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# Potential anti-obesity impact of green seaweed; *Enteromorpha intestinalis*

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**Abstract**

Obesity is a global disease that threatens millions of people causes death every year as result of overweight complications. Furthermore, obesity is a main cause of cardiovascular disease and type 2 diabetes. Preventing and treating obesity has been a prevailing concern for many countries also the uptake of natural remedies become a primary focus of researchers to get safer and more efficient drugs. In this article we evaluate the effect of green seaweed; *Enteromorpha intestinalis* as a therapeutic supplement for obesity and obesity-related diseases. Female albino rats were divided into four groups; control negative (CN), control positive (CP) and positive treated with *E. intestinalis* crude extract (M) and *spirulina* powder supplement (SP). Female rats were fed by algal extract for 4 weeks and weight gain change was determined, at the end of the treatment period blood was collected and different biochemical parameters were measured to evaluate the anti-hyperlipidemic, anti-inflammatory, and antiobesity effects of the *E. intestinalis* crude extract compared to *spirulina* microalgae powder supplement on rats fed a high-fat diet. *E. intestinalis* extract (M) showed a significant effect in suppressing weight gain. Also, the M group showed a significant effect in suppressing plasma total cholesterol and triglycerides recording about 118 $mmolL^{-1}$ and 62 $mmolL^{-1}$, respectively which is nearly the same as the control negative group. Also, low-density lipoprotein, leptin, glucagon-like peptide-1 and peptide y y recorded about 60.55 $mmolL^{-1}$, 8.63 $mmolL^{-1}$ 11.93 $mmolL^{-1}$, and 102.8 $mmolL^{-1}$so usage of *E. intestinalis* crude extract has positively effect in treatment and prevention of obesity as well as *Spirulina* supplement.

*Keywords:Enteromorpha intestinalis*, *Spirulina*, anti-obesity, anti-inflammatory.

## 1. Introduction

Obesity is defined as an increase in body weight due to excessive accumulation of adipose tissue that presents a health risk [1]. Obesity is a global disease with at least 2.8 million people dying each year as a result of being overweight or obese according to the World Health Organisation (WHO) figures [2].Due to its high expansion and association with chronic diseases (cardiovascular diseases, insulin resistance, atherosclerosis,.etc.), WHO considers obesity to be one of the most obvious public health problems. Also, endocrinological studies of the adipose tissue reveal that there is a great relationship between obesity and immunity as adipose tissue secretes bioactive molecules and immunomodulators so, obese individuals if attacked by different illnesses are more likely to need more hospitalization or ICU admission than slim individuals[3]. Treatments of obesity include changing lifestyle by eating low-caloric healthy food with high vitamin contents and practicing cardio exercises as well as pharmacological therapy [4],

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[5].Remedies for obesity have been changed over years where, lots of drugs have been excluded due to their serious side effects [5]. Recently, researchers affirmed on development of natural obesity medicines to overcome the side effects of synthetic drugs [6]. Natural polyphenols, alkaloid, saponin and flavonoid-rich sources in addition to fiber-rich natural sources are being used as a complementary natural remedy for obesity [6]–[8]. Over the last decades, marine algae have been considered as an alternative source of healthy food owing to their high vitamins, polyunsaturated fatty acids, polysaccharides and mineral contents in addition to low lipid content [9]. Marine algae are widely distributed along shallow water of Mediterranean and Red-Sea shores [10].*Enteromorpha*species green seaweed, contains many bioactive compounds which attracted extensive interest due to its multiple biological activities [11]. The richness of carotenoids, fucoidans and phlorotannins reinforces seaweed to play a vital role in pharmaceutical, nutraceutical and cosmeceutical industries [12], [13].Previous studies of a species of*Enteromorpha*genus; *E. prolifera*which has high polysaccharides contents proved that it has high hypolipidemic activity in rats as it decreased body weight, triglycerides level, cholesterol level and low-density lipoprotein level (LDL) [14]. Previous studies showed that *spirulina* multicellular cyanobacteria has a positive effect on obesity [15]. In our study, a comparison between *spirulina* cyanobacteria supplement purchased from local market and *Enteromorpha intestinalis* extract as a supplement in treatment of obesity; a main cause of lots of metabolic syndromes by feeding algal extract dose orally using tube (gavage) daily for 4 weeks to high-fat diet female albino rats and monitoring weight change and different biochemical parameters.

## 2. Materials and methods

### *2.1. Materials*

*Enteromorpha intestinalis (Linnaeus) Nees* green seaweed was collected in March 2020, from coastal area of Abou-Quier bay, Alexandria, Egypt. The sample was microscopically identified at the National Institute of Oceanography and Fisheries, Egypt. A commercial *Spirulina* microalgae supplement was purchased from Viva Natural. Each gram of Spirulina powder (*Arthrospira platensis*) contains proteins, carbohydrate, lucine, valine, lysine, phenyl alanine, methionine, lipids, β-carotene, c-phycocanin, c-phycoerthrin, vitamin E, vitamin B1, vitamin B3, vitamin B6 and vitamin B12[16], [17].

### *2.2. Processing of marine algae*

Fresh algae was washed with distal water to remove wastes and epiphytes, and then the sample was allowed to dry in shade then powdered to get about 111.0 g powder, then successively extracted at 40° C with petroleum ether to remove fats (400 ml × 5 times), then extracted with a mixture of $CH_2Cl_2$/MeOH (1: 1) several times (400 ml × 5 times) until complete extraction to get petroleum ether extract (PT) and crude extract (M), respectively. After filtration, petroleum ether extract was discarded while crude extract was concentrated under reduced pressure at 40°C using a rotary evaporator to get about 7.34 g. Six grams of crude extract (M) was dissolved in 100 ml of 5 % tween emulsifier solution (Wt. /v) to get 6% algal solution that will be ready for experiment. Dosage fed orally was 5ml/kg weight of *E. intestinalis* extract solution and spirulina treatment group.Total flavonoids and total phenolic of*E. intestinalis* extract (M) were determined spectrophotometrically using rutin and gallic acid standards, respectively.

### 2.3. *Quantitative estimation of total flavonoids/ phenolic content of E. intestinalis*

The total flavonoids content (TF) of *E. intestinalis* crude extract (M) were determined by spectrophotometric method by preparing 6 different concentrations of rutin standard (1000, 500, 250, 150, 100 and 50 µg/ml). A sample solution is prepared by dissolving 1mg of (M) in 1 ml of methanol. Each of 6 standards and sample were added to 96-well plate in 6 replicates. Then measured at 510 nm using (FLUO star Omega**),** a multi-mode microplate reader. A calibration curve is then plotted. Total flavonoid is calculated by using equation $y= 0.0011x-0.0131$, $R^2= 0.9946$ [18].

Total phenolic content of *E. intestinalis* crude extract (M) was determined by spectrophotometric method by preparing gallic acid stock solution of 1mg/ml (standard)in methanol and 7 serial dilutions were prepared in the concentrations of 500, 250, 125, 62.5, 31.2, 15.6, and 7.8 µg/ml. A solution of the sample was prepared in concentration of 5mg/ml in methanol. Each of the 7 standards and sample were pipetted in the 96-Well plate in 6 replicates. Then measured at 630 nm using (FLUO star Omega**),** a multi-mode microplate reader. A calibration curve is then plotted. The total phenolic is calculated by using equation $y= 0.0027x-0.0143$, $R^2= 0.9986$ [19].

### *2.4. Animal and experimental diet*

Thirty healthy adult female albino rats of the Wistar strain weighing around 200- 230 g were procured from the central animal house of the Medical Research Institute, “Smouha” Alexandria University for the

study and kept in separate cages. The rats were housed in the animal house of the Faculty of Pharmacy, Pharos University under laboratory conditions (relative humidity 85 ± 2%), temperature 22 ± 1°C and 12h light and 12h dark cycle), in plastic cages in a well-ventilated animal house. Rats were randomly assigned to (4) separate groups (n = 6 each), first group was the control negative (CN) which was fed on a normal diet (normal diet contents: 18 % protein, 10 % sucrose, 5 % corn oil, 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibres) [20], Second group is control positive (CP) fed on high-fat diet without treatment; high-fat diet content composed of (normal diet: abdominal fat) (2:1)[21], [22]. Third group; *E. intestinalis* extract treatment group (Group III) fed on high-fat diet and fed orally by 5ml/kg weight of *E. intestinalis* extract solution and spirulina treatment group (Group IV) fed on high-fat diet and fed orally by 5ml/kg weight of *spirulina* extract. The algal extract dose was orally delivered using an oral tube daily for 4 weeks. Body weight (g) of investigated rats was measured weekly during the experiment using analytical top balance and at the end of the treatment period (4 weeks), rats were subjected to fasting for 12 hours and were sacrificed using diethyl ether anesthesia, and blood samples were collected.

Animal Utilisation Protocol was applied according to institutional guidelines and approved by the National Institute of Oceanography and Fishers (NIOF) committee (NIOF-IACUC) and the certificate was coded as (NIOF-ME9-O-23-R-007).

### *2.5. Microscopic examination of liver tissue*

Semithin section of liver tissue stained with toluidine blue (TB) (Magnification about 1000) was examed using a light microscope. also, the section of liver tissue of different states during experiment was examined using electron microscope (Magnification about 2000-2500) to monitor changes in liver cell [21, 22].

### *2.5. Biochemical parameters and statistical analysis*

Plasma Triglycerides (TG), total cholesterol (cholesterol), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were determined. Plasma anti-obesity hormones (leptin, insulin, glucagon-like peptide-1 and peptide y y) were also determined**.** Catalase (CAT), Superoxide dismisses (SOD) and Plasma glutathione (GSH) which are enzymatic antioxidants were also determined. Total plasma protein, Total antioxidant capacity (TAC) as well as Vitamin C Plasma levels were also determined**.** Plasma electrolytes; copper ($Cu^{+2}$); zinc ($Zn^{+2}$); (sodium ($Na^{+1}$); potassium ($K^{+1}$) and magnesium ($Mg^{+2}$) were also determined. Anti-inflammatory Biomarkers; C - reactive protein (CRP) and Tumor necrosis factor α (TNF α) were also determined. Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level. F-test (ANOVA) was used to assess the amount of variability between the group means in the context.

## 3. Results

### *3.1. Flavonoid and Phenolic content*

Figure 1, shows the total flavonoids content of *E. intestinalis* crude extract was calculated using rutin standard deviation curve and recorded 71.77 mg/g extractand was expressed as mg rutinequivalent (RE). Also figure 2 shows Gallic Acid standard deviation curve, the total phenolic content was expressed by mg Gallic Acid Equivalent (GAE) recorded 25.98 mg / g crude extract (M).

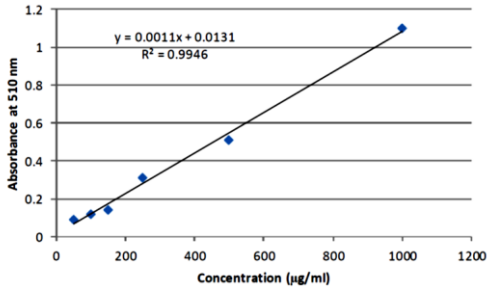



Figure 1: Rutin standard deviation

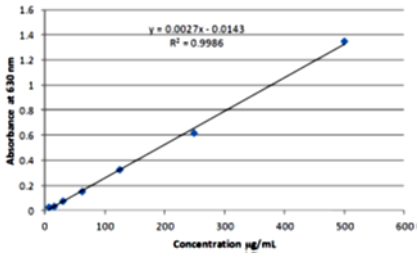



Figure 2: Gallic Acid standard deviation curve

In table 1, obese female rats in the positive control group showed the highest weight gain compared with their initial body weight, while the obese rats treated with *E. intestinalis* crudeextract (M) showed the highest decrease in weight gain during the experiment. However, obese rats treated with *Spirulina extract* resulted in a decrease in weight gain after 2 weeks of the experiment.

Table 1

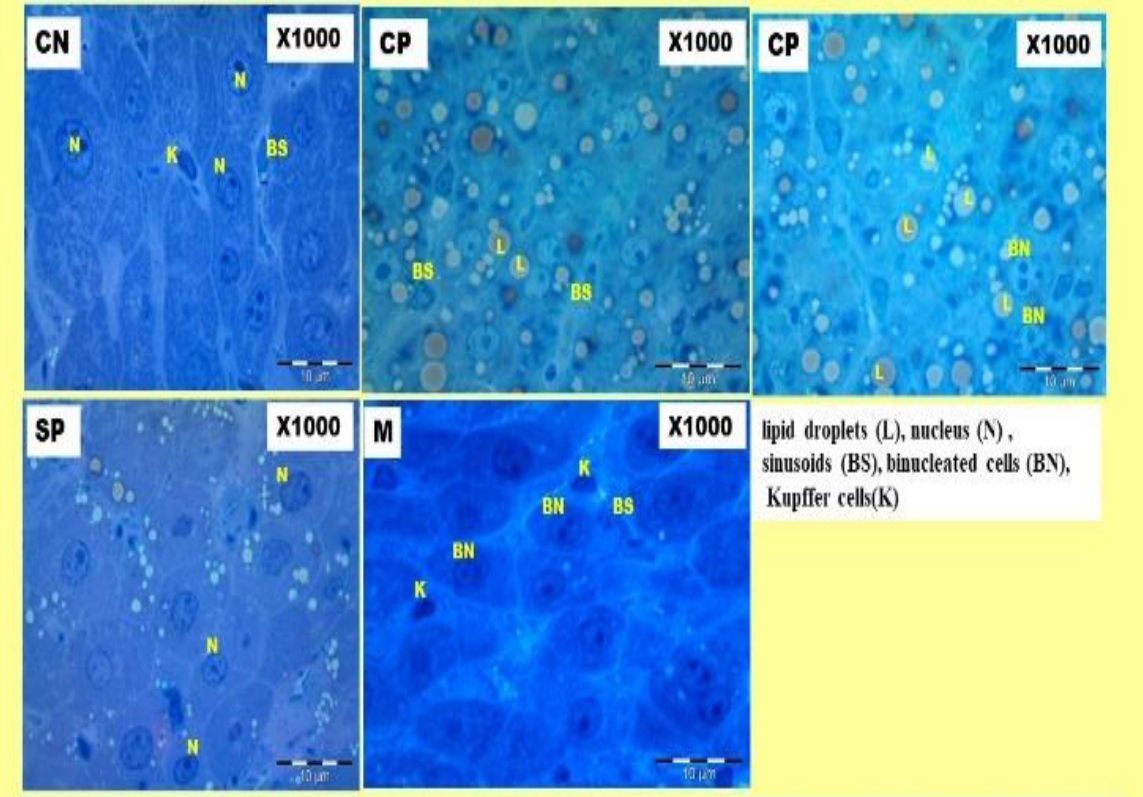
The weight gain change of female rats treated with *E. intestinalis* crude extract (M) and *Spirulina* microalgae (SP) compared with CN and CP groups.

| Body Weight (g) | CN | CP | M | SP |
|---|---|---|---|---|
| Initial (Mean ± SD) | 227.4 ± 12.30 | 270.5 ± 20.10 | 268.8 ± 26.90 | 270.5 ± 18.83 |
| 1st week (Mean ± SD) | 235.4 ± 11.91 | 268.2 ± 6.97 | 266.8 ± 28.87 | 267.7 ± 14.46 |
| 2nd week (Mean ± SD) | 235.4 ± 9.42 | 277.8 ± 11.36 | 263.8 ± 27.61 | 265.5 ± 12.65 |
| 3rd week (Mean ± SD) | 238.8 ± 7.69 | 286.8 ± 11.55 | 261.3 ± 29.01 | 263.3 ± 13.37 |
| 4th week (Mean ± SD) | 238.2 ± 6.83 | 290.0 ± 11.80 | 255.7 ± 25.31 | 260.2 ± 13.98 |

Values are mean ± SD (n=6 rats in each group), nagative control (fed with normal diet)(CN) , positive control (fed on high fat diet without treatment)(CP), positive treated with *E. intestinalis*crude extract (M) and positive treated with *spirulina* extract(S).

### *3.2. Microscopic examination*

In figure 3, Semithin Section of liver stained with toluidine blue (TB) (X1000). In control liver **(CN)**; hepatocytes are arranged in strands separated by blood sinusoids **(Bs)**, nuclei round or elliptical chromatin as dense granular bodies along nuclear membrane as irregular masses also nuclei exhibited eccentric nucleoli **(N)**, homogenous cytoplasm &plasma membrane fairly uniform. In control positive fatty liver **(CP)**; alternations include cells possess disorder arrangement some degenerated hepatocytes & degenerated binucleated cells **(BN)**. Also, cells pleomorphic in shape &size, enlarged nuclei, lipid droplets **(L)** increased inside hepatocytes, intercellular space & blood sinusoids **(BS)**. Treated fatty liver with spirulina **(SP)** showed cell pleomorphic in shape & size also, nuclei exhibited different shapes and sizes **(N)**, slight decrease of lipid droplets, clear obvious cells, a granular appearance of cytoplasm. Treated fatty liver with green algae **(M)** showing irregularly arranged hepatocytes, an increase in the number of binucleated cells **(BN)**, enlarged Kupffer cells **(K)**, decreased lipid droplets **(L)**.




Figue3 (Light microscope section of rat liver (L.M), Magnification (1000)); (CN) L.M section of control negative, (CP) L.M section of control positive(fatty liver (CP) L.M section of control positive(fatty liver), (SP) L.M section of rat liver SP., (M) L.M section of rat fed Green algae.

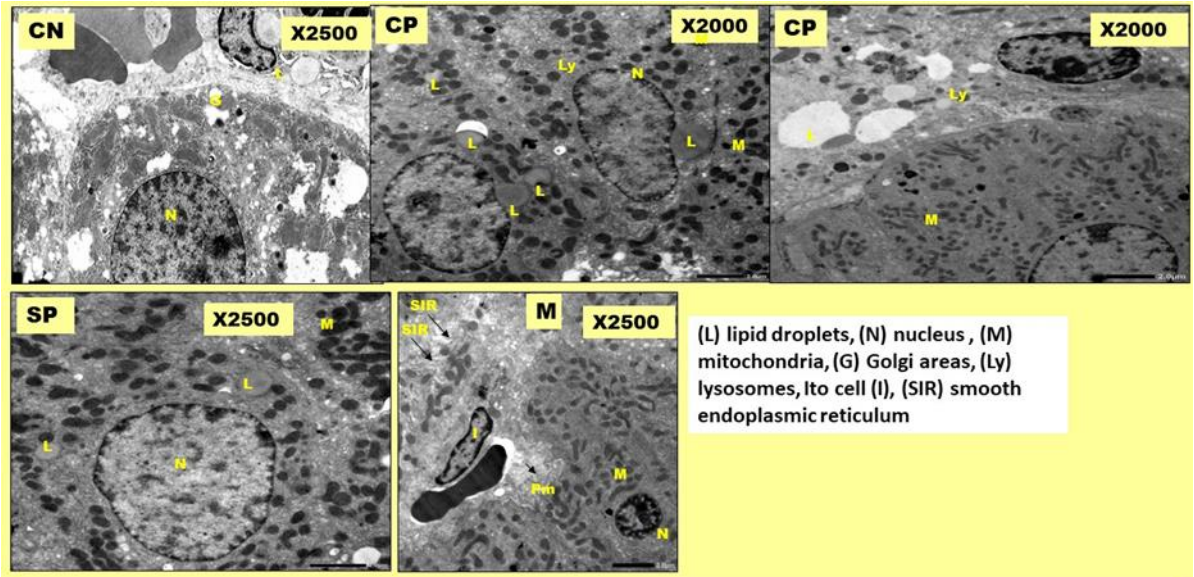



**Figure4 (Electron microscope section of rat liver (E.M), Magnification (2000-2500)) ; (CN) E.M section of control negative, (CP) E.M section of control positive(fatty liver), (CP) E.M section of rat liver (fatty liver), (SP) E.M section of treated rat liver with spirulina, (M) E.M section of treated rat fatty liver with Green algae.**

Figure 4 shows electron microscope (EM) section of rat liver in different stages (Magnification; 2000-2500). Figure 4; EM section of control negative rat liver **(CN)** shows a regular cell membrane, Golgi areas **(G)**, and a part of the spherical nucleus with two nucleolus (N).

EM section of rat fatty liver **(CP)**, shows an increase in the lipid droplets **(L)**, irregular shaped nucleus **(N)** and mitochondria **(M)**. EM section of treated rat liver with spirulina **(SP)** shows decreased number of lipid droplets **(L)** compared with control positive **(CP)**, nucleus with small vacuoles absence of nucleolus **(N)**& irregular shaped mitochondria **(M)** while treated rat liver with Green algae **(M)** shows irregular shaped plasma membrane **(Pm)** dilated smooth endoplasmic reticulum, numerous mitochondria **(M)**, perisinusoidal cells; Ito cells **(I)** in space of Disse& small vacuoles inside the hepatocyte nucleus.

### *3.3. Biochemical Parameters*

Table 2 shows serum lipid profile where the percent of triglyceride of treated group with green seaweed is lower than control negative group and Spirulina group compared with the control positive group while cholesterol content is the same in groups **M** and **SP** which are higher than that in **CN** group. Also, LDL content is approximately the same in **M** and **SP** groups but much lower than **CN** group. HDL is approximately the same in CN, M and SP groups. Serum lipid profile levels of each normal, obese untreated, obese treated female rats with *E. intestinalis* crude extract (M)and *spirulina* microalgae (SP) after 4 weeks of experiment showed that obese rats treated with *E. intestinalis* extract showed the greatest decrease in serum cholesterol and TG levels followed by results of that treated with *Spirulina* extract. Also plasma anti-obesity hormones of each group which is mentioned in table 3 showed an increase of insulin sensitivity and glucagon-like peptide-1 compared with the positive control group, but lower than those in rats treated with *Spirulina* extract which showed the highest improvement. Table 4 shows the effect of four weeks experiment on inflammatory markers; C-reactive protein and tumor necrosis factor α of treated groups. Treatment of obese rats with *E. intestinalis* extract showed the highest reduction in serum TNF-α.The CRP level was most decreased in the obese rats treated with *Spirulina* followed by that in the group treated with *E. intestinalis* extract. As mentioned in table 5 and 6 which showed the effect of our experiment on oxidative stress parameters and none enzymatic antioxidants, a decrease in MDA was recorded in group M which was treated with *E. intestinalis* extract, while the highest increase in glutathione peroxidase (GPx) was recorded in the group treated with *Spirulina* (SP). Also,an increase in the levels of liver glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activities in group SP were recorded. Table 7 shows the difference in electrolyte content in the serum of rats; where M and SP groups are almost the same as the normal group (CN) while that of high-fat diet group (CP) is much higher than recorded in normal groups this confirms that there is no disruption of electrolyte absorption function of cells compared with high-fat diet rats which affected.

Table 2

Serum lipid profile levels of treated female rats with *E. intestinalis* crude extract (M) and *spirulina* microalgae(S) compared with CN and CP groups, after 4 weeks experiment.

| Metabolic parameters | CN | CP | M | SP | F | p |
|---|---|---|---|---|---|---|
| TG | 64.5±9.15 | 149.8±21.78 | 62.25±8.96 | 79.80±13.50 | 24.113* | <0.001* |
| Cholesterol | 97.0±11.63 | 204.0±19.12 | 118.3±17.84 | 119.6±15.21 | 32.349* | <0.001* |
| LDL | 35.85±11.82 | 148.2±22.94 | 60.55±19.12 | 59.04±16.10 | 34.921* | <0.001* |
| HDL | 48.25±4.86 | 25.80±3.70 | 45.25±4.86 | 44.60±6.66 | 17.013* | <0.001* |

**F**: **F for ANOVA test,** p: p value for comparing between the studied groups**,** *: Statistically significant at p ≤ 0.05, CN: Control negative, CP: Control positive, M: 5ml/kg body weight of *E. intestinalis* andS:5ml/kg body weight of *spirulina* Table 3

Table 3

Plasma anti-obesity of treated female rats with *E. intestinalis* crude extract (M) and *spirulina* microalgae (SP) compared with CN and CP groups, after 4 weeks experiment.

| Metabolic parameters | CN | CP | M | SP | F | p |
|---|---|---|---|---|---|---|
| Leptin | 7.88±1.10 | 20.82±2.39 | 8.63±1.54 | 9.22±2.13 | 30.745* | <0.001* |
| Insulin | 2.98±0.46 | 0.60±0.23 | 1.31±0.32 | 1.90±0.28 | 35.570* | <0.001* |
| Glucagon | 11.50±1.59 | 24.26±7.01 | 11.93±2.25 | 13.88±3.23 | 8.896* | <0.001* |
| Peptide y y | 91.50±15.42 | 157.6±21.48 | 102.8±15.02 | 97.60±19.15 | 7.333* | <0.001* |

**F**: **F for ANOVA test,** p: p value for comparing between the studied groups**,** *: Statistically significant at p ≤ 0.05 CN: Control negative, CP: Control positive, M: 5ml/kg body weight of *E. intestinalis* andSP:5ml/kg body weight of *spirulina*

Table 4

Inflammatory markers of treated female rats with *E. intestinalis* crude extract (M) and *spirulina* microalgae(S) compared with CN and CP groups, after 4 weeks experiment.

| Anti-inflammatory | CN | CP | M | SP | F | p |
|---|---|---|---|---|---|---|
| (CRP) | 2.55 ± 0.55 | 37.0 ± 7.97 | 8.78 ± 2.75 | 6.12 ± 1.78 | 2.876* | 0.038* |
| (TNF α) | 54.75 ± 8.34 | 210.2±66.44 | 76.25±15.48 | 76.80±8.70 | 17.486* | <0.001* |

**F**: **F for ANOVA test,** p: p value for comparing between the studied groups**,** *: Statistically significant at p ≤ 0.05 CN: Control negative, CP: Control positive, M: 5ml/kg body weight of *E. intestenalis*andSP:5ml/kg body weight of *spirulina***,** CRP: C-reactive protein and TNF α: Tumor necrosis factor α.

Table 5

Oxidative stress parameters of treated female rats with *E. intestenalis*crude extract (M) and *spirulina* microalgae(S) compared with CN and CP groups, after 4 weeks experiment.

| | CN | CP | M | SP | F | P |
|---|---|---|---|---|---|---|
| MDA | 5.83 ± 0.76 | 25.06 ± 6.99 | 9.73 ± 1.67 | 11.14 ± 2.11 | 20.106* | <0.001* |
| GPx | 66.0 ± 6.58 | 25.40 ± 5.32 | 49.0 ± 3.74 | 51.80 ± 9.15 | 13.896* | <0.001* |

CN: Control negative, CP: Control positive, M: 5ml/kg body weight of *E. intestinalis* andS:5ml/kg body weight of *spirulina* MDA: Malondialdehyde, GPx: Glutathione peroxidase

Table 6

None enzymatic antioxidants parameters and electrolyte content of female rats treated with *E. intestinalis* crude extract (M) and *spirulina* microalgae (SP) compared with CN and CP groups, after 4 weeks experiment.

| Antioxidant | CN | CP | M | SP | F | p |
|---|---|---|---|---|---|---|
| Catalase | 40.0±5.35 | 13.28±2.85 | 29.0±5.35 | 32.40±5.41 | 17.603* | <0.001* |
| SOD | 63.50±7.85 | 30.20±3.56 | 55.50±6.03 | 57.20±3.70 | 9.626* | <0.001* |
| GSH | 43.25±5.12 | 15.80±3.11 | 32.75±7.41 | 38.20±6.22 | 14.146* | <0.001* |
| Plasma protein | 7.30±0.34 | 6.40±0.43 | 7.10±0.57 | 7.22±0.25 | 3.511* | 0.017* |
| TAC | 44.75±6.24 | 14.40±2.97 | 33.75±4.86 | 42.60±3.58 | 17.131* | <0.001* |
| Vitamin C | 14.90±3.74 | 5.62±2.0 | 9.20±1.59 | 11.80±3.18 | 6.492* | 0.001* |
| $Cu^{+2}$ | 124.0±8.12 | 165.0±14.37 | 129.5±11.27 | 133.4±12.38 | 8.709* | <0.001* |
| $Zn^{+2}$ | 218.8±17.33 | 149.2±20.79 | 201.5±11.68 | 202.2±20.14 | 7.437* | <0.001* |
| $Na^{+}$ | 138.5±7.72 | 121.8±8.79 | 133.8±7.37 | 139.2±7.79 | 3.991* | 0.010* |
| $K^{+}$ | 4.08 ± 0.31 | 5.80 ± 0.41 | 4.50±0.57 | 4.23 ± 0.60 | 9.012* | <0.001* |
| $Mg^{+2}$ | 3.20 ± 0.50 | 1.76 ± 0.24 | 2.30 ± 0.23 | 2.74 ± 0.45 | 7.563* | <0.001* |

**F**: **F for ANOVA test,** p: p value for comparing between the studied groups**,** *: Statistically significant at p ≤ 0.05 CN: Control negative, CP: Control positive, M: 5ml/kg body weight of *E. intestenalis*andSP:5ml/kg body weight of *spirulina***,** SOD:Superoxide dismisses, GSH: Glutathione, TAC: Total anti-oxidant capacity, $Cu^{+2}$: copper, $Zn^{+2}$: **Zinc,** $Na^{+}$: sodium, $K^{+}$: potassium, $Mg^{+2}$: magnesium.

## 4. Discussion

Recently, great attention worldwide towards the management and prevention of human diseases using seaweeds has been paid. Throughout our experiment, it was observed that the usage of greenseaweed; *E. intestinalis* crudeextract on the body weight of obese female albino rats are positively effective as it suppressed body weight gain as shown in our results compared to *Spirulina* supplement (SP) which suppressed body weight gain of obese rats and this agrees with reported before in 2017 and 2018 that *Spirulina maxima* has valuable effect in reducing weight [23], [24]. As is shown in our results; greenseaweed *E. intestinalis* crudeextract rich with phenolic compounds and flavonoid this accounts for the antiobesity effect of *E. intestinalis* crudeextract[25]. Also, studying the histological change in liver tissue of control negative rat liver **(CN)**, control positive fatty liver **(CP)**, treated rat liver with *spirulina* **(SP)** and treated rat liver with Green seaweed extract **(M)** using a light microscope and transmission electron microscope which confirmed that both treated rat liver with *spirulina* **(SP)** and treated rat liver with green seaweed extract **(M)** has less lipid droplets than in control positive **(CP)** also, nucleus with small vacuoles, absence of nucleolus and irregular shaped mitochondria in treated rat liver with *spirulina* **(SP)** while treated rat liver with green algae extract **(M)** shown irregularly shaped plasma membrane, dilated smooth endoplasmic reticulum, numerous mitochondria,perisinusoidal cells; Ito cell (I) in space of Disse& small vacuoles inside the hepatocyte nucleus and these finding support our earlier results.Excessive fat accumulation is a consequence of a positive energy imbalance. The Biochemical parameters of hyperlipidemic patient characterized by elevation of serum TC, a decrease in HDL cholesterol and an increase in low-density lipoprotein (LDL) cholesterols. An association between hyperlipidemia and serum triglycerides has also been reported [26].In our experment, group M showed the greatest decrease in serum cholesterol and TG levels followed by results of group SP and these results are in agreement with theprevious studies which reported the effect of *Enteromorphaprolifera*, on triglycerides, total cholesterol (cholesterol), low density lipoprotein (LDL), there was marked inhibition of serum TC, TG and LDL-C content while There was an increase in high density lipoprotein (HDL), which indicated that *E. prolifera* might be beneficial to individuals with some degenerative diseases[27]. Previous studies showed that, LDL-C and HDL-C levels were inversely correlated with C-reactive protein (CRP) levels which agree with our results[28]. Group SP of treated rats with *Spirulina* powderextract also showed a decrease in serum LDL level which in agreement with a previous study in which a significant reduction in LDL:HDL ratio was found in 15 diabetic patients given *Spirulina*[29]. Obesity is associated with a chronic inflammatory response, characterized by abnormal adipokine production and the activation of some proinflammatory signaling pathways, resulting in the induction of several biological markers of inflammation. Measuring the level of CRP produced and released by the liver is the most common type of blood test used to determine the level of inflammation followed by abnormal production of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α). TNF-α activates NF-KB which is one of the transcription factors involved in mediating cellular inflammatory response [30]. Although it is well accepted that inflammation in obesity can induce insulin resistance, recent evidence suggests that the opposite is also true, i.e., insulin resistance by itself can also induce inflammation [31]. The richness of phenolic compounds and flavonoid contents in *E. intestinalis* crude extract explains the anti-inflammatory and antioxidant effect of crude extract. Group M (obese treated rats with *E. intestinalis* extract) showed the highest reduction in serum TNF-α, these results are agreed with the previous studies where, CRP level was most decreased in group SP than in group M [32]. Antioxidants are recognized for promoting health and lowering the risk of cancer, hypertension and heart disease. The uses of natural antioxidants from plant extracts have generated increasing interest due to health professionals, and consumers' concern about the safety of synthetic antioxidants in foods[33]. Malondialdehyde (MDA) is one of the final products of lipid peroxidation so it's a marker of free radical production and consequent tissue damage [34]. In our study, a decrease in MDA was recorded in the group which treated with *E. intestinalis* extract, while the highest increase in glutathione peroxidase (GPx) was recorded in the group treated with *Spirulina*. These results agree with research reporting *Spirulina* significantly increases the levels of liver glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione S- transferase (GST) activity through its antioxidant potential thereby decreasing the level of lipid peroxidation[35]. The protective role of *Spirulina* may be related to its contents of vitamins E, C, β-carotene, as well as the enzyme superoxide dismutase, selenium and brilliant blue polypeptide pigment phycocyanin[17]. SOD is a metalloenzyme that catalyzesdismutation of superoxide anion into oxygen and hydrogen peroxide. Such enzymes provide defence system for the survival of aerobic organisms. *E. intestinalis* showed a significant increase in SOD level and a highly significant increase in all antioxidant parameters compared with the untreated group. Richness of phenolic compounds and flavonoid contents in *E. intestinalis* crude extract explain the anti-inflammatory effect of crude extract.

As final conclusion, usage of *E. intestinalis* crude extract has positively effect in treatment and prevention of obesity as well as *Spirulina* supplement and we recommend to make clinical trials.

## 5. Abbreviations

- CN control negative
- CP control positive
- M treated group with E. intestinalis crude extract
- S Treated group with spirulina extract
- COVID-19 Coronavirus disease of 2019
- SARS-CoV2 Sever acute respiratory syndrome-related coronavirus
- TG triacylglycerol
- TC total cholesterol
- PT petroleum ether extract
- TF Total flavonoids content
- NIOF National institute of oceanography and fishers
- LDL Low density lipoprotein
- HDL High density lipoprotein
- CAT Catalase
- SOD Superoxide dismisses
- GSH Plasma glutathione
- TAC Total antioxidant capacity
- MDA Malondialdehyde
- GPx glutathione peroxidase
- Cu copper
- Zn zinc
- Na sodium
- K potassium
- Mg magnesium
- CRP C - reactive protein
- TNF- α Tumor necrosis factor- α
- RE Rutin
- GAE Gallic Acid

## 6. Conclusions

Usage of *E. intestinalis* crude extract has positively effect in treatment and prevention of obesity as well as *Spirulina* supplement and we recommend to make clinical trials.

## 7. Conflicts of interest

The authors have declared no conflict of interest

## 8. Formatting of funding sources

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## 9. Ethics approval and consent to participate

This study is approved from National institute of oceanography and fishers (NIOF) committee for ethical care and use of animals/ aquatic animals (NIOF-IACUC) with ethical approved code; (NIOF-ME9-O-23-R-007).

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