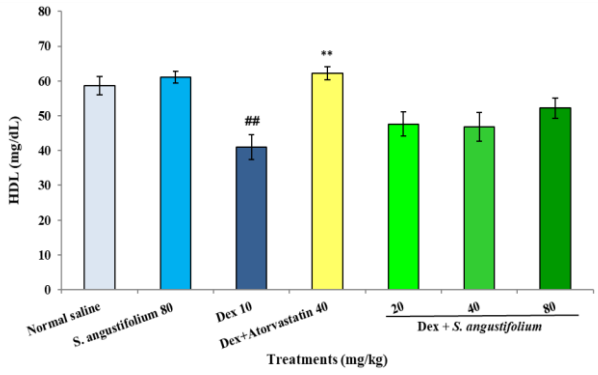

Figure 4. Effects of *Sargassum angustifolium* extract (20, 40 and 80 mg/kg) and atorvastatin (40 mg/kg) on serum HDL level in dexamethasone (Dex)-induced dyslipidemia. Values are means±SEM (n=6). $^{\#\#}$p<0.01 versus normal control, and **p<0.01 versus Dex

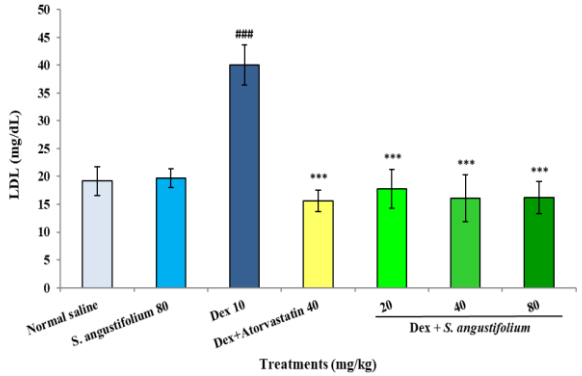

Figure 5. Effects of *Sargassum angustifolium* extract (20, 40 and 80 mg/kg) and atorvastatin (40 mg/kg) on serum LDL level in dexamethasone (Dex)-induced dyslipidemia. Values are means ± SEM (n=6). ###p<0.001 versus normal control, and ***p<0.001 versus Dex

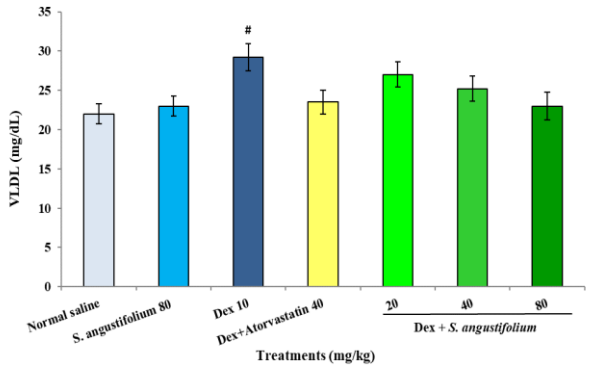

Figure 6. Effects of *Sargassum angustifolium* extract (20, 40 and 80 mg/kg) and atorvastatin (40 mg/kg) on serum VLDL level in dexamethasone (Dex)-induced dyslipidemia. Values are means ± SEM (n=6). #p<0.05 versus normal control

Sargassum subrepandum extract was able to decrease leptin concentration and increase serum adiponectin level and also alleviate inflammation through reducing nitric oxide and tumor necrosis factor (TNF)-alpha levels [28]. In another study by Yu et al., adding brown algae *S. fusiforme* to rats' diet significantly lowered blood lipids and activities of lipoprotein lipase, hepatic lipase and hepatic hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase in rats with hyperlipidemia [29]. Kwon and coworkers showed the anti-obesity property of *S. horneri* ethanol extract by regulating the expression of adipogenic transcription factors such as peroxisome proliferator-activated receptor (PPAR)-γ and inhibiting the differentiation of adipocytes [30]. Inhibition of LCAT and hepatic triglyceride lipase has been reported from *S. polycystum* extract (200 mg/kg) in a model of hepatotoxicity and hyperlipidemia caused by acetaminophen in rats [31].

Phytochemical analysis has revealed that *Sargassum* plants comprise saponins, sterols, triterpenes, tannins and some exclusive metabolites like chromanols and plastoquinones [32]. They are reach sources of various polysaccharides and their sulfated forms such as fucoidan and alginates [33]. Preetha et al. showed that one week administration of sulphated polysaccharides from *S. wightii* in high fat-induced hyperlipidemic rats led to the prominent decrease in total cholesterol (60.75%), triglycerides (39.52%), LDL (69.65%) and VLDL (58.29%) levels and significant increase in HDL (38.17) level. They also found anti-inflammatory activity from *S. wightii* sulphated polysaccharides by alleviating inflammatory complications associated with hyperlipidemia [34]. Antihyperlipidemic effects have been reported from *S. vulgare* sulphated polysaccharides in high fat-induced hyperlipidemic and obese rats through inhibition of lipase activity and modification of lipid profile [35]. The beneficial effects on blood glucose level and lipid profile have also been reported from sodium alginate rich extract of *S. crassifolium* and *S. polycystum* extract in diabetic rats [36,37]. Moreover, antihyperlipidemic activities have been proven for other bioactive components of seaweeds including fucoxanthin and phlorotannins through inhibiting activity of HMG-CoA reductase and acyl-CoA:cholesterol acyltransferase in intestinal and liver cells [38].

Exposure to dexamethasone (10 mg/kg) caused a significant decrease in food intake and body weight gaining in rats compared to the normal control animals (p=0.035 and p=0.000, respectively). Administration of *S. angustifolium* at the doses of 20 and 40 mg/kg significantly increased food intake in rats (p=022 and p=0.008, respectively) however atorvastatin (p=0.096) and dose of 80 mg/kg from *S. angustifolium* (p=0.116) could not compensate the reduction in food consumption. However none of the treatments could prevent body weight loss caused by dexamethasone (Table 1). Dexamethasone administration results in weight loss in animals due to the metabolic alterations such as muscles atrophy and fat catabolism. Husni et al. reported notable increase in body weight in streptozotocin-induced diabetic rats receiving *S. polycystum* extract (150 mg/kg) [37]. While in the study of Kolsi et al., no effect on appetite and food intake of the high fat diet-induced

hyperlipidemic rats was observed after administration of *S. vulgare* sulphated polysaccharide [35].

Our results showed that the liver/body weight ratio was increased in dexamethasone-induced dyslipidemic rats (p=0.036). Treatment with atorvastatin and *S. angustifolium* (80 mg/kg) prevented liver weight gaining (p=0.001 and p=0.000, respectively) (Table 1).

As shown in Figures 7 and 8, significant increase in serum ALT and AST levels was observed in dexamethasone-induced dyslipidemic group (p=0.000 and p=0.003, respectively). Treatment with atorvastatin and *S. angustifolium* at all doses decreased these liver function markers. It is well known that treatment with dexamethasone is associated with elevation of liver function enzymes due to the enhanced enzymes gene expression [20,39]. There are several reports about the protective effect of some *Sargassum* sp. and their bioactive constituents on liver dysfunction by reducing enzyme biomarkers towards normal values [34,31].

Histopathological evaluation of the liver tissues in animals which were exposed to dexamethasone for one week showed lipid accumulation, fatty degeneration, diffused steatosis and cellular swelling compared to the normal architecture of the liver cells in normal control group (Figures 9A and 9B). Notable decrease in micro- and macro-vesicular steatosis was found in rats' livers that were treated with atorvastatin (Figure 9C). There was partial alleviation in lipid accumulation and steatosis after treatment with *S. angustifolium* at the doses of 20 mg/kg (Figure 9D), 40 mg/kg (Figure 9E) and 80 mg/kg (Figure 9F). Liver tissues in animals that received only *S. angustifolium* (80 mg/kg) showed normal structure (Figure 9G).

Steatosis and fatty liver histopathological alterations including vacuolation and disseminated lipid accumulation are considered as the time- and dose-dependent hepatic adverse effect of dexamethasone [26, 39]. Hepatoprotective effect and improvement of histological changes have been reported from some plants of genous *Sargassum* during acute and chronic liver damages [34,40].

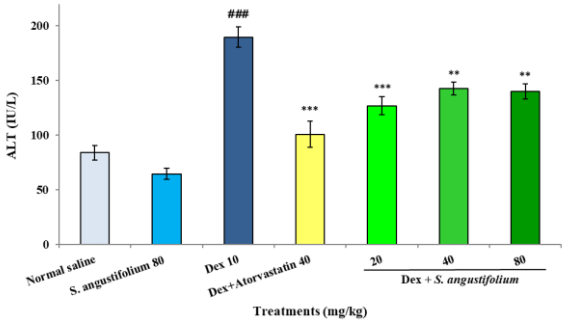

Figure 7. Effects of *Sargassum angustifolium* extract (20, 40 and 80 mg/kg) and atorvastatin (40 mg/kg) on serum ALT level in dexamethasone (Dex)-induced dyslipidemia. Values are means + SEM (n=6). ###p<0.001 versus normal control, and **p<0.01 and ***p<0.001 versus Dex

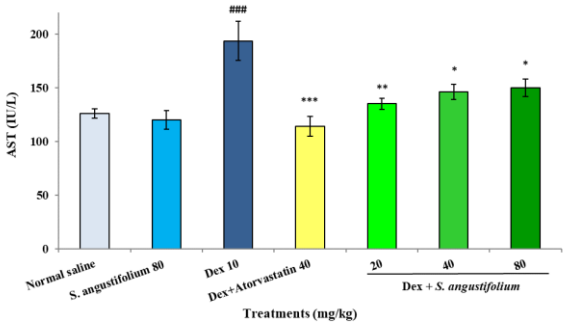

Figure 8. Effects of *Sargassum angustifolium* extract (20, 40 and 80 mg/kg) and atorvastatin (40 mg/kg) on serum AST level in dexamethasone (Dex)-induced dyslipidemia. Values are means + SEM (n=6). ###p<0.001 versus normal control, and *p<0.05, **p<0.01 and ***p<0.001 versus Dex

Table 1. Effect of *Sargassum angustifolium* ethanol fraction on food intake, body and liver weight in dexamethasone-induced dyslipidemia

Groups	Food intake (g/100g BW/day)	BW changes (%)	Liver weight (BW%)
Normal control	7.26±0.55	+1.36±0.24	4.98±0.066
S. angustifolium control (80 mg/Kg)	6.74±0.43	+1.62±0.15	4.79±0.081
Dexamethasone-induced dyslipidemia control	5.13±0.32$^{\#}$	-18.43±2.95$^{\#\#\#}$	5.38±0.039$^{\#}$
Dexamethasone + Atorvastatin (40 mg/Kg)	6.90±0.23	-17.51±3.43$^{\#\#\#}$	4.84±0.103**
Dexamethasone + *S. angustifolium* (20 mg/Kg)	7.36±0.44*	-18.52±2.13$^{\#\#\#}$	5.06±0.066
Dexamethasone + *S. angustifolium* (40 mg/Kg)	7.62±0.64**	-17.35±2.03$^{\#\#\#}$	5.03±0.087
Dexamethasone + *S. angustifolium* (80 mg/Kg)	6.83±0.46	-17.86±4.16$^{\#\#\#}$	4.47±0.016***

Values are means±SEM (n=6). Tukey post hoc analysis, $^{\#}$p<0.05, $^{\#\#\#}$p<0.001 versus normal control, *p<0.05, **p<0.01 and ***p<0.001 versus dexamethasone-induced dyslipidemia control. BW: body weight

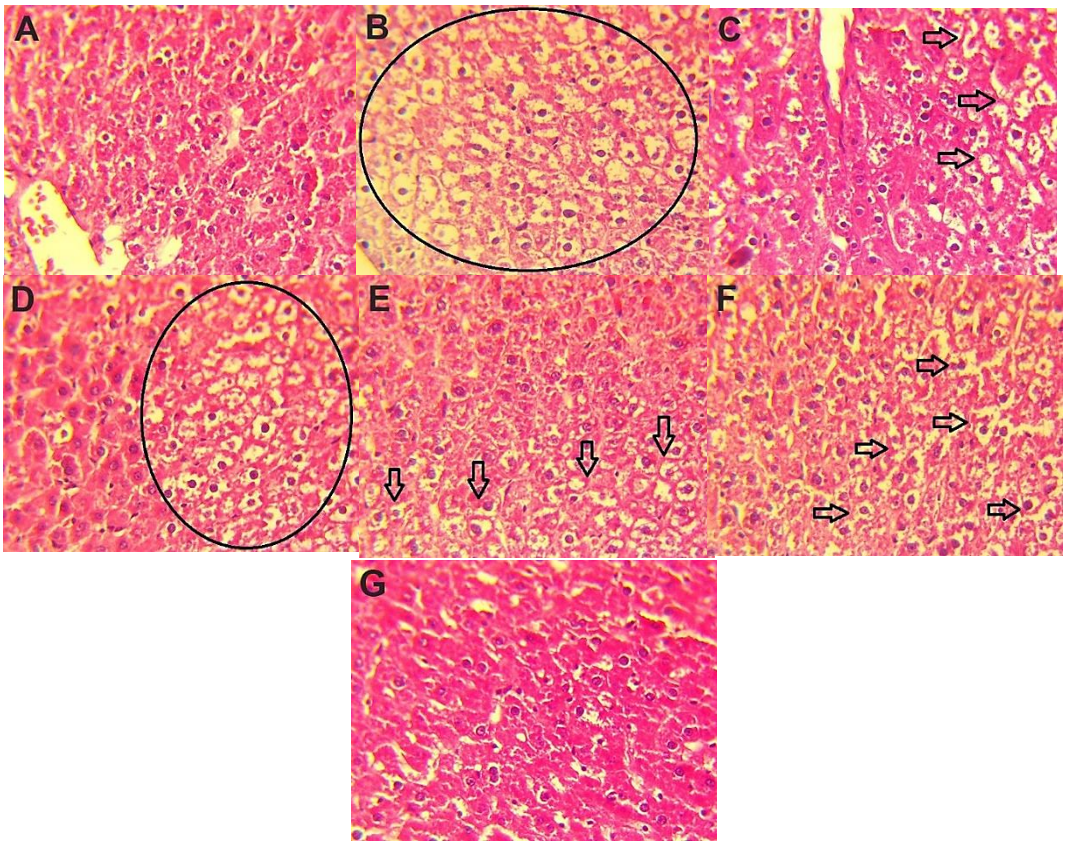

Figure 9. Representative H & E histological sections of the liver tissue of normal control group showing normal hepatocytes appearance (A); dexamethasone-induced dyslipidemic group showing diffused steatosis, fatty degeneration and cellular swelling (B); atorvastatin treated group showing mild vesicular steatosis (C); *Sargassum angustifolium* extract treated groups with doses of 20 mg/kg (D); 40 mg/kg (E) and 80 mg/kg showing moderate vesicular steatosis (F); *Sargassum angustifolium* extract alone treated group showing normal liver cells architecture (G); ×400 magnification; ovals indicate diffused steatosis, and arrows indicate partial lipid accumulation.

Oxidative stress is also taken into account as one of the principal issues involved in the pathogenesis of glucocorticoid-induced dyslipidemia [26]. Our results showed significant reduction in serum concentration of MDA as a marker of lipid peroxidation in dexamethasone-induced dyslipidemic rats after treatment with atorvastatin and different doses of *S. angustifolium* (p=0.000). Ethanol fraction of *S. angustifolium* at the dose of 80 mg/kg revealed highly potent anti-lipid peroxidative activity (Figure 10).

There are many reports for potent antioxidant actions of *S. angustifolium* through iron chelating, inhibiting lipid peroxidation, increasing total antioxidant capacity and scavenging free radicals due to the various phytochemicals especially high content of phenolic constituents [17,32].

The major limitation of the present study included the lack of investigation of the mechanisms of antihyperglycemic and antihyperlipidemic effects of *S. angustifolium* ethanol fraction. However, to our knowledge, this is the first study to effort to show the effect of this specie in a model of dyslipidemia.

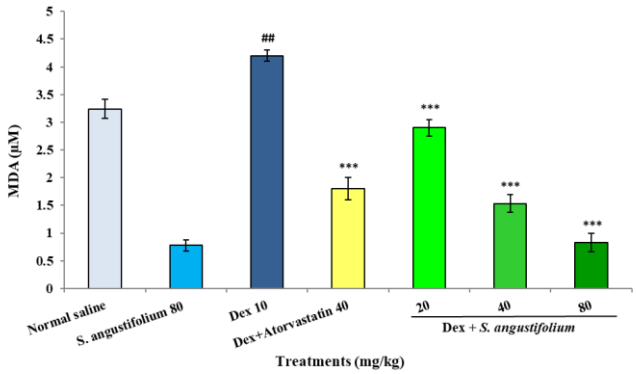

Figure 10. Effects of *Sargassum angustifolium* extract (20, 40 and 80 mg/kg) and atorvastatin (40 mg/kg) on serum MDA level in dexamethasone (Dex)-induced dyslipidemia. Values are means ± SEM (n=6). $^{\#\#}p<0.01$ versus normal control, and $^{***}p<0.001$ versus Dex

Conclusion

The results of the present study revealed the anti-hyperlipidemic, anti-hyperglycemic and antioxidant properties of the ethanol fraction of *S. angustifolium* through decreasing serum triglycerides, total cholesterol, LDL, blood glucose and MDA level and preventing histopathological alterations of liver tissues in dexamethasone-induced dyslipidemic rats. Regarding these findings and various bioactive constituents with beneficial cardiovascular activities, more investigations are suggested to clarify the precise mechanism of action and to establish the clinical value of this marine medicine for human dyslipidemic diseases.

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Author contributions

Leila Safaeian was responsible for the research plan, designing the animal studies, analyzing the data and editing the manuscript; Afsaneh Yegdaneh planned the herbal experiments; Mahnaz Halvaei-Varnousfaderani and Saeed Bazvand were involved in the animal treatments and preparation of the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

ALT: alanine aminotransferase; AST: aspartate aminotransferase; GAE: gallic acid equivalents; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; VLDL: very low density lipoprotein cholesterol; MDA: malondialdehyde