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Hypolipidemic influence of *Sargassum subrepandum*: mechanism of action

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Abstract. – OBJECTIVES, This study aimed to elucidate the role and mode of action of *Sargassum subrepandum* methanolic extract in management of dyslipidemia in adult female rats.

MATERIAL AND METHODS, Forty adult female Sprague Dawley rats were assigned into four groups: (1) lean control rats fed on standard diet, (2) dyslipidemia control fed on the atherogenic diet, (3) lean rats orally administered with 100 mg/kg b. wt of *Sargassum subrepandum* methanolic extract and (4) dyslipidemia rats orally administered with *Sargassum subrepandum* methanolic extract. Plasma lipid profile, serum MDA, NO, leptin, TNF-alpha and adiponectin levels were demonstrated in the all studied groups.

RESULTS, The results showed that feeding of rats with athrogenic diet caused significant elevation in plasma cholesterol, triglyceride, LDL, serum MDA, NO, leptin and TNF-alpha levels while, it produced significant decline in plasma HDL and serum adiponectin levels compared with lean control rats. However, treatment of dyslipidemia rats with *Sargassum subrepandum* methanolic extract induced significant improvement of plasma lipid profile, marked decrease in serum MDA, NO, leptin, TNF-alpha level in concomitant with remarkable increase in serum adiponectin level.

CONCLUSIONS, These results indicated that *Sargassum subrepandum* extract plays a vital role in ameliorating dyslipidemia and its complications particularly oxidative stress and implication. This could be attributed to the hypolipidemic effect, antilipidperoxidative activity and antinflammatory property of *Sargassum subrepandum* methanolic extract.

Key Words:

Dyslipidemia, Oxidative stress, Inflammation, Adipokines, *Sargassum subrepandum.*

Introduction

Hyperlipidemia is defined as a condition of a normally elevation of any or all lipids and/or lipoprotein in the blood[1]. This is one of the most common diseases found all over the world. Hyperlipidemia is a major risk factor for insulin resistance, type 2 diabetes, heart diseases, orthopedic problems and many other chronic diseases. The incidence of obesity has dramatically increased and has become epidemic in the western as well as eastern world[2].

Seaweeds have long been used in the Japanese and Chinese diet. In 600 B.C., Sze Teu wrote in China, "Some algae are a delicacy fit for the most honored guests, even for the King himself." Around 21 species are used in everyday cooking in Japan, six of them since the eighth century, thus seaweeds accounts for some 10% of the Japanese diet[3].

Seaweeds contain large amounts of polysaccharides, notably cell wall structural polysaccharides that are extraded by the hydrocolloid industry namly alginate and fucoidans from brown seaweeds. Brown seaweeds also contain storage polysaccharides, notably laminarin (b-1, 3-glucan)[4]. When faced with the human intestinal bacteria, most of these polysaccharides are not digested by humans and, therefore, can be regarded as dietary fibres[4]. Water-soluble and water-insoluble fibres have been associated with different physiological effects. Many viscous soluble polysaccharides have been correlated with hypocholesterolemic and hypoglycemic effects, whereas water-insoluble polysaccharides are mainly associated with a decrease in digestive tract transit time[5]. Among polysaccharides, fucoidans were particularly studied as they showed interesting biological activities (anti-thrombotic, anti-coagulant, anticancer, anti-proliferative, anti-viral, and anti-inflammatory). It has been reported extract of *Sargassum oligocystum* has antibacterial[6] as well as antitumor activities[7].

The main goal of the current study was to explore the role and the possible mode of ac-

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tion of *Sargassum subrepandum* methanolic extract in management of dyslipidemia in adult female rats.

Materials and Methods

Extensive survey and collecting visits to the studied sites (El-Kantara, Ismailia and Suez) were done. The richest areas for seaweeds were undoubtedly subtidal regions where water movement is moderate to very great. Field collecting equipments included implements to remove the seaweeds, container to hold them, labels, and containers for transport to the laboratory.

Field Containers

These were plastic containers, but for intertidal collection, buckets and bags with various sizes were used, and for the subtidal collection by SCUBA, diving perforated plastic bags were used. The algal materials were transported to the laboratory in an ice box.

Algal Materials

Seven kg_s of *Sargassum subrepandum* were collected from Ismailia to Al Kantara from the banks of the navigation way of Suez Canal at depth 2-4 m.

Species Identification

The collected samples had been identified and compared with the materials in the Marine Botany Laboratory, Marine Science Department, Suez Canal University, by Dr. Muhammad Mosaad Ibrahim Hegazi. Associate Professor of Botany.

Algal Extract Preparation

Fresh algal sample was washed with fresh water and cleaned from any epiphytes by using fine brush. Then, it was mixed with methanol and homogenized using electrical blender and extracted three times with 80% methanol each time. The extract was filtered using Buchner funnel under suction. The filtrate was concentrated using rotary evaporator at 40°C till it became free of methanol. The yield of the extract was weighed (50 g) and kept in deep freeze for subsequent step.

Experimental Animals

Forty adult female Sprague Dawley rats 3 month old weighting 130-140 g body weight were enrolled in the current study. The animals were obtained from the Animal House Colony of the National Research Centre, Dokki, Cairo, Egypt. All animals were acclimated in specific pathogen free plastic cages for two weeks before starting the experiment. The animals were maintained under controlled conditions and received human care in compliance with the guidelines of the Ethical Committee of Medical Research of National Research Centre.

After the acclimation period, the rats were assigned into four groups (10 rat/ group) as follows: gp^1 Lean control rats fed on standard diet, gp^2 dyslipidemia control rats fed on atherogenic diet[8] for 8 months, gp^3 Lean rats fed on standard diet for 8 months, then they were orally administrated with 100 mg/kg b.wt of *Sargassum subrepandum* methanolic extract for[9] four months. gp^4 dyslipidemic rats fed on atherogenic diet for 8 months, then they were orally administrated with *Sargassum subrepandum* extract in a dose of 100 mg/kg b.wt for four months.

Blood Sampling

At the end of the experimental period, fasting blood samples were collected from retro-orbital venous plexus under diethyl ether anesthesia. Blood samples were divided into two tubes, one containing EDTA for obtaining plasma and the other dry clean centrifuge tubes to obtain sera. Blood samples were centrifuged at 1800 × g for 15 min at 4°C. The resulting supernatants were collected. Serum and plasma samples were stored at -20ºC in clean plastic Eppendorff tubes till analysis.

Biochemical Analysis

Cholesterol in plasma was determined by colorimetric method using Bio-diagnostic kit (Cairo, Egypt) according to the method described by Allain et al[10]. Plasma triglycerides were estimated by colorimetric method using Bio-diagnostic kit (Cairo, Egypt) according to the method described by Fassati and Prencipe[11]. Low density lipoprotein (LDL) cholesterol in plasma was assayed by colorimetric method using Bio-diagnostic kit (Cairo, Egypt) according to the method described by Wieland and Seidel[12]. High plasma density lipoprotein (HDL) cholesterol was determined by colorimetric method using Bio-diagnostic kit (Cairo, Egypt) according to the method described by Burstein et al[13]. Serum malondialdehyde (MDA) as a product of lipid peroxidation was detected by colorimetric method using Bio-diag-

nostic kit (Cairo, Egypt) according to the method described by Ohkawa et al[14]. Serum nitric oxide (NO) was estimated by colorimetric method using Bio-diagnostic kit (Cairo, Egypt) according to the method described by Montgomery and Dymock[15]. Serum leptin was assayed by enzyme linked immunosorbent assay (ELISA) technique using BioSource kit (Nivelles, Belgium) according to the method of Keim[16]. Tumor necrosis factor-alfa (TNF-α) was estimated in serum ELISA technique using Orgenium kit (Vantaa, Finland) according to the method described by Seriolo et al[17]. Adiponectin was determined in serum using an ELISA kit provided by Linco Research (St. Charles, MO, USA) according to the method of Ryan et al[18].

Statistical Analysis

All results in the present study were expressed as mean ± SE of the mean. Statistical Pakage for the Social Sciences (SPSS, Inc., Chicago, IL, USA) program, version 11.0 was used to compare significance between each two groups. Differences was considered significant when $p \leq 0.05$.

Results

The present results revealed that feeding rats with atherogenic diet for 8 months led to significant increase in plasma cholesterol, triglycerides and LDL levels associated with significant decrease in HDL level in comparison with the lean control rats. Lean rats treated with *S. subrepandum* extract showed significant decrease in plasma cholesterol, triglycerides and LDL levels accompanied with significant increase in HDL level as compared to lean control rats. The dyslipidemic rats treated with *S.subrepandum* showed significant decrease in plasma cholesterol, triglycerides and LDL levels associated with significant increase in HDL level in comparison with the dyslipidemic control group (Table I).

Also, the current results showed that feeding of rats with atherogenic diet for 8 months induced significant elevation in serum MDA and NO levels in comparison with the lean control rats. Lean rats treated with *S.subrepandum* extract showed insignificant decrease in serum MDA level but it showed significant decrease in serum NO level in comparison with the lean control rats. The dyslipidemic rats treated with *S.subrepandum* extract showed significant reduction in both MDA and NO serum levels in comparison with the dyslipidemic control rats (Table II).

The current data revealed that dyslipidemia produced by feeding rats with atherogenic diet resulted in significant increase in serum leptin and TNF-α levels associated with significant decrease in adiponectin level in comparison with the lean control rats. Lean rats treated with *S. subrepandum* extract showed insignificant decrease in serum leptin level but it showed significant decrease in serum TNF-α level. However, treatment of lean rats with *Sargassum subrepandum* extract caused insignificant increase in serum adiponectin level as compared with the lean control rats. Treatment of dyslipidemic rats with *S. subrepandum* extract led to significant depletion in each of serum leptin and TNF-α levels associated with significant elevation in serum adiponectin level in comparison with the dyslipidemic control group (Table III).

Discussion

The present study revealed that feeding of female rats with atherogenic diet resulted in significant increase in plasma cholesterol, triglycerides (TG) and LDL accompanied with significant de-

Table I. Effect of *Sargassum subrepandum* methanolic extract on plasma lipid profile in dyslipidemic rats.

HDL mg/dl	LDL mg/dl	Triglycerides mg/dl	Cholesterol mg/dl	Parameters/groups
28.39 ± 0.5	15.93 ± 0.3	85.69 ± 2.2	70.93 ± 0.8	Lean control
15.67 ± 0.4[a]	25.17 ± 0.4[a]	106.43 ± 3.8[a]	162.32 ± 4.8[a]	Dyslipidemic control
31.55 ± 0.2[a]	13.68 ± 0.3[a]	77.16 ± 1.2[a]	62.99 ± 1.1[a]	Lean + *S. subrepandum*
23.64 ± 0.4[b]	16.26 ± 0.1[b]	83.37 ± 1.4[b]	100.32 ± 1.8[b]	Dyslipidemic + *S. subrepandum*

[a]Significant change at $p < 0.05$ in comparison with lean control group. [b]Significant change at $p < 0.05$ in comparison with dyslipidemic control group.

Table II. Effect of *Sargassum subrepandum* methanolic extract on serum MDA and NO in dyslipidemic rats.

NO µmol/L	MDA nmol/ml	Parameters/groups
50.37 ± 0.6	2.4 ± 0.1	Lean control
79.99 ± 0.9[a]	7.2 ± 0.1[a]	Dyslipidemic control
47.75 ± 1.3[a]	2.1 ± 0.2	Lean + *S. subrepandum*
60.41 ± 0.9[b]	4.0 ± 0.1[b]	Dyslipidemic + *S. subrepandum*

[a]Significant change at $p < 0.05$ in comparison with lean control group. [b]Significant change at $p < 0.05$ in comparison with dyslipidemic control group.

crease in HDL level. The increased plasma cholesterol level as a result of high fat diet may be due to an enhancement of *de novo* synthesis in the liver cell and exchange with plasma lipids or both[19]. Nabel[20] has demonstrated that hypercholesterolemia induced by high fat diet (HFD) is attributed to disruption of LDL-receptor pathways which are necessary for cholesterol synthesis and excretion pathways in the liver. This highlighted the molecular targets for regulating plasma cholesterol levels.

Concerning plasma level of TG, the present finding is well agreed with the study of Yugarani et al[21] who demonstrated that plasma TG level increased significantly after feeding rats with HFD. This indicates that the increase in TG is of dietary origin. Kritchevsky et al[22] supported this finding as they stated that the TG composition, structure as well as chain length of fatty acids in dietary fat are important determinants of atherogenicity.

Nabel[20] reported that the elevated plasma levels of LDL are attributed to impairing the activity of hepatic LDL receptors, which normally clear LDL from the plasma. Low-density lipoprotein (LDL) molecules are composed of a cholesteryl ester core surrounded by a coat made up of phospholipid and apolipoprotein B-100. The liver secretes LDLs as larger precursor particles called very-low-density lipoproteins, which contain triglycerides and cholesterol esters. Capillaries in muscle and adipose tissue remove the triglycerides, and the lipid particle is modified into an LDL, with its cholesteryl ester core and apolipoprotein B-100 coat. LDLs circulate in the plasma, and the apolipoprotein B-100 component binds to LDL receptors on the surface of hepatocytes. Through receptor-mediated endocytosis, receptor-bound LDLs enter hepatocytes and undergo degradation in lysosomes, and the cholesterol remnants enter a cellular cholesterol pool. A negative-feedback loop regulates the number of LDL receptors. A rise in the hepatocyte cholesterol level suppresses the transcription of LDL-receptor genes, and LDL is retained in the plasma. Moreover, the deficiency of lipoprotein transport abolishes transporter activity, resulting in elevated cholesterol absorption and LDL synthesis. Thus, plasma concentration of LDL is largely dependent on the rates of its production and removal from the circulation[23].

The results concerning plasma HDL level in rats fed on atherogenic diet, the present results are well documented by the study of Yugarani et al[21]. It has been reported that cholesterol transport to extra hepatic tissues is primarily ensured by LDL while HDL has an important role in reversing the cholesterol transport process[24]. Therefore, HDL exerts a protective effect against coronary heart disease induced by hypercholesterolemia[25].

Table III. Effect of *Sargassum subrepandum* methanolic extract on serum leptin, TNF-α and adiponectin in dyslipidemic rats.

Adiponectin µg/dl	TNF-α Pg/ml	Leptin ng/ml	Parameters/groups
3.95 ± 0.1	66.43 ± 0.3	16.21 ± 0.2	Lean control
1.66 ± 0.1[a]	105.89 ± 0.2[a]	56.74 ± 0.9[a]	Dyslipidemic control
4.54 ± 0.1[a]	61.48 ± 1.00[a]	14.15 ± 0.4	Lean + *S. subrepandum*
3.23 ± 0.1[b]	79.97 ± 0.9[b]	36.95 ± 0.4[b]	Dyslipidemic + *S. subrepandum*

[a]Significant change at $p < 0.05$ in comparison with lean control group. [b]Significant change at $p < 0.05$ in comparison with dyslipidemic control group.

Feeding of rats with HFD induced significant increase in serum nitric oxide (NO) and malondialdhyde (MDA) levels. These results in agreement with those of Haghjooyjavanmard et al[26]. Hyperlipidemia increases superoxide formation which is responsible for increasing peroxynitrite[27] that resulted from the reaction of singlet oxygen radical O_2^- and nitric oxide radical (NO)[28]. Also, increased caloric intake is an important factor in decreasing the mitochondrial membrane fluidity and increasing the generation of reactive oxygen species (ROS) and reactive nitrogen species[29].

Although ROS are essential for certain physiological processes, when their concentrations are raised, the body's antioxidant defences may be unable to cope. The result is a condition called oxidative stress, an imbalance between the oxidants and antioxidants systems[30]. It has been demonstrated that ROS production was increased in parallel with fat accumulation in a NADPH oxidase-dependent manner. This indicates that in accumulated fat, elevated levels of fatty acids activate NADPH oxidase and induce ROS production. ROS itself augmented mRNA expressions of NADPH oxidase subunits, including NADPH oxidase 4 (NOX4) and Transcription factor PU.1 in adipocytes. In summary, fat accumulation due to HFD and elevated ROS appear to upregulate mRNA expression of NADPH oxidase, establishing a vicious cycle that augments oxidative stress in the blood[31]. Thus, it is possible that increased ROS production as well as MCP-1 secretion from accumulated fat should cause infiltrated of macrophages and oxidative stress[32].

The current results indicated that there is significant increase in serum leptin level due to feeding with HFD. In fact, the excess of circulating leptin for a given level of adiposity may reflect resistance to leptin, which may result in energy imbalance. Therefore, relative leptin levels may be hypothesized to predict adiposity changes over time[33]. Flier[34] reported that the mechanisms posited to explain the state of leptin resistance in hyperlipidemia include impaired leptin transport across the blood-brain barrier and the presence of negative regulators of leptin signalling in CNS neurons that express leptin receptors and their downstream targets.

The current results demonstrated significant increase in serum TNF-α level in rats fed on HFD. This result supports that of Margoni et al.[35] who stated an increase in serum TNF-α level in subclinical dyslipidemia. More than one study has shown a positive correlation between adiposity and adipose content of TNF-α. Hotamisligil et al[36] stated that TNF-α mRNA is over expressed in adipose tissue of hyperlipidemic mice. Barzilai et al[37] found that surgical removal of visceral fat reverses insulin resistance in mature Sprague-Dawley rats and decreases expression of TNF-α mRNA in subcutaneous fat. Also, the finding of Xu et al[38] showed that diet-induced hyperlipidemia is accompanied by increased TNF-α in skeletal muscle. Moreover, Stephen and Christine[39] reported that diet-induced hyperlipidemia is accompanied by increased TNF-α protein in both adipose tissue and muscle.

The present result showed significant decrease in serum level of adiponectin in rats fed with HFD. It was reported that peroxisome proliferator-activated receptor gamma (PPARγ) positively regulates the transcription of the adiponectin gene *via* PPARγ responsive element in the promoter[40]. Oxidative stress has been found to suppresses PPARγ mRNA expression in adipocytes and the nuclear translocation of PPARγ has been found to be inhibited by nitration associated with oxidative stress[40]. Therefore, down regulation of adiponectin expression may be partially attributed to the decreased gene expression and diminished amount of nuclear PPARγ under condition of oxidative stress due to hyperlipidemia. Also, decreased expression of adiponectin correlate has been found to with insulin resistance that associated with hyperlipidemia[41].

The current results revealed that administration of methanolic extract of *Sargassum subrepandum* extract in dyslipidemic rats resulted in significant decrease in plasma cholesterol, triglycerides and LDL levels in concomitant with significant increase in plasma HDL level. In accordance with our results the ethanolic extract of *S. subrepandum* have been found to have hypolipidemic activities, as indicated by decreasing serum cholesterol, triglycerides, and LDL levels in rats[42]. The major active principle of *Sargassum sp*. is fucoxanthin. Fucoxanthin is a type of non-provitamin a carotenoid and it belongs to xanthophyll. Japanese researchers have shown that fucoxanthin may cause up to a 10% weight loss in experimental animals by direct effects on shrinking abdominal fat stores[43]. Moreover, uncoupling protein 1 (UCP1) is a key molecule for antihyperlipidemic. Fucoxanthin induces both protein and mRNA expression of UCP1[44]. Furthermore, Woo et al[45] showed that fucoxanthin significantly lowered the levels of triglycerides and cholesterol, as fucoxanthin is beneficial for the suppres-

sion of the hepatic lipogenesis with an increase in hepatic lipolysis. Also, fucoxanthin has been found to produce significant depletion in the activities of hepatic fatty acid synthesis. Reduction in the hepatic lipogenic enzyme activity probably limits the availability of long chain fatty acids required for hepatic triglycerides synthesis. Interestingly, fucoxanthin significantly lowered the peroxisome proliferator activated receptor gamma (PPAR γ) mRNA expression as well as the activity of hepatic PAPR, a rate-limiting enzyme in the triglycerides synthesis[45]. Additionally, it has been shown that, the consumption of fucoxanthin significantly increased feces weight and fecal lipids, i.e, lead to increases in fecal excretion of fat[46] and cholesterol[47]. Also, Matsumoto et al[48] demonstrated that marine carotenoids inhibit lipase activity in the gastrointestinal lumen *in vitro* and suppress triglyceride absorption in lymph duct-cannulated rats. The carotenoids could not directly block the absorption of cholesterol from rat jejunum but could selectively inhibit the activity of pancreatic lipase as a competitive inhibitor[46].

Considering that the liver is the major site of fatty acid metabolism, all the metabolic actions of fucoxanthin, including the reduction of hepatic lipogenic enzyme activity, elevation of the hepatic β oxidation activity and the fecal lipids excretion, seemed to be contributed to the prevention of dyslipidemia in high-fat fed mice[49]. Lastly, fucoxanthin supplementation seemed to inhibit hepatic HMG-CoA reductase as well as ACAT activity, which may reduce the hepatic cholesterol pool and, thus, cholesterol accumulation[50].

Sargassum extract has been shown to prevent the elevation of total cholesterol and triglycerides in both serum and tissue, which indicates its protective nature against cholesterolemia. This protective nature may be due to the presence agents like sulphated polysaccharide that could delay the intestinal absorption of cholesterol or hasten the cholesterol excretion[5]). Also the elevated level of LDL has been found to be significantly reduced in brown seaweed extract treated rats which may be due to the antioxidant property of this extract, which is capable of inhibiting LDL peroxidation[52]. It has been reported that hypolipidemic drugs with antioxidant properties, may prevent LDL peroxidation and retard the LDL accumulation[53].

Plasma HDL level was significantly increased in hyperlipidemic rats treated with *Sargassum subrepandum* HDL promotes the reverse cholesterol transport, in which HDL induces the efflux of excess accumulated cellular cholesterol[54]. It seems that fucoxanthin enhances the efflux of excess cholesterol accumulated by high-fat diet via the increased plasma HDL[45]. It has been reported that rats pretreated with *Sargassum polycystum* extract showed an improvement in HDL level which could be attributed to the ability of this extract to hasten the decomposition of the generated free radical species[9].

Our results revealed that the methanolic extract of *Sargassum subrepandum* had an antioxidant effect as indicated by the significant reduction in serum MDA and NO levels in hyperlipidemic rats. Yan et al[55] reported that *Sargassum sp*. possess powerful antilipid peroxidative property. The antioxidant and free radical scavenger activity of fucoxanthin as carotenoids' are well known. This could be due to its singlet oxygen-quenching activity[55,56].

Fucoxanthin has been reported to suppress NO production[57]. Chang et al[58] reported that the induction of COX-2 activity and subsequent generation of PGE2 are closely related to the NO production. Fucoxanthin has been found to inhibit lipopolysaccharide (LPS) induced NO and PGE2 production. Also, it has been demonstrated that fucoxanthin inhibited NO production via the suppression of iNOS expression[59,60].

The present results revealed that administration of methanolic extract of *S.subrepandum* produced significant decrease in proinflammatory markers such as leptin and TNF-α in hyperlipidemic rats. Since leptin is secreted from adipose tissues, fucoxanthin's activity to lower the serum leptin concentration is believed to be due to the ability of fucoxanthin to reduce white adipose tissue[61].

Fucoxanthin may somehow affect monocytes and macrophages that produce TNF-α, thereby revealing its anti-inflamamtory action[57]. Fucoxanthin could also downregulate TNF-α mRNA expression in white adipose tissue[44]. In accordance with our findings Heo et al[60] reported that fucoxanthin significantly inhibited the production of pro-inflammatory cytokine TNF-α. The inhibition of iNOS/NO pathway by fucoxanthin may be associated with the attenuation of TNF-α formation[60]. Moreover, fucoxanthin exhibits anti-inflammatory properties by down regulation of mitogen activated protein kinase (MAPK) signal pathways and the inhibition of nuclear factor kappa (NF-κB) activation i.e, fucoxanthin blocks the pathway of TNF-α formation[62]. NF-κB is a mam-

malian transcription factor that controls number of genes, including iNOS, COX-2, TNF-α, IL-1β, and IL-6, all of which are relevant to immunity and inflammation[63]. Fucoxanthin inhibits the cytoplasmic degradation of (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha) (IκB-α) and the nuclear translocation of p50 and p65 proteins, resulting in lower levels of NF-κB transactivation. This finding implies that the effect of fucoxanthin on the production of inflammatory mediators and cytokines are mediated, at least in part, via the suppression of the NF-κB signaling pathway[62].

The current result revealed that the treatment of hyperlipidemic rats with *Sargassum subrepandum* extract resulted in significant decline in serum adiponectin level. This finding could be attributed to fucoxanthin content of *Sargassum subrepandum* where it has been reported that fucoxanthin has a regulatory effect on adipokines mRNA in white adipose tissue[64]. The present study provided detailed mechanisms for the influence *Sargassum subrepandum* methanolic extract as a promising agent for management of dyslipidemia and its serious complications particularly oxidative stress and inflammation.

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