ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Bioactivity of *Enteromorpha intestinalis* Polysaccharide Algae Against Hypercholestrolemic Diabetic Rats.

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**ABSTRACT**

The aim of this study was to evaluate the bioactivity of the polysaccharide obtained from aqueous extract of *Enteromorpha intestinalis* algae on hyperlipidemia, hypercholesterolemia and hyperglycemic rats fed with high fat and high cholesterol diets. Type 2 diabetes was induced by introduction of high fat diet as well as daily oral injections of cholesterol for twelve consecutive weeks, followed by induction of insulin insufficiency achieved by injection of low dose of streptozotocin. Lipid profile, diabetic and inflammatory markers, liver, kidney and heart functions besides oxidative stress were determined before and after treatment with the algal polysaccharide and compared with reference drugs (glibenclamide along with fenofibrate). These biochemical studies were supported by the histopathological investigation of liver, kidney, pancreas and heart tissues. The obtained results showed that oxidative stress and inflammatory markers associated with hypercholesterolemia and hyperglycemia were significantly increased in rats fed high fat diet. The histopathological examination showed alteration in structure of liver, kidney, pancreas and heart tissues. Treatment with the polysaccharide extract or with reference drugs improved both biochemical and histological alterations. In conclusion the present study confers the potential effect of the extract to improve lipid profile, reduce hyperglycemia and enhance insulin secretion. Its antioxidant activity may protect against cardiovascular diseases, as well as improvement in liver and kidney disorders.

**Keywords:** *Enteromorpha intestinalis*, hyperglycemia, hypercholesterolemia, diabetes, oxidative stress.

https://doi.org/10.33887/rjpbcs/2019.10.4.13

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ISSN: 0975-8585

## INTRODUCTION

Hypercholesterolemia (HC) caused by obesity is a polygenic condition that has become a very concerning public health problem and may consequently result in many disorders [1]. It is frequently associated with oxidative stress and release of inflammatory cytokines and results in the formation and accumulation of plaque deposits in the arteries, where it produces numerous functional and structural alterations in the vascular wall that lead to the development of atherosclerosis or coronary heart disease (CHD) [2].

Diet-induced HC has been recorded to adversely influence the health of humans and animal species. High level of blood cholesterol is usually associated with an increased risk for the development and progression of coronary artery disease and consequently ischemic heart disease [3].

Cardiovascular diseases, type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease and cancer stand out among the main health problems that are accountable for morbidity associated with obesity. Obesity management and the treatment of its associated complications has led to considerable cost increases in healthcare [4].

Fibrates (fenofibrate) are a class of lipid-lowering drugs that trigger the peroxisome proliferator-activated receptor-alpha (PPAR-alpha). Fibrates decrease triglyceride levels and increase high density lipoprotein-cholesterol (HDL-C) levels while the effect on low density lipoprotein-cholesterol (LDL-C) levels varies [5].

Glibenclamide also known as glyburide, is a class of medications known as sulfonylureas, used in treatment of non-insulin dependent diabetes mellitus. It promotes insulin secretion through inhibition of ATP-sensitive $K^+$ (KATP) channels in the pancreatic cells [6].

The medical treatment and reduction of the effects of diabetes and hyperlipidemia are key modalities in the prevention of heart disease. There is an urgent need for more effective and alternative treatment options that ameliorate the adverse effects accompanied fenofibrate and glibenclamide therapy in hyperlipidemic diabetic patients [7].

In the last decades, sulfated polysaccharides of algal origin have attracted much attention as functional additives in the pharmaceutical field, in food and cosmetic industries [8].

The polysaccharides isolated from brown and green seaweeds have been shown to have therapeutic properties for health and disease management, such as anticancer, antiobesity, antidiabetic, antihyperlipidemic, antioxidant, antinflammatory, immunomodulatory and others [9].

Scientific researches have revealed various hypolipidemic and fat-lowering compounds from natural sources, such as medicinal plants algae that are now being studied as prospective sources of these products [10].

*Enteromorpha intestinalis* (*E. intestinalis*) is a green alga species, frequently found in the coastal zone of seas and oceans. As a cosmopolitan species, it is settling a majority of habitats connected with salty and slightly salty waters [11]. *E. intestinalis* polysaccharide exhibits significant hypolipemic and anti-aging activities and could promote both cellular and humoral immunity to inhibit the tumor growth [12].

This current study confers on seaweed potency to be used in reduction of lipid profile parameters as well as in lowering hyperglycemia, protecting from cardiovascular diseases and enhancing insulin secretion.

## MATERIAL AND METHODS

### Collection of the algal sample

*E. intestinalis* (family: *Chlorophyceae*) was collected from the coast of Abukir, Alexandria, Egypt. Herbarium specimens of the algae were identified by Dr. Shaalan S. A, Professor of Phycology, Faculty of Science, Alexandria University and have been deposited under no. at Pharmacognosy Department, NRC, Egypt.

**Extraction and purification of aqueous soluble polysaccharide**

Extraction and purification of the aqueous polysaccharide was carried out according to the method of Matloub *et al*. [13].

**Chemicals**

All chemicals and reagents were purchased from Biodiagnostic Company for diagnostic and research reagents (Egypt) and Randox diagnostic kits (United Kingdom). Standard drugs (Fenofibrate) and (Glibenclamide) were purchased from Novartis Pharmaceuticals.

**Experimental design**

Fifty male Wister rats, aged 8-12 weeks and weighing 110 - 120 g, provided by the Animal House of the National Research Centre were used. This study was approved by the Ethical Committee of the National Research Centre (NRC), Egypt (ethical approach number:10 133). Animals were randomly divided into five groups of ten animals each as follow:

**Group 1**: (control group), receiving normal diet.
**Group 2**: (control + extract), receiving normal diet and a daily oral dose of 200 mg/kg of aqueous *E. intestinalis* polysaccharide extract for four weeks.
**Group 3:** Hyperglycemia associated with hypercholesterolemia module (HC-HG group), receiving high fat diet and oral cholesterol at a dose of 30 mg/kg body weight five times a week for 12 weeks [14] then injected once (i.p) with low dose of streptozotocin (STZ) (40 mg/kg) [15]. Glucose was measured after 72 hours for development of hyperglycemia.
**Group 4**: (HC-HG + Ext.), HC-HG rats orally administered polysaccharide extract.
**Group 5**: (HC-HG + Reference drugs), HC-HG rats receiving daily an oral dose of the standard antihyperlipidemic reference drug, fenofibrate (10 mg/kg body weight) [16] and antihyperglycaemic reference drug glibenclamide (10 mg/kg) for four weeks [17].

**Blood collection and tissue sampling**

Upon completion of the study, rats were fasted overnight, anaesthetized, blood samples collected from retro-orbital sinus, serum separated and kept at −80°C till used for biochemical investigations of lipid profile, liver, kidney functions and inflammatory markers. The rats were then sacrificed by cervical dislocation using diethyl ether anesthesia. The liver was rapidly removed, washed in saline, dried on filter paper and weighed. Each liver was then cut into two parts; one part was immediately preserved in 10 % buffered formalin at 4°C for histopathological examination; the other part was homogenized in phosphate buffer and stored at – 80 °C for further biochemical analysis. Also, the kidney, heart and pancreas tissues were rapidly excised, immediately preserved in 10% buffered formalin, embedded in paraffin and stained by hematoxylin & eosin stain according to Bancroft and Layton [18].

**Biochemical examination**

Both alanine aminotransferase (ALT) and aspartate aminotransferase (AST), activities were assayed according to the method of Reitman and Frankel [19], gamma glutamyl aminotransferase (GGT) by the method of Szasz [20]. Alkaline phosphatase (ALP) activity, total bilirubin (TB), total protein (TP) and albumin (ALB) were determined colorimetrically according to the methods of Belfield and Goldberg [21], Walters and Gerade [22], Bradford [23] and Doumas *et al*. [24] respectively.

Urea and creatinine levels were measured according to the method of Patton and Crouch [25] and Henry [26] respectively.

Serum TC [27], TG [28], HDL-C [29] and total lipids (TLs) [30] levels were determined. Very low-density lipoprotein-cholesterol (VLDL-C) and LDL-C were calculated using standard method of Schriewer *et al.* [31].

Fasting blood glucose level was estimated according to method of Trinder [32] and insulin level was measured by Ultrasensitive rat insulin ELISA kit from Mercodia (Sweden) according to the method of Korner *et al*. [33].

Serum total lactate dehydrogenase (LDH) activity was measured according to Babson and Babson [34] and serum creatine kinase (CK-MB) by Wicks *et al*. [35]

Serum level of interleukin-6 (IL-6) and C-reactive protein (CRP) were estimated by ELISA (Kamiya biomedical) company (USA) kits using methods suggested by Hirano [36] and Tracy *et al.* [37] respectively.

Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and nitric oxide (NO) were measured according to Nishikimi *et al.* [38], Aebi [39], Griffith [40] and Allen *et al*. [41] respectively.

Biochemical data were statistically analyzed by one-way analysis of variance (ANOVA), using the Statistical Package for Social sciences (SPSS for windows, version 22.0) followed by non-parametric post hoc test (LSD) (least significant difference) using CoStat Computer programme (CoHort software, version 6.303, Monterey, United States), where unshared superscript letters indicate that these groups are significant at $P \leq 0.05$.

## RESULTS

The total carbohydrates, free sugars and total polysaccharides as well as polysaccharide of *E. intestinalis* were recorded in table (1). In addition, chemical characterization of polysaccharide isolated from aqueous extract of *E. intestinalis* (ECEM) were recorded in table (2).

The FT–IR spectrum data of ECEM scanned between wave number 4000 and 400 $cm^{-1}$ revealed the characteristic absorption bands of polysaccharide at 3403.74 and 2928.38 $cm^{-1}$ attributed to the hydroxyl group and alkyl group, respectively. Meanwhile, the FT–IR spectrum data showed absorption bands at 1646.91& 1635.34 $cm^{-1}$ assigned for C=O stretching in secondary amides.

The band at 1448.28 $cm^{-1}$ attributed to the symmetric stretch vibration of COO− and the stretch vibration of C-O within COOH. Whereas, the bands at 1145.51 $cm^{-1}$ assigned to the glycosidic linkage stretch vibration of C–O–C and C–O–H. While, the absorption band at 1253.50 $cm^{-1}$ was attributed to S=O stretching vibration indicating the presence of esterified sulfate. Moreover, the band at 870.70 $cm^{-1}$ was attributed to β-configuration of glycosidic linkage. While, the signal at 755.959 cm−1 might correspond to the bending vibration of C–O–S of sulfate in equatorial position.

This amalgamation indicates that the isolated polysaccharide from *E. intestinalis* was sulphated polysaccharide that is associated with protein and containing sulfate ester.

In the present study the effects of aqueous polysaccharide extract on the levels of serum ALT, AST, GGT, ALP, TB, TP, ALB, urea and creatinine were presented in table (3). Whereas its effects on the levels of TC, TG, TLs, LDL-C, HDL-C, VLDL-C, atherogenic index (AI), CK-MB and total LDH were presented in table (4).

Data recorded in table (5), demonstrated the effect of aqueous polysaccharide extract on the level of serum glucose and insulin during the experiments time. Its effects on the level of hepatic GPx, SOD, NO, CAT together with serum IL-6 and CRP were presented in table (6).

The effects of aqueous polysaccharide extract on liver, kidney, pancreas and heart tissues were presented in figures (1A-1E, 2A-2E, 3A-3E, 4A-4E).

## DISCUSSION

Hyperlipidemia is a common disease with the rapid improvement of economy and quick changes in diet. It involves abnormally elevated levels of one or more of lipids and/or lipoproteins in blood. The incidence of hyperlipidemia is threatening people's health. A number of studies indicated that hyperlipidemia is an independent risk factor for stroke, coronary heart disease and sudden cardiac death [42].

Diabetes mellitus is a metabolic disease resulting from defects in insulin secretion or insulin action or both. This disease is often associated with high blood glucose (hyperglycemia), increased levels of TC, TG, LDL-C (hyperlipidemia), and decreased levels of HDL-C. The hyperglycemia can increase the incidence of many complications in diabetic patients, while hyperlipidemia contributes to the development of coronary heart diseases, vascular endothelial dysfunctions, increased inflammatory cells and atherosclerosis [43]. Therefore, correcting the level of blood lipids efficiently has significant value to improve the severity, deliberate the development and reduce the existence of complications of T2DM mellitus and coronary heart disease [44].

Marine algae produce large amounts of sulfated polysaccharides, as a component of their cell walls, and are widely used in the food and pharmaceutical industry because of their rheological properties as thickening agents [45].

In the present study data revealed that the total polysaccharides of *E. intestinalis* were 19.40 % of dried powder. These results are in agreement with Matloub *et al.* [13]. The total sugar and ash contents were 56.77 % and 18.64% respectively in consistent with Matloub *et al.* [13] and Nguyen *et al*. [46].

Infrared spectrum data of aqueous *E. intestinalis* extract were in consistent with the result of Matloub *et al.* [13], who studied and estimated the composition and chemical analysis of a variety of polysaccharides extracts.

In the current study induction of type II diabetes by high fat diet and low dose of STZ (HC-HG) caused a significant increase in serum enzyme activities (ALT, AST, GGT and ALP) while caused reduction in TP and ALB when compared to control rats. Elevated levels of ALT and AST may be due to high cholesterol diet which induce hypercholesterolemia consequently enhance ALT and AST enzyme activities in serum, resulting in increased enzyme leakage from hepatocytes. The excess of stored fat affects liver functions and increased free radicals lead to liver damage of hypercholesterolemic rats [47] and [48]. While, increased GGT activity can be justified by either obesity and deposition of visceral fat along with insulin resistance induced non-alcoholic fatty liver [49] or as a response to chronic inflammation and oxidative stress associated with diabetes mellitus [50]. The results are in agreement with those of Soliman *et al*. [51]. In the present study serum ALP enzyme activity exhibited significant derangement in diabetes mellitus in consistent with the results of Bora *et al.* [52]. While, the significant reduction in serum TP and ALB content in HC-HG rats may be justified by the anabolic effect of insulin on protein metabolism as it stimulates protein synthesis [53]. These results are in agreement with those of Hamed *et al*. [54], who observed that serum TP and ALB decreased in HFD-STZ rats due to increase in protein catabolism and increase in albumin excretion in urine.

In the present study HC-HG treated rats with aqueous polysaccharide extract as well as reference drugs caused a significant reduction in ALT, AST, GGT and ALP enzyme activities and significant elevation of serum ALB when compared to untreated HC-diabetic rats but with better improvement with treated extract than with reference drugs. This ameliorative effect emphasis the hepato-protective activity of *E. intestinalis*. These findings are in agreement with the results of Rizk *et al.* [55], who showed the hepato-protective effect of sulfated polysaccharide from green alga *Ulva fasciata* extract, consequently polysaccharide extract expected to be effective in recovery of hepatic function by restoring lipid metabolism and delaying hepatic disordered.

Results of HC-HG rats in the current study showed significant elevation in serum urea and creatinine levels as compared to control rats. This may be contributed to increased protein catabolism and accelerated amino acid deamination for gluconeogenesis [56]. These findings were in agreement with the results of Krishnasamy *et al*. [57], who observed an increase of both serum urea and creatinine levels in STZ- induced diabetic rats which could be explained on basis of occurrence of renal dysfunction or dehydration. In the present study administration of algal polysaccharide extract to HC-HG rats results in significant decrease of urea and creatinine levels compared to HC-HG untreated rats. This results are in accordance with the results of Rizk *et al.* [55], who declared that polysaccharide of green alga *U. fasciata* caused significant decrease in serum urea and creatinine levels.

In this study HC-HG rats administered high fat diet accompanied with a daily oral injection of cholesterol resulted in a significant increase in TC, TG, TLs, LDL-C, VLDL-C and AI levels and significant decreased in HDL-C as compared to control rats which is in harmony with the findings of Antony *et al*. [58] and Abdel-Rahman *et al*. [59] whom attributed the effect of hypercholesterolemia to the increased dietary cholesterol intake which in

turn, increased rate of intestinal cholesterol absorption. The increased LDL-C level is one of the most important risk factors for CVD in which, LDL-C particles are taken up by macrophage cells after oxidized then deposited in the arterial intimae leading to formation of atheroma [60]. Elevated level of LDL-C found in HC-HG rats may be due to a down regulation in LDL-C receptors by cholesterol and saturated fatty acids included in the diet [61]. Treatment with aqueous polysaccharide extract as well as glibenclamide along with fenofibrate caused significant decrease in the levels of serum TC, TG, TLs, LDL-C, VLDL-C and AI level and a significant increase in HDL-C level compared to HC-HG group. These results suggested that polysaccharide extract exhibit hypolipidemic activity almost as potent as fenofibrate. These results are in agreement with the previous results on some polysaccharides extracts in which, they exhibited anti-hyperlipidemic effect [62] or attributed to its antioxidant potential to decompose free radicals species generated during cholesterol administration [63].

In the present study HC-HG rats showed a significant increase in the activity of serum CK-MB and total LDH when compared to the normal rats, in accordance with the results of Ding *et al.* [64]. These findings could be explained by the fact that reduced muscle mitochondrial content function with increasing obesity would reduce the total cellular ATP yield, which would result in increased mitochondrial volume, and increased glycolytic enzymes requiring increased activity of creatine kinase, as this enzyme is responsible for rapidly transferring high-energy phosphate groups from the site of production to the site of use [65].

The present high activity of serum CK and LDH demonstrated that the cellular membranes integrity of myocardial tissues might be disturbed. Moreover, several studies showed that the levels of CK and LDH were significantly increased in diabetic rats [66] and may indicate incidence of CVD as consequence of obesity [67].

Treatment of HC-HG rats with aqueous polysaccharide extract as well as reference drugs (glibenclamide along with fenofibrate) caused a significant lower level of CK-MB and total LDH compared to HC-HG untreated rats, which confirms that the extract under study possess potent activities against heart injury and may lead to reduction in the risk of developing heart diseases. These results are coincidence with the results of Mahmoud *et al*. [68].

The present study simulated a distinct hyperglycemic state where the fasting blood glucose (FBG) levels in HC-HG rats reached 4.8 times the control levels and significantly decreasing in serum insulin level as compared to control, simulating diabetes mellitus due to induction of insulin resistance condition by high fat diet for 12 weeks followed by controlled destruction of β-cells by inflammation due to low dose of STZ. This can be justified by systemic insulin resistance induced by prolonged high fat diet then insulin insufficiency induced by low dose STZ that induced alkylation of β-cells DNA along with its affinity to act as an intracellular nitric oxide (NO) donor, as well as increased oxidative stress and activated inflammatory responses (cytokine activation) [69].

After four weeks of treatment by aqueous polysaccharide extract significant decrease in FBG levels and increase in insulin level were observed compared to HC-HG rats. Also, a significant decrease in FBG was shown after treatment with reference dugs compared to HC-HG untreated rats, in consistence with several publications that studied glibenclamide's effect in diabetic rats as reported by Ahmadi *et al.* [70]. This glucose lowering effect of sulfated polysaccharide may be attributed to gluconeogenesis and the regulation of lipid levels [71]. The reduction in blood glucose levels exhibited by glibenclamide was also altered by the presence of fenofibrate since cytochrome $P_{450}$ $3A_4$ ($CYP3A_4$) is the major enzyme in the metabolism of glibenclamide, its metabolic process could have been altered by some CYP inducers or inhibitors [72]. Therefore, aqueous polysaccharide extract of *E. intestinalis* seems to have promising value for the development of potent hypoglycemic phytomedicine.

The results of the present study elucidated that the induction of diabetes by high fat diet and low dose of STZ displayed a significant elevation in hepatic GPx, SOD, NO and CAT levels compared to control rats. This result was in conformation with the findings of Salmanoglu *et al*. [73]. This significant increase in $GP_X$ can be attributed to $GP_X$ being an enzyme whose function is to protect erythrocyte membrane against oxidation. Therefore, it regulates concentration of hydrogen peroxide in tissues that do not contain catalase or show its insufficient activity [73]. Previous report suggested that the increased activity of SOD was accompanied by the increase in the activity of CAT [74]. This finding can be explained by compensatory adaptation of organism to oxidative stress in high fat diet induced obesity, different body organs might response to oxidative stress selectively by decrease or increase in the concentrations of markers of oxidative stress [75]. Findings of the present study were in parallel with the experiment previously performed by Rizk *et al.* [55], where induction of

diabetic condition through high fat diet and low dose STZ caused a significant increase in hepatic NO level. Also, in agreement with results of Morsy *et al*. [76], who declared that HFD-STZ induced elevation of NO in diabetic rats. This results can be understood by the hypothesis that generated NO maintains the hepatic microcirculation and endothelial integrity, while inducible NO synthase (iNOS)-governed NO production which can either potentiates the hepatic oxidative injury in ischemia/reperfusion or iNOS expression that protects against hepatic apoptotic cell death [77]. On the other hand, treatment with aqueous polysaccharide extract as well as reference drugs induced significant reduction in hepatic GPx, SOD, NO and CAT compared to HC-HG untreated rats which represented that algal extract exhibit antioxidant activity. These results can be fortified by the fact that many other members of the green and brown algae were proved to possess an antioxidant activity. The antioxidative effect of *ulvan* (green algae) sulphated polysaccharide cause increased HDL-C level and decreased LDL-C level [55]. This improvement in oxidative stress could be explained by the predominant component sugars in the hydrolysates of polysaccharide extract (rhamnose, xylose and galactose). Therefore, these species may be of potential sources of natural antioxidants for treatment or prevention of diseases.

The present study showed that induction of rats by high fat diet and low dose STZ caused a significant elevation in serum IL-6 and CRP levels compared to control rats which gave an indication about the existence of pro-inflammatory signaling due to adiposity and pancreatic inflammation [78]. The mechanism of T2DM induction through stimulation of insulin resistance via high fat diet could be explained by excess circulating FFA (free fatty acids) which cause inhibition of insulin signaling through the activation of serine kinases, which in turn encourage the mechanism of serine phosphorylation of insulin receptor substrates (IRS) causing disturbance in insulin receptor (IR) signaling where it has been proved that chronic elevated IL-6 inhibit IR signaling [79]. Treatment of HC-HG rats with aqueous extract caused significant reduction in IL-6 and CRP levels compared to HC-HG rats, these results may be attributed to the interaction of algal sulphated polysaccharide with the complement system suggesting that it might influence the innate immunity to reduce the pro-inflammatory state [60]. The present results were in accordance with the findings of Albuquerque *et al.* [80]. The extract of *E. intestinalis* contained saponins, steroids, terpenoids and alkaloids in which, saponins are known to produce inhibitory effect on inflammation [81].

The histopathological findings showed that the hepatocytes of control rat liver were arranged with well distinct cytoplasm and nuclei, in which sinusoids radiated from the central vein, which agreed with Desai *et al*. [82]. HC-HG rats caused inflammation with fat vacuoles, congestion in the portal vein, focal hemorrhage, apoptosis and fatty changed hepatocytes in the parenchyma. These effects could be atttributed to lipid accumulation in the hepatocytes cell cytoplasm, in coincidence with findings of Wang *et al*. [83] and Rizk *et al.* [55]. Hepatocytes of HC-HG rats treated with aqueous extract as well as reference drugs were improved with fewer endothelium injuries and reduction in fat vacuoles, showing considerable reduction in pathological changes and decrease signs of fatty liver. These findings may be attributed to their hepatoprotective activity due to polysaccharide contents of algal extract [84].

Control kidney revealed normal histology of the glomerulus, well-spaced tubules and normal orientation of nephrons with adequate glomeruli. These findings are in agreement with Matboli *et al*. [85]. Histopathological examination of untreated diabetic kidney tissue showed degeneration in lining epithelium in some of cortical tubules and necrosis with congestion in the glomerlui accompanied with hemorrhage in the corticomedullary portion. These observations were in coincidence with those of Suman *et al*. [86]. However, HC-HG rats treated with aqueous polysaccharide extract and reference drugs, showed normal histology of glomerulus. This result may be attributed to the antinflammatory, antioxidant and hypolipidemic effect of sulphated polysaccharide of algal extract [55].

HC-HG pancreatic tissue sections showed degeneration and atrophy in the islets of Langerhans cells accompanied with congestion in the blood vessels. These observations were in agreement with the findings of Nie *et al.* [87]. HC-HG rats treated with polysaccharide extract and reference drugs caused remarkable amelioration of the pancreatic tissue and islets of Langerhans, in agreement with Lin *et al*. [71].

Control heart revealed normal histology of the myocardial bundles. Histopathological examination of HC-HG heart tissue showed severe congestion in the myocardial blood vessels, oedema with inflammatory cells infiltration in the subendocardium and focal hemorrhage in between the myocardial bundles, these observations were in coincidence with those of Wu *et al*. [88]. However, HC-HG diabetic rats treated with polysaccharide algal extract and reference drugs, showed normal histopathological myocardium. The present findings indicate the

cardiovascular safety profile of algal extract. These results may be attributed to antioxidant, antinflammatory and hypolipidemic effect of sulphated polysaccharide algal extract [2].

**Table (1): Chemical characterization of sulfated polysaccharide obtained from aqueous extract of *E. intestinalis***

| Characters | %Yield content of dried powdered of *E. intestinalis* |
|---|---|
| Total Carbohydrate | 20.34±0.10 |
| Free Sugars | 0.94± 0.009 |
| Total polysaccharide | 19.40 |
| Yield % of cold-water extract | 4.93 |

**Table (2): Chemical characterization of the isolated polysaccharide from tested algae.**

| Characters | % of isolated polysaccharides |
|---|---|
| Total carbohydrate | 56.77 ±0.5 |
| Moisture | 4.88±0.1 |
| Ash | 18.64±0.1 |
| C | 15.50 |
| H | 2.40 |
| S | 10.80 |
| N | 1.20 |
| Protein | 7.50 |
| Degree of sulfation | 8.84 |

**Table (3): Effect of polysaccharide extract on some liver and kidney function parameters.**

| Groups / Parameters | Control ($G_1$) | Control + Ext. ($G_2$) | HC-HG ($G_3$) | HC-HG + Ext. ($G_4$) | HC-HG + Ref. drugs ($G_5$) |
|---|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| **ALT(U/ml)** | $86.13^{d}$ ±4.29 | $85.62^{d}$ ±5.26 | $109.25^{a}$±6.15 | $93.25^{c}$±2.25 | $100.87^{b}$±4.88 |
| **AST (U/ml)** | $125.00^{bc}$ ±2.56 | $122.75^{cd}$ ±5.54 | $240.38^{a}$±10.40 | $115.75^{de}$±3.28 | $131.00^{b}$ ±5.50 |
| **GGT (U/ml)** | $3.50^{e}$ ±0.07 | $3.48^{e}$±0.196 | $5.51^{a}$±0.17 | $3.39^{e}$ ±0.04 | $4.25^{bc}$ ±0.09 |
| **ALP (IU/ml)** | $305.25^{d}$ ±3.37 | $307.00^{d}$ ±5.23 | $509.50^{a}$±3.70 | $312.38^{d}$ ±4.17 | $416.50^{b}$±5.97 |
| **TB (mg/dl)** | $0.34^{ab}$ ±0.06 | $0.33^{ab}$ ±0.03 | $0.36^{ab}$±0.03 | $0.32^{ab}$ ±0.02 | $0.34^{ab}$ ±0.05 |
| **TP (g/L)** | $7.42^{bcd}$ ±0.19 | $7.63^{ab}$ ±0.31 | $7.08^{e}$±0.07 | $7.19^{de}$±0.05 | $7.75^{a}$0.31 |
| **ALB (g/dl)** | $4.34^{b}$±0.14 | $4.74^{a}$ ±0.37 | $3.54^{c}$ ±0.26 | $4.45^{b}$±0.18 | $4.71^{a}$±0.26 |
| **Urea (mg/dl)** | $44.77^{g}$±1.86 | $47.42^{g}$±3.79 | $85.16^{b}$±2.05 | $60.84^{e}$±6.33 | $78.31^{c}$±6.36 |
| **Creatinine (mg/dl)** | $0.62^{d}$ ±0.13 | $0.76^{c}$ ±0.09 | $1.40^{a}$ ±0.08 | $1.14^{b}$±0.08 | $1.36^{a}$±0.09 |

- *Data expressed as mean ± SD (n=10).*
- *Unshared superscript letters are significant values between groups at P≤0.05.*

ISSN: 0975-8585

**Table (4): Effect of polysaccharide extract on lipid profile and some heart function parameters.**

| Groups / Parameters | Control ($G_1$) | Control + Ext. ($G_2$) | HC-HG ($G_3$) | HC-HG + Ext. ($G_4$) | HC-HG + Ref. drugs ($G_5$) |
|---|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| TC (µg/dl) | $87.8^{b}$±4.77 | $89.48^{b}$±10.86 | $237.50^{a}$±39.7 | $95.53^{b}$±4.36 | $66.85^{cd}$ ± 6.03 |
| TG (µg/dl) | $74.50^{c}$±15.29 | $82.30^{c}$±10.61 | $197.13^{a}$±29.03 | $153.80^{b}$±41.06 | $74.00^{c}$±20.48 |
| TLs (mg/dl) | $267.04^{c}$±52.53 | $254.64^{c}$±11.63 | $668.70^{a}$±227.50 | $422.04^{b}$±136.73 | $323.80^{bc}$±74.84 |
| LDL-C (µg/dl) | $48.64^{b}$±6.38 | $46.81^{b}$± 9.35 | $193.81^{a}$±35.17 | $40.40^{bc}$±14.37 | $24.04^{cd}$±13.7 |
| HDL-C (mg/dl) | $24.26^{b}$± 2.43 | $26.25^{b}$± 3.20 | $4.26^{a}$±7.70 | $24.38^{b}$ ±6.25 | $28.00^{b}$±7.89 |
| VLDL-C | $14.90^{c}$±3.06 | $16.42^{c}$ ±2.21 | $39.43^{a}$±5.80 | $30.75^{b}$±8.21 | $14.80^{c}$± 4.1 |
| AI | $2.65^{bc}$±0.48 | $2.42^{bc}$±0.41 | $54.72^{a}$±1.07 | $3.10^{b}$±1.11 | $1.58^{cd}$± 0.78 |
| CK-MB (U/L) | $81.38^{d}$± 3.81 | $74.48^{d}$± 0.60 | $246.01^{a}$ ± 31.58 | $144.50^{c}$± 3.74 | $164.63^{c}$± 6.61 |
| Total LDH (U/L) | $130.50^{e}$± 6.41 | $114.13^{g}$± 1.14 | $239.38^{a}$± 8.34 | $135.83^{d}$± 2.11 | $140.62^{c}$± 0.77 |

- *Data expressed as mean ± SD (n=10).*
- *Unshared superscript letters are significant values between groups at P≤0.05.*

**Table (5): Effect of polysaccharide extract on the levels of serum glucose and insulin.**

| Groups / Parameters | | Control ($G_1$) | Control + Ext. ($G_2$) | HC-HG ($G_3$) | HC-HG + Ext. ($G_4$) | HC-HG + Ref. drugs ($G_5$) |
|---|---|---|---|---|---|---|
| | | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| Serum Glucose (mg/dL) | 1st week before injection with STZ | $104.50^{a}$±21.01 | $95.38^{a}$±10.35 | $98.37^{a}$±13.93 | $104.37^{a}$±20.47 | $98.00^{a}$±12.35 |
| | After 72 hrs of STZ injection | $94.75^{b}$±13.04 | $94.87^{b}$±12.90 | $450.87^{a}$± 49.66 | $435.63^{a}$±74.29 | $475.88^{a}$±61.59 |
| | After 1 week of treatment | $102.50^{c}$±13.69 | $102.75^{c}$±22.70 | $442.00^{a}$±36.66 | $415.88^{a}$±84.68 | $464.63^{a}$±92.21 |
| | After 2 weeks of treatment | $89.00^{c}$±7.71 | $93.88^{c}$±10.94 | $427.50^{a}$±52.61 | $297.80^{b}$±46.54 | $381.13^{a}$±116.28 |
| | After 3 weeks of treatment | $89.92^{c}$±11.83 | $101.49^{c}$±9.82 | $470.53^{a}$±33.62 | $111.40^{c}$±17.66 | $207.13^{b}$±57.64 |
| | After 4 week of treatments | $91.25^{c}$±7.57 | $92.25^{c}$±6.90 | $443.12^{a}$±36.37 | $101.62^{c}$±15.10 | $133.75^{b}$±32.22 |
| Serum insulin (µIU/ml) | | $19.91^{c}$±0.56 | $19.24^{c}$±0.35 | $9.17^{a}$±0.05 | $14.81^{b}$±0.63 | $9.25^{a}$ ±0.29 |

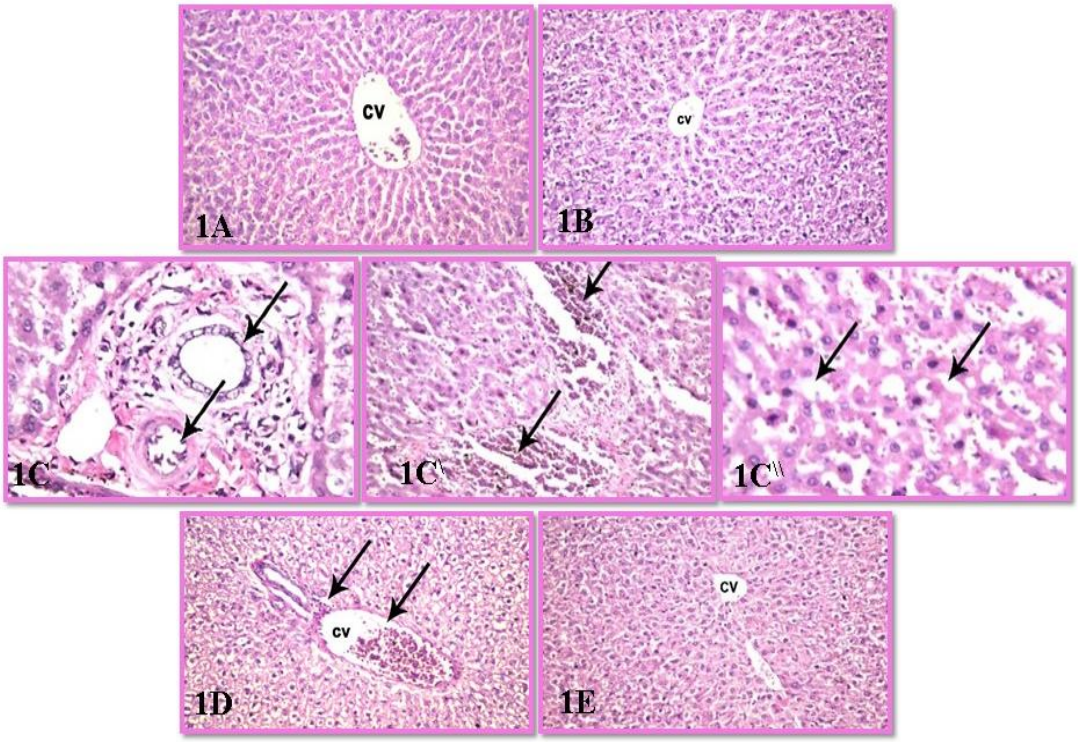
- *Data expressed as mean ± SD (n=10).*
- *Unshared superscript letters are significant values between groups at P≤0.05.*

ISSN: 0975-8585

**Table (6): Effect of polysaccharide extract on some hepatic antioxidants and proinflammatory parameters.**

| Groups / Parameters | Control ($G_1$) | Control + Ext. ($G_2$) | HC-HG ($G_3$) | HC-HG + Ext. ($G_4$) | HC-HG + Ref. drugs ($G_5$) |
|---|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| GPx (U/g tissue) | $758.44^{c}$±20.29 | $422.39^{c}$±38.09 | $7923.80^{a}$±96.37 | $821.94^{c}$±9.23 | $815.42^{c}$±16.53 |
| SOD (U/g tissue) | $193.63^{b}$±5.33 | $56.55^{d}$±2.56 | $574.25^{a}$±35.83 | $136.75^{c}$±7.42 | $188.00^{b}$±2.97 |
| NO (mg/g tissue) | $44.70^{c}$±1.43 | $47.18^{c}$ ±1.48 | $112.59^{a}$ ±1.49 | $53.48^{b}$±1.62 | $54.01^{b}$±1.63 |
| CAT (U/g tissue) | $143.80^{e}$ ±2.08 | $140.73^{e}$±6.23 | $279.64^{a}$±5.01 | $157.65^{d}$±3.54 | $156.76^{d}$±3.51 |
| IL-6 (pg/ml) | $22.84^{g}$±2.08 | $23.75^{g}$±1.80 | $109.25^{a}$±4.59 | $86.45^{b}$±6.11 | $67.24^{d}$±4.31 |
| CRP (ng/ml) | $5.04^{e}$±1.05 | $4.14^{e}$±0.62 | $53.18^{a}$±5.03 | $41.06^{b}$±1.87 | $31.29^{c}$±4.27 |

- *Data expressed as mean ± SD (n=10).*
- *Unshared superscript letters are significant values between groups at P≤0.05.*




**Figures (1A, 1B): Micrograph of control liver (1A) and rat liver treated with extract (1B) showing normal structure of hepatic lobule. Figures (1C,1C/,1C//): Micrograph of liver of rat in HC-HG group showing congestion in portal vein with periductal inflammatory cells infiltration and oedema surrounding the bile duct in portal area (1C), focul hemorrhage in hepatic parenchyma (1C\) and apoptosis in some of hepatocytes (1C\\). Figures (1D, 1E): Micrograph of HC-HG rat liver treated with extract (1D) and reference drugs (glibenclamide along with fenofibrate) (1E) showing normal structure of hepatic lobule (all H&E, X 40 except 1C, 1C\, 1C\\ X 80).**

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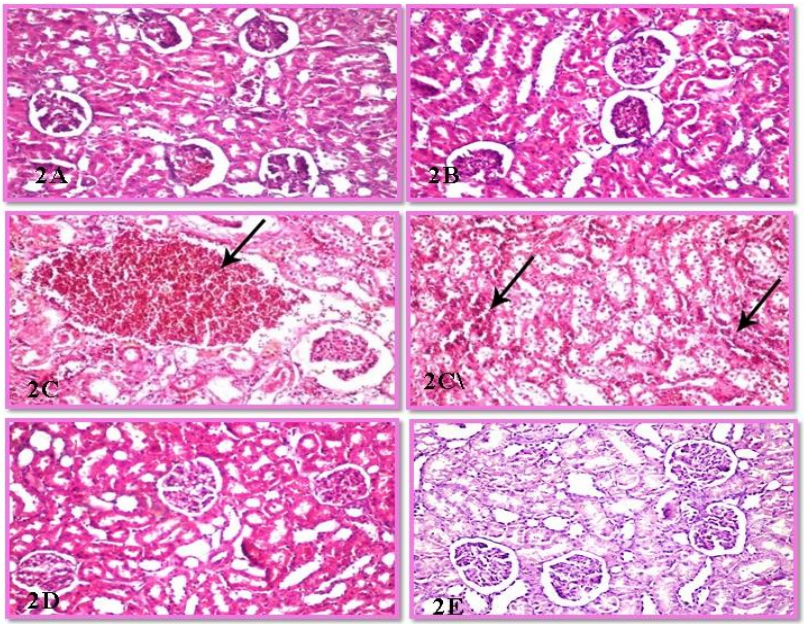
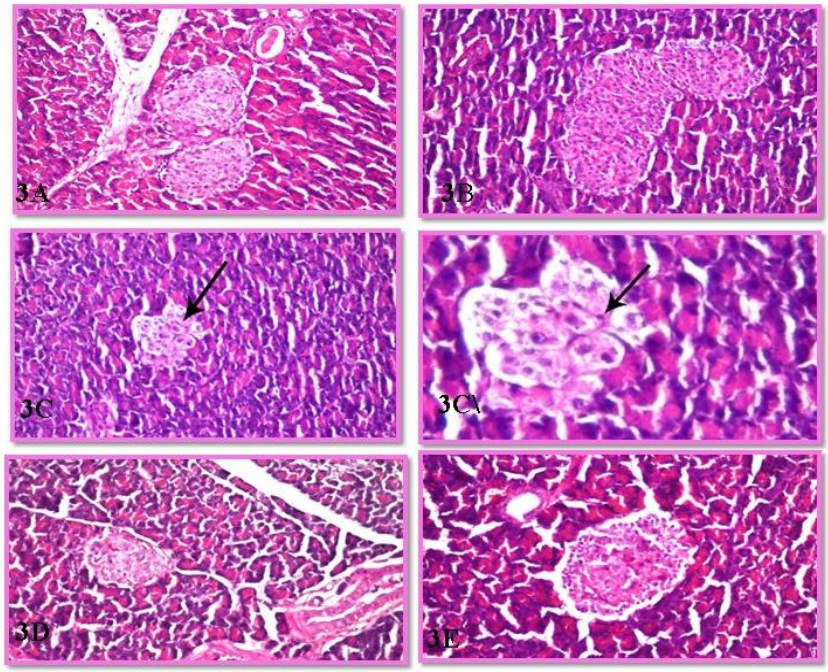
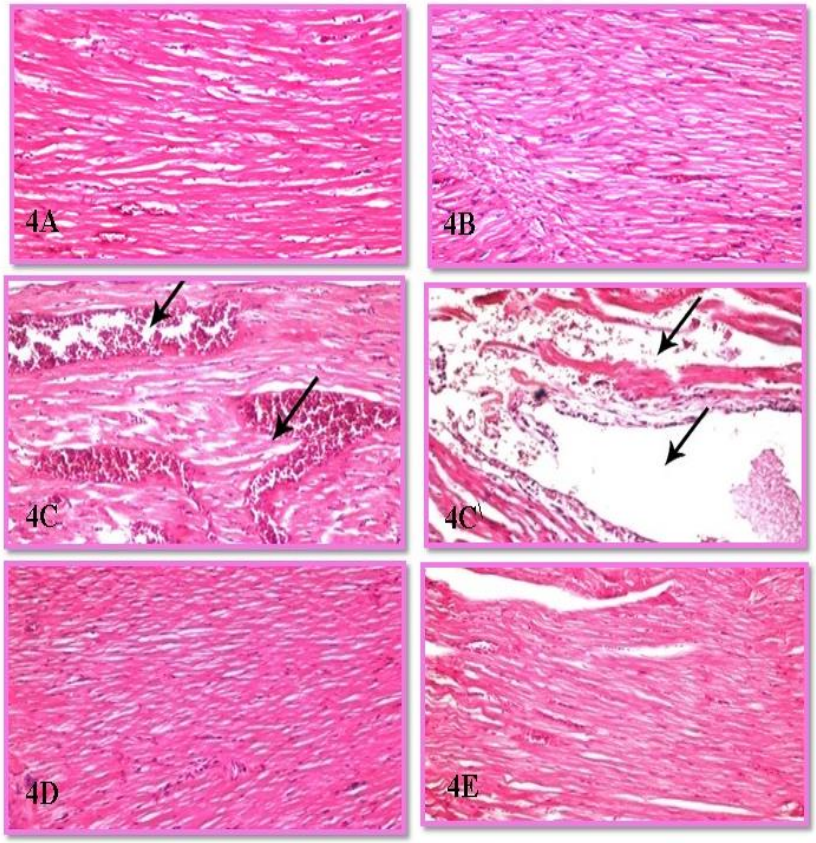



**Figures (2A, 2B): Micrograph of control kidney (2A) and rat kidney treated with extract (2B) showing normal histological structure of glomerlui and tubules at the cortex. Figures (2C, 2C\): Micrograph of rat kidney HC-HG group showing severe congestion of intertubules blood vessels at the cortex (2C) and focal hemorrhage in between the degenerated tubules at corticomedullary portion (2C\). Figures (2D, 2E): Micrograph of rat kidney HC-HG treated with extract (2D) and reference drugs (glibenclamide along with fenofibrate) (2E) showing normal histological structure. (H&E, X 40).**




**Figures (3A, 3B): Micrograph of pancreas of normal rats (3A) and rat pancreas treated with aqueous extract (3B) showing normal histological structure of islands of Langerhans cells as endocrine portion and the acini as exocrine one. Figures (3C, 3C\): Micrograph of rat pancreas HC-HG group showing atrophy in islands of Langerhans (3C) and the magnification of 3C to identify the atrophy in islands of Langerhans cells (3C\). Figures (3D, 3E): Micrograph of rat pancreas HC-HG treated with extract (3D) and reference drugs (3E) showing normal histological structure. (all H&E X 40 except 3C\ X 80).**

ISSN: 0975-8585




**Figures (4A, 4B): Micrograph of heart of normal rats (4A) and heart rat treated with aqueous extract (4B) showing normal histological structure of the myocardial bundles. Figures (4C, 4C\): Micrograph of rat heart HC-HG group showing severe congestion of myocardial blood vessels. (4C), subendocardial oedema and inflammatory cells infiltration (4C\). Figures (4D, 4E): Micrograph of rat heart HC-HG treated with extract (4D) or reference drugs (4E) showing normal histological structure. (all H&E X 40).**

## CONCLUSION

The current study was designed to examine the efficiency of lowering cholesterol and glucose levels together with ameliorating antioxidant activity. Polysaccharide from green algae have been shown to possess significant hepatoprotective, renoprotective, hypocholesterolemic, atheropreventive, cardioprotective, hypoglycemic, antioxidant activities and antinflamatory effects. The present results also indicated that, green alga *E. intestinalis* had a free radical scavenging activity which probably provides organ protection against hypercholesterolemia. This was supported by histopathological examination of hepatocytes, kidney, myocardium and pancreatic tissues.

These results suggest that sulphated polysaccharide algal extract possess the previously mentioned multiactivity through inhibition of ROS. Therefore, marine algae derived sulphated polysaccharide may have great potential for further development as neutraceutical and pharmaceutical products.

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