Caulerpa prolifera ameliorates the impact of dyslipidemia – induced oxidative stress and inflammation.

Hanna, H. Ahmed[1,*], Mohga, S. Abdalla[2], Emad, F. Eskander[1], Manal, F. Al-Khadragy[3], Mary, N. Massoud[1]

1: Hormones Department , National Research Centre, Dokki, Cairo, Egypt.
2: Chemistry Department, Faculty of Science, Helwan University, Helwan, Egypt.
3: Zoology and Entomology Department Faculty of Science, Helwan University, Helwan, Egypt.
hanaaomr@yahoo.com, mary_nasif@yahoo.com

Abstract: This study was undertaken to investigate the potential role of *Caulerpa prolifera* methanolic extract in management of dyslipidemia and its complications in female rats. Forty adult Sprague Dawley rats were enrolled in the present study and they were assigned as lean control group; dyslipidemic control group; lean rats treated with C.prolifera methanolic extract (50 mg/kg b.wt), and dyslipidemic rats treated with C.prolifera methanolic extract for 4 months. The results revealed that the treatment of dyslipidemic rats with C.prolifera resulted in significant reduction in plasma cholesterol, triglycerides, LDL level accompanied with significant elevation in plasma HDL level. Also, C.prolifera extract induced significant deacrease in serum MDA and inorganic free radical (NO) levels in dyslipidemic rats. Furthermore, Administration of C.prolifera extract in dyslipidemic animals produced significant depletion in serum leptin and TNF-α level associated with significant rise in serum adiponectin level. These results indicated that C.prolifera extract has played a vital role in ameliorating dyslipidemia and its complications particularly oxidative stress and inflammation. These findings may provide new concept for development of effective natural therapy for dyslipidemia and its associated serious complications.
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1. Introduction

The term hyperlipidemia refers to increased concentrations of lipids (triglycerides, cholesterol, or both) in the blood (Xenoulis and Steiner, 2010) . Hyperlipidemia can be either primary or secondary to other diseases. Secondary hyperlipidemia is the most common form and can be a result of endocrine disorders, pancreatitis, cholestasis, protein-losing nephropathy, obesity, and high fat diets. Primary hyperlipidemia is less common and usually associated with certain breeds. Hypertriglyceridemia is the most common type of primary hyperlipidemia , and appears to have a genetic basis although its etiology remains unknown. Management is achieved by administration of low fat diets with or without administration of lipid-lowering agents such as omega-3 fatty acids, gemfibrozil, and niacin. (Xenoulis and Steiner, 2010).
For thousands of years, algae and seaweeds have been used for human food in many countries including Australia, England, Greece, Italy, Japan and Egypt as well. Among these algae the green algea Caulerpa which contains over 75 species including *Caulerpa Prolifera.*

Many algae have important secretions which are generally used for defensive purposes. These secretions have caught the attention of a number of researchers, as to whether or not these metabolites can be used in medical research. Among these metabolites, caulerpenyne (CYN) which is the main metabolite of the *Caulerpa* species, has had an important position in *Caulerpa* research since the beneficial effects related to its determined properties such as cytotoxicity, antiviral, antiproliferative and apoptotic which have been demonstrated in many scientific reports (Rebah *et al.*, 2008). Recent study made by Cengiz et al. (2010) proved that *C. prolifera* derived metabolites can be promising material for treatment of diabetes, obesity and other related diseases. Therefore, the current study

aims at investigating the potential therapeutic role of green macroalga *(Caulerpa prolifera)* that is widely spread among the Egyptian coasts mainly Red sea and Suez Canal in management of dyslipidemia and its complications in adult female rats.

2. 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.

2.1 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 materials were transported to the laboratory in an ice box.

2.2 Algal materials*:*

Four and half kg_s of *C. prolifera* were collected from Ismailia to Al Kantara from the banks of the navigation way of Suez Canal at depth 2-4m.

2.3 Species Identification:

All 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.

2.4 Algal extract preparation:

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

2.5 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 Center, Dokki, Cairo, Egypt. The 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.

The animals were assigned into four groups (10 rat/ group) as follows: gp(1) Lean control rats fed on standard diet, gp(2) dyslipidemic control fed on atherogenic diet (Auger et al.,2002) for 8 months, gp(3) Lean rats fed on standard diet for 8 months then they were orally administrated with 50 mg/Kg b.wt of *Caulerpa Prolifera* extract for four months. gp(4)dyslipidemic rats fed on atherogenic diet for 8 months then they were orally administrated with *Caulerpa Prolifera* extract in a dose of 50 mg/Kg b.wt of (*Harda and Kami,1998*) for four months .

2.6 Blood sampling

At the end of the experimental period, fasting blood samples were collected from retro-orbital venous plexus under diethyl ether anesthia. 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 xg for 15 min at 4 °C. The resulting supernatants were collected. Serum samples were stored at -20°C in clean plastic eppindorff tubes till analysis.

2.7 Biochemical analyses:

Cholesterol in plasma was determined by kinetic method using Bio-diagnostic kit (Egypt) according to the method described by Allain et

al.(1974). Plasma triglyceride was estimated by kinetic method using Bio-diagnostic kit (Egypt) according to the method described by *Fassati* and Prencipe **(**1982**).** Low density lipoprotein **(**LDL) cholesterol in plasma was assayed by kinetic method using Bio-diagnostic kit (Egypt) according to the method described by Wieland and Seidel **(**1983**).** High density lipoprotein **(**HDL) cholesterol in plasma was determined by kinetic method using Bio-diagnostic kit (Egypt) according to the method described by Burstein. (1970). Serum malondialdehyde (MDA) as a product of lipid peroxidation was detected by kinetic method using Bio-diagnostic kit (Egypt) according to the method described by Ohkawa et al. **(**1979**).**Serum nitric oxide (NO) was estimated by kinetic method using Bio-diagnostic kit (Egypt) according to the method described by Montgomery. ***(1961).*** Serum Leptin was assayed by enzyme linked immunosorbent (ELISA) technique using BioSource kit (Belgium) according to the method of *Keim et al.* (1998). Tumor necrosis factor –alfa (TNF-α) was estimated in serum by enzyme linked immunosorbent (ELISA) technique using Orgenium kits (Finland) according to the method described by Seriolo. (2006).Adiponectin was determined in using an ELISA kit provided by Linco Research (USA) according to the method of Ryan et al.(2003).

2.8 Statistical analysis:

In the present study, all results were expressed as mean ± S.E of the mean. Statistical pakage for the social Sciences (SPSS) program, version 11.0 was used to compare significance between each two groups. Differences was considered significant when $p \leq 0.05$.

3. Results:

The present results revealed that feeding rats with atherogenic diet for 8 months led to significant increase in plasma cholesterol, triglyceride and LDL levels associated with significant decrease in HDL level in comparison with the lean control group. Lean group treated with *C.prolifera* extract showed insignificant decrease in plasma cholesterol and LDL levels accompanied with insignificant increase in HDL level as compared to lean control group. Significant decrease in plasma triglycerides level was detected in lean group treated with *C.prolifera extract* in comparison with control group. The dyslipidemic group treated with *C.prolifera* 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 1).

Feeding of rats with atherogenic diet for 8 months induced significant elevation in serum MDA and NO levels in comparison with the lean control group. Lean group treated with *C.prolifera* extract showed insignificant decrease in serum MDA level but it showed significant deacrease in serum NO level in comparison with the lean control group. The dyslipidemic group treated with *C.prolifera* extract showed significant reduction in both MDA and NO serum levels in comparison with the dyslipidemic control group. (Table 2).

The current results 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 group . Lean group treated with *C.prolifera* extract showed insignificant decrease in serum leptin and TNF-α levels, while it showed insignificant increase in serum adiponectin level as compared with the lean control group. Treatment of dyslipidemic group with *C.prolifera* 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 3).

Table (1): The effect of *Caulerpa prolifera* methanolic extract on lipid profile of dyslipidemic rats .

Groups / Parameter	Cholesterol mg/dl	Triglyceride mg/dl	LDL mg/dl	HDL mg/dl
Lean control	70.93±0.8	85.69±2.2	15.93±0.3	28.39±0.5
Dyslipidemi control	162.32±4.8[a]	106.43±3.8[a]	25.17±0.4[a]	15.67±0.4[a]
Lean + *C.prolifera*	65.20±1.1	80.46±1.5[a]	14.94±0.6	30.04±1.2
Dyslipidemic + *C.prolifera*	106.36±2.9[b]	90.07±1.5[b]	18.58±0.3[b]	21.07±0.3[b]

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 (2): The effect of *Caulerpa prolifera* methanolic extract on serum MDA and NO of dyslipidemic rats .

Groups / Parameters	MDA nmol/ml	NO µmol/L
Lean control	2.4±0.1	50.37±0.6
Dyslipidemic control	7.2±0.1[a]	79.99±0.9[a]
Lean + *C.prolifera*	2.2±0.1	49.18±1.9[a]
Dyslipidemic + *C.prolifera*	4.6±0.1[b]	63.89±1.1[b]

Table (3): The effect of *Caulerpa prolifera* methanolic extract on serum leptin, TNF-α, and adiponectin of dyslipidemic rats.

Group / Parameter	Leptin ng/ml	TNF-α Pg/ml	Adiponectin µg/dl
Lean control	16.21±0.2	66.43±0.3	3.95±0.1
Dyslipidemic control	56.74±0.9[a]	105.89±0.2[a]	1.66±0.1[a]
Lean + C.prolifera	15.23±0.2	64.49±1.00	4.13±0.2
Dyslipidemic + *C.prolifera*	41.88±0.8[b]	88.41±0.6[b]	2.97±0.2[b]

4. Discussion

The present study revealed that feeding of rats with atherogenic diet resulted in significant increase in plasma cholesterol, triglycerides and LDL accompanied with significant deacrease in HDL level. The quantity and quality of fats present in the diet play an important role in the regulation of the synthesis of cholesterol and triglycerides-rich lipoproteins, bile acid secretion, and intestinal output of cholesterol as well as its metabolites. In agreement with our results, Yugarani et al. (1992) observed the same effect of feeding high fat diet in rats. This finding could be explained as that the feeding with atherogenic diet leads to increasing cholesterol absorption and hence plasma cholesterol increment (Yugarani et al., 1992).

Concerning serum triglycerides level, the present finding is well agreed with the study of Yugarani et al. (1992) who demonstrated that plasma triglycerides level increased significantly after feeding rats with HFD indicating that the increasing in triglycerides is of dietary origin.

Regarding to plasma low density lipoprotein (LDL) and high density lipoprotein (HDL) levels in rats fed with HFD, the current results are in good agreement with those of Shanmugasundaram et al. (1986). The increment of plasma LDL level after HFD consumption could be explained via involvement of two enzymes namely cholesterol ester hydrolase (CEH) and cholesterol ester synthetase (CES). These enzymes balance the cholesterol levels in the blood. Hence, it is logical to assume that the elevation in plasma cholesterol is mediated through increased cholesterol turnover and influenced by the relative balance between CEH and CES activity. With increased estrifying activity (when CEH:CES is lowered) cholesterol will be predominantly in its ester form (as in LDL) and can lead to the development and progression of atherosclerosis. Kritchevsky et al. (1982) observed an increase in each of CES and CEH activities during hyperlipidemia but the synthetase activity has been found to be much more pronounced causing CEH:CES to decline sharply. Therefore, under conditions favoring increased cholesterol turnover, cholesterol ester tends to accumulate leaving plasma LDL levels high (Shanmugasundaram et al., 1986).

The results concerning plasma HDL level in hyperlipidemic group, the present result is well documented by the study of Yugarani et al. (1992). It has been reported that cholesterol transport to extrahepatic tissues is primarily ensured by LDL while HDL has an important role in reversing the cholesterol transport process (Gurr et al., 1989). Hypercholesterolemia is an important etiological factor in coronary heart disease(CHD). Studies have shown that the risk of developing CHD is linearly related to serum cholesterol concentration and low density lipoprotein cholesterol (LDLC), while high density lipoprotein cholesterol (HDLC) exerts a protective effect (Mattson and Grundy,1985).

With respect to the effect of HFD on the markers of oxidative stress, the present study showed significant increase in serum NO and MDA levels. 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. Therefore, 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 (Esposito et al., 1999).

Due to fat accumulation as a result of HFD, it has been found that mRNA expression levels of NADPH oxidase subunits increased, and mRNA expression levels and activities of antioxidant enzymes decreased. Also, a high level of mRNA expression of the transcription factor PU.1, which up regulates the transcription of the NADPH oxidase gene in adipose tissue has been detected. Weisberg (2003) reported that macrophages infiltrated the adipose tissues and were an important source of inflammatory cytokines. Macrophages are also known to produce ROS, and it is possible that infiltrated macrophages are involved in augmenting NADPH oxidase and elevating ROS. Moreover, it has been shown that ROS could increase the expression of MCP-1, a chemoattractant for

monocytes and macrophages in adipocytes. Byproducts of lipid peroxidation by ROS, such as *trans*-4-hydroxy-2-nonenal and malondialdehyde, are themselves potent chemoattractants. Therfore, it is possible that increased ROS production and MCP-1 secretion from accumulated fat should cause infiltration of macrophages oxidative stress and inflammation.

Concerning the effect of HFD on serum NO level, The present results are agreed well with those of Haghjooyjavanmard et al. (2009). Hyperlipidemia increases superoxide formation which is responsible for increasing peroxynitrite that resulted from the reaction of singlet oxygen radical O_2^- and nitric oxide radical (NO) (Moran et al., 2010).

Adipocytokines such as leptin, tumor necrosis factor alpha (TNF-α) and adiponectin, synthesized and secreted by adipocytes, have been found to be linked to hyperlipidemia (Schuldiner et al., 2001). Dysregulated production of "offensive" adipocytokines is critically involved in pathogensis of metabolic syndrome, the serious complication of hyperlipidemia (Furukawa et al., 2004).

The current results indicated that there is significant increase in serum leptin level due to feeding of rats with HFD. This finding coincides with that of Schwartz et al. (1996). 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. The mechanism for the development of peripheral leptin resistance remains to be determined. In general leptin resistance in hyperlipidemia could be due to receptor defects, post-receptor defects or disruption of any of the integrative neuronal circuits necessary for leptin action (English and Wilding, 2006).

The present results showed significant increase in serum TNF-α in rats fed with HFD. This result is greatly supported by that of Margoni et al. (2010) who reported that the increase in serum TNF-α level in subclinical dyslipidemia suggests that TNF-α level is useful indicator of inflammation and a tool to optimize therapeutic management and reduce hyperlipidemic complications. More than one study have shown a positive correlation between adiposity and adipose content of TNF-α. Xu et al. (2002) have shown that that TNF-α mRNA is over expressed in adipose tissue of Zucker fatty rats.

The results of the present study showed significant deacrease in serum level of adiponectin in rats fed with HFD. Consumption of HFD. is associated with reduced adiponectin RNA expression accompanied by deacreased protein levels in adipose tissue. (Furukawa et al.,2004). Fat accumulation closely correlated with markers of systemic oxidative stress. They also demonstrated that adiponectin levels correlated inversely with systemic oxidative stress. Increased oxidative stress due to accumulated fat leads to dysregulated production of adipocytokines. Also, the selective increase in ROS production in accumulated fat, leads to elevation of systemic oxidative stress that leads to more and more inhibition to adipocytokines including adiponectin. In cultered adipocytes, it has been demonstrated that excess of oxidative stress supressed mRNA expression and secretion of adiponectin (Furukawa et al., 2004).

The current results revealed that administration of methanolic extract of *Caulerpa prolifera* extract in rats resulted in significant deacrease 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 *C. prolifera* have been reported to have hypolipidemic activities, as indicated by decreasing serum cholesterol, triglyceride, and LDL levels in rats. These findings could be explained as, the macroalgal polysaccharides including caulerpyne have a hypolipidemic effect due to the reduction of cholesterol absorption in the gut (Kim et al., 2006).

Caulerpa also contains carotenoids which are well known as powerful antioxidants, as well as vitamins C , E. Murillo (1992) reported that β−carotene had significant hypocholesterolemic effect as it has been found to reduce cholesterol biosynthesis, increased HDL level and decreased LDL level. The decrease in the oxidation of these lipid-carriers reduce the risk of atherosclerosis and coronary heart disease. The possible mechanism for lowering plasma cholesterol in animals fed on diet containing carotenoids is the

inhibition of cholesterol synthesis through the inhibition of β-hydroxy-β-methylglutaryl CoA (HMG-CoA) synthesis. This enzyme involves in cholesterol biosynthesis, which is expected to parallel to the activity of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis. Also, β−carotene has been found to have hypotriglyceridemic effect, through increasing the activity of triglyceride lipase and phospholipase. These are key lipolytic enzymes in the metabolism of triglyceride and phospholipids. Vitamins C and E with a consequent great inhibition of MDA, might explain the significant decline in plasma triglycerides, total cholesterol, and LDL as they could preserve LDL-cholesterol from the peroxidation phenomenon. Furthermore, these vitamins have a direct effect on plasma cholesterol levels because they contribute to inhibiting cholesterol biosynthesis (Naziroglu,2003). Elevated HDL levels due to vitamins C and E supplementation are associated with the lowering levels of very low-density lipoprotein cholesterol (VLDL) as well as triglycerides levels but low-density lipoprotein (LDL) cholesterol levels may be within the reference range.

The present data showed that oral administration of *C. Prolifera* extract in hyperlipidemic rats led to significant decrease in serum MDA and NO levels as markers of oxidative stress. Oxidative stress refers to the imbalance between the generation of highly reactive nitrogen and oxygen species and their removal through the antioxidant defence systems. The current result led to the conclusion that this extract possess an antioxidant activity and free radical scavenging property. This finding could be attributed to its content of terpenoid compounds (Abdel-Wahab et al., 2005) as well as minerals and antioxidative vitamins. Caulerpa species also contains polyphenols (flavonoids) besides terpenoids (Glombitza and Keusgen, 1995) Terpenoids have been shown to possess antioxidative properties in different situations, particularly against lipid peroxidation as a result of their high lipophilicity.

Green algae also provided a worthwhile source of vitamin C and E, the average content of vitamin C in green algae is between 500 to 3000 mg/kg. . Vitamin C is well known apotent free radical scavenger and a powerful antioxidant agent. Therefore, it is evident that vitamin C and vitamin E play a major role in the protection against free radical-mediated tissue damage through inhibiting the production of MDA a product of lipid peroxidation. (Stadtman, 1991).

Additionally, these marine algae contain a specific type of marine sesquiterpenoid compound which has antiinflammatory activity beside its ability to inhibit inducible nitric oxide syntheses (iNOS) activity (Lucas et al., 2003).

Phenolic compounds or falavonoids in Caulerpa play an important role in plant resistance and defence against microbial infections which are intimately connected with ROS since these compounds possess potent antioxidant properties. These properties include the inhibition of the production of ROS, i.e. they function as “down”-regulators of activated leukocytes, inhibitors of leukocyte activation or inhibitors of oxygen activating enzymes such as xanthin oxidase or myeloperoxidase. Phenolics are also able to act as radical scavengers or radical-chain breakers, thus extinguishing strongly oxidative free radicals such as the hydroxyl radicals. Moreover, they also may react with non-radical species such as hypochlorous acid or ONOOH yielding products with much low oxidative capacities (Gotoh et al., 1996). Finally it has been shown that caulerpenyne, the indole derivatives, could supress NO production by inhibiting inducible nitric oxide synthase (iNOs) expression in activated macrophages (Dey et al., 2006).

The present results revealed that administration of methanolic extract of *C.prolifera* resulted in significant deacrease in proinflammatory markers such as leptin and TNF-α in hyperlipidemic rats. In agreement with our results, caulerpenyne in Caulerpa species possesses antiinflamatory activity. This effect of caulerpenyne may be mediated via inhibition of cycloxygenases and/or lipoxygenases and other inflammatory mediators. De Souza (2009).
caulerpenyne could decrease the neutrophil counts in relation to other leukocytes suggesting that it is capable to suppress neutrophil recruitment to the inflammatory sites (De Souza ,2009). It is well-known that reactive oxygen species, nitric oxide and prostaglandin (PGE2) are considered as inflammatory factors, and play

important roles in damage of tissues by inflammation . It has been suggested that inhibition of endogenously generated prostaglandins is one of the mechanisms by which the pro-inflammatory effects of the neutrophils are limited by caulerpin (Oktar, 2004).

C. prolifera with high level in vitamin C is responsible for the decreasing serum leptin level as vitamin C causes a dramatic concentration dependent fall in leptin secretion. Vitamin C has aspecific effect in isolated rat adipocytes on glucose and fat metabolism and on secretion/expression of important obesity-related proteins. (Garcia-Diaz et al., 2010)

The anti-inflammatory activity of caulerpin probably involves an antioxidant effect and The indole group of caulerpin probably is responsible for the anti inflammatory activity (De Souza et al., 2009). Indole derivatives have been reported to deacrease TNF-α production and interleukin 1β (1L-1β) mRNA expression in activated macrophages (Dey et al., 2006)

Gamma tocopherols in *C. prolifera* have been shown to regulate cell signalling and gene expression, to affect inflammation and to play a role in the preservation of endothelial function. Some of the beneficial effects of vitamin E is the inhibition of protein kinase C, possibly through nuclear factor-*κ*B inhibition (Evans et al., 2002). Therfore γ-tocopherol in *Caulerpa* also possesses anti-inflammatory effects. it has been reported that tocopherol has an effect on signal transduction pathway of the proinflammatory stimulations including TNF-α (Muller et al., 2004)

Regarding the elevated adiponectin level in hyperlipidemic rats trated with *C.prolifera* extract, it has been suggested that, vitamin E (tocopherol) in *C. prolifera* is responsible for this effect. Tocopherol *in vivo* has been confirmrd as tocopherols could act as peroxisome proliferator activated receptor gamma (PPARγ) ligands (De Pascale et al., 2006) via induction of PPRγ expression in adipocytes (De Pascale et al., 2006). The increase of PPARγ expression together with an activation of PPARγ could be responsible for adiponectin induction. Also the direct effect of tocopherols on the induction of adiponectin in mice of both mRNA and protein levels has been reported by Landrier et al. (2009).

5. Conclusion

In conclusion, the present study provides an obvious evidence that *Caulerpa prolifera* extract hold the promise of being useful hypolipidemic, antilipid peroxidative and anti inflammatory agent. Tus, this work may lead to the development of novel and cheap natural raw materials that may possess vital applications for treatment of dyslipidemia and its serious complications. On the whole, this study highlights the need to include many overlooked marine macroalgae in ongoing and planned research on the development of health promoting diets.

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Correspondence to:

Hanna Hamdy Ahmed

National Reasearch center, Dokki, Cairo, Egypt

Phone: +202-22628439; Cell phone: 0123661935

Email: Hannaomr@yahoo.com

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