Brown Algae (Sargassum Subrepandum) from Egypt Exhibited High Nutritional Composition and Bioactive Constituent's Content: A Biological Application on Obesity and its Complications in Experimental Rats

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Abstract

Obesity is a medical problem that increases the risk of other diseases and health problems known as complications of obesity. In view of the side effects that resulted from the use of drug therapy and surgical interventions in the treatment of obesity, some of which posed a threat to health, which called for the search for safe and alternative alternative/natural methods. Therefore, the current study was conducted with the aim of exploring the effect of dietary intervention using marine algae (Sargassum subrepandum) on obesity and its complications in experimental rats. Sargassum subrepandum powder (SSP) showed a high nutritional composition through its high of essential nutrients (carbohydrates, fiber, protein and ash), minerals (K, Na, Ca, Zn, Fe, Cu and Mn) and vitamins (A, C, B2, B3, B9, E). It also contains many of the following bioactive constituents: polyphenols, flavonoids, carotenoids, tannins, terpenoids, triterpenoids, polysaccharides, anthocyanin's and kaempferol, which resulted in high antioxidant activity. On the other side, biological experiment indicated that rats of the model obese group recorded 335.01 g i.e. increased by the rate of 115.60% when compared to the base line. Intervention with SSP by 1.5, 3.0, 4.5 and 6.0% lead to significant (p≤0.05) decreasing on liver serum enzymes (AST and ALT). Also, SSP was effective in protecting against obese complications including inhibit liver disorders through liver serum enzymes-lowering activity, improvement of the serum antioxidant status (increase the glutathione fractions and decrease the formation of malonaldehyde and reactive oxygen species). The histological examinations of the heart and adipose tissue confirmed these results. In conclusion, we recommended SSP by a concentration up to 6% to be included in daily diets, drinks and food supplementation of normal and obese people.

Keywords: Sargassum subrepandum; Minerals; Vitamins; Antioxidant; Body weight; Glutathione fractions; Malonaldehyde; reactive oxygen species.


1. Introduction

Algae are a group of living organisms that are able to capture the energy of light through the process of photosynthesis, converting inorganic substances (mostly water + carbon dioxide) into organic substances (sugars) in which they store energy [1]. They are also a name that denotes a group of diverse plants belonging to more than 20,000 thousand species, and these algae are found in different forms in terms of shape, size, and way of living [2]. The scholars are unanimously agreed that the word algae may denote plant groups that share a number of characteristics, the most important of which is that they do not have roots, stems, flowers, or real leaves, but rather they are a group of cells standing next to the other, living mostly in salt water (seas and oceans) and fresh (rivers), and also contain chlorophyll, and thus the ability to carry out the process of photosynthesis [3]. Some types of algae are also familiar to most people, for example seaweed, pond scum, or algae blooms in lakes. Despite this, there is a vast and diverse world of algae that not only help us with life, but are essential to our existence in it. In general, Marine algae are classified as brown, red, or green algae [4].

Brown algae (BA) belong to Family, Phaeophyceae are a large group of mostly marine multicellular algae, including many seaweeds located in different countries around the world including Egypt. Most BA contain the pigment fucoxanthin, which gives them their name and gives them their unique greenish-brown hue [5]. In the littoral zone of the Egyptian coast, BA is currently the most dominant group. Members of Sargassum genus represent valuable sources of a several compounds including proteins, lipids, minerals, essential fatty and amino...
acids, and bioactive compounds [6-8]. Also, BA consists mainly of water (90 %) in the native state. Polysaccharides are major components and comprise alginites, cellulose, and sulfated polysaccharides such as fucoidans and laminarins. Other components include proteins, free mannitol, minerals such as iodine and arsenic (inorganic and organic), polyphenols, peptides, fatty compounds, and various pigments [6, 9, 10]. Alginites, probably the most widely used of the algal extracts, are composed of block copolymers of mannuronic and guluronic acid sugars and have been adopted by the food industry as thickening agents and by the pharmaceutical industry as binders, gelling agents, and wound absorbents [10].

From a nutritional and therapeutic point of view, brown alga such as Sargassum genus is used dried in condiment and soup bases or eaten fresh in salads, rolls, or stews, or with rice. It is thought that the overall content of certain traditional Asian diets contributes to the low incidence of cancer, particularly breast cancer [11, 12]. Also, it is apparent that the unique levels of seaweed intake contribute to the variance in the levels of breast cancer [13]. There is a nine fold lower incidence of breast cancer in the Japanese population and an even lower incidence in the Korean population compared to the incidence in the West [14]. The relative longevity and health of Okinawan Japanese populations has been attributed in part to dietary algae in studies [15]. In Brazil, a dietary intervention for 10 weeks study, 3g of decosahexaenoic acid, 5g of seaweed (wakame) powder, and 50 mg of isoflavonoids from soybean (Glycine soja) were given daily to immigrants, at high risk for developing diseases. This combination reduced blood pressure and cholesterol levels, suppressed the urinary markers of bone resorption, and attenuated a tendency toward diabetes. Recently, our studies indicated that consuming of BA (L.Sargassum subrepandum ) powder up to 4% of the diet was effective in protecting of some obese complications including oxidative stress, immunodeficiency and bone disorders, and liver dysfunctions [6, 7].

Obesity defines as abnormal or excessive fat accumulation that presents a risk to health [16]. A body mass index (BMI) BMI ≥ 30 kg/m² is generally considered obesity. It is well known that obesity is a growing problem globally with high rates in both developed and developing countries [16-18]. Obesity is an increasing, global public health issue. It is associated with a significant increase in mortality, with a life expectancy decrease of 5–10 years [19]. Patients with obesity are at major risk for developing a range of comorbid conditions. The health risks associated with being overweight and obese include a range of conditions, including diabetes, cardiovascular disease (CVD), hypertension, dyslipidemia, sleep apnea, some cancers, musculoskeletal disease, infertility, disability, dementia, and mortality [19].

In Egypt, the study which was conducted through the “100 Million Health" campaign, confirmed that 39.8% of adult Egyptians suffer from obesity, according to a survey study in 2019, which included 49.7 million people . The study also indicated that the results of the survey indicated that obesity is more prevalent among women, at a rate of 49.5%, compared to 29.5% among men. The same study also explained that the 13 diseases resulting from obesity constitute a huge burden on the health and economic systems in Egypt, including high blood pressure, diabetes, sleep apnea, fatty liver, hyperlipidemia, heart disease and depression. Additionally, for the economic burden, the study indicated that obesity costs Egyptians about 50 billion pounds annually to treat associated diseases attributed to it, and this figure includes direct medical costs for treating patients who suffer from these diseases due to obesity. And with the escalation of the numbers of obese patients in Egypt, the methods of confronting them also increased, the most prominent of which was slimming surgeries of all kinds. The Egyptian Society for Obesity Surgery (ESOS), also indicated nearly 30,000 obesity surgeries are performed annually. However, there are many cases in which these surgeries fail, in addition to the serious medical complications that may result from these surgeries [20-22]. In another obesity treatment/prevent strategy, a number of pharmacological approaches have been investigated in recent years, but few therapeutically effective and safe products have been developed [23]. In another meaning, the modern pharmacological therapy is costly and associated with multiple side effects resulting in patient non-compliance. Thus, there is a need to explore alternative therapies particularly from natural sources as these are cost effective and possess minimal side effects. In this attention, some plant parts have been utilized as anti-obesity agents in our previous study [24-30]. These plant parts initially took the form of crude drugs such as powders, extracts and other formulae which exhibited effective roles in preventing and/or curative obesity and its complications in experimental animals. Therefore, the results of previous studies were very encouraging, and even a strong motivation to expand in this field, by searching for different plant parts that are widespread in the global and local environment, and are suitable for treating obesity and its complications.

For the above reasons, the present study aims to determine the chemical composition, bioactive compounds and biological activities of brown alga species (Sargassum subrepandum L.) collected from Mediterranean Sea coasts in Egypt. Also, the possibility of using such algae in the strategy of obesity disease treatment and its complications in experimental rats will be in the scope of this investigation.

2. Materials and Methods
2.1. Materials
2.1.1. Collection and Taxonomy of the Brown Algae (Sargassum Subreperandum)

The Sargassum subreperandum samples were collected from the coasts of Mediterranean Sea, Alexandria, Alexandria Governorate, Egypt during late December, 2021. The identification/ verification of the algae samples were carried out by the Department of Agricultural Plant specialists, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Fresh algal samples were thoroughly washed with distilled water to remove epiphytes, encrusting materials, and excess salt and stored in a deep freezer at -20°C.
2.1.2. Chemicals and Kits

Bioactive compounds standard [gallic acid (GA), catechin (CA), α-tocopherol, linalool, ursolic acid and butylated hydroxytoluene (BHT)], were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals (Except as otherwise stated), reagents and solvents were of analytical grade were purchased from El-Ghomhorya Company for Trading Drug, Chemicals and Medical Instruments, Cairo, Egypt. Kit's assays for Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), malondialdehyde (MDA) were purchased from BIODIAGNOSTIC, Dokki, Giza, Egypt. GSH and GSSG were assayed by the kits provided by MyBioSource, Inc., San Diego, CA, USA. Triglycerides (TG), Total cholesterol (TC), HDL-Cholesterol and LDL-Cholesterol were purchased from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt.

2.2. Methods

2.2.1. Preparation of Sargassum Subrepandum Samples

The collected samples of Sargassum subrepandum were cleaned up from epiphytes and non-living matrix in running water, and rinsed many times in distilled water. The samples were then spread on string nets and allowed to dry in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at 55 °C until arriving by the moisture in the final product to about 10%. The dried samples were ground into a fine powder in high mixer speed (Moulinex Egypt, ElAraby Co., Benha, Egypt). The material that passed through an 80 mesh sieve was retained, kept in suitable closed bottles, stored at room temperature until use.

2.2.2. Nutritional Composition of Sargassum Subrepandum

Sargassum subrepandum samples were analyzed for nutritional composition including moisture (using oven method at 105°C for 4 h), protein (T.N. × 6.25, using Kjeldahl method through oxidation, distillation, and titration, semi-automatic apparatus, Velp company, Italy), fat (soxhelt semi-automatic apparatus Velp company, Italy), petroleum ether solvent), ash (dry ashing method, muffle furnace at 600°C up to material becoming ash), and fibers contents were determined using the official methods of analysis of the Association of Official Analytical Chemists [31]. Carbohydrates calculated by difference using the following formula: Carbohydrates (%) = 100 – (% protein + % fat + % Ash + % fiber).

2.2.3. Minerals

Minerals content of SSP samples were determined according to the method mentioned by Singh, et al. [32]. In brief, 0.5 g of defatted sample i.e. left behind for lipid estimation were transferred into a digested glass tube and 6 ml of tri-acids mixture (containing nitric acid: perchloric acid: sulfuric acid in the ratio of 20 : 4 : 1 v/v respectively) were added to each tube. The tubes content were digested gradually as follow, 30 min at 70 °C; 30 min at 180 °C and 30 min at 220 °C. After digestion i.e. until the mixture becomes colorless, the mixture was cooled, dissolved in distilled water, and the volume was increased to 50 ml in volumetric beaker. The mixture samples were filtration in ashless filter paper and aliquots were analyzed for minerals (K, Na, Ca, Zn, Fe, Cu and Mn) using of atomic absorption spectrophotometer (Perkin – Elmer, Model 2380, Waltham, MA, USA).

2.2.4. Vitamins

Fat-soluble vitamins (A and E) were extracted from the SSP samples according to the methods described by Epler, et al. [33] and Hung, et al. [34] while water-soluble vitamins (B and C) according to Moeslinger, et al. [35], and analyzed by using HPLC techniques. SP Thermo Separation Products Liquid Chromatography (Thermo Separation products, San Jose, CA, USA) was used with a Consta Metlic 4100 pump, a Spectra Series AS100, Spectra System UV 1000 UV/Visible Spectrophotometer Detector, Spectra System FL 3000 and a PC 1000 system software. The columns used (Alltech, Deerfield, IL, USA) were a reversed-phase water Adsorbosil C18 (5 µM, 100 mm × 4.6 mm I.d.) for water-soluble vitamins; and normal Ultrasphere Si (5 µM, 250 mm × 4.6 mm I.d.) for analysis of fat-soluble vitamins. Under the chromatographic conditions used in these methods, mean values ±SD of vitamins A, C, E, B2, B3, and B6 recoveries were 92.15± 1.65, 89.02 ±2.03, 90.25±1.57, 87.66± 2.21, 86.09 ±1.44, and 88.32 ±2.06 and respectively.

2.2.5. Bioactive Constituents

Throughout this study absorbance for different assays were measured using UV-160A; Shimadzu Corporation, Kyoto, Japan. Total phenolics in SSP were determined using Folin-Ciocalteu reagent according to Singleton and Rossi [36] and Wolfe, et al. [37] and were expressed as gallic acid equivalents (GAE). The total carotenoids were determined by using the method reported by Lichtenthaler [38]. Total flavonoids contents were estimated using colorimetric assay described by Zhisen, et al. [39] and expressed as catechin equivalent, CAE. Total polysaccharides were measured according to the method of Vazirian, et al. [40] and were expressed as mg of starch equivalents. Tannins were determined by the method of Van-Burden and Robinson [41] and expressed as catechines equivalents. Total terpenoids were measured according to the method of Ghorai, et al. [42] and were expressed in mg of linalool equivalents. Total triterpenoids were measured according to the method of Schneider, et al. [43] and were expressed in mg ursolic acid equivalents. Kaempherol was measured according to the method mentioned in Fouda, et al. [44].
2.2.6. Antioxidant Activity

SSP samples were extracted by the method of Gharib, et al. [45] and antioxidant activity (AA) of the samples and standards (α-tocopherol and BHT) was determined according to the BCB assay following a modification of the procedure described by Marco [46].

2.2.6. Biological Experiments

2.2.6.1. Animals

Animals used in this study, adult male albino Sprague-Dawley rats (150-160 g per each) were obtained from Helwan Station, Ministry of Health and Population, Helwan, Cairo, Egypt.

2.2.6.2. Diets

The basal diet (BD) was prepared according to the following formula as mentioned by Reeves, et al. [47] as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The high fat diet (HFD) prepared according to Research Diets, Inc. NJ, as follow: casein (23.3%), L-cystine (0.35%), corn starch (8.48%), maltodextrin (11.65%), sucrose (20.14%), soybean oil (2.91%), lard fat (20.69%), mineral mixture (1.17%), dicalcium phosphate (1.52%), calcium carbonate (0.64%), potassium citrate.1 H2O (1.92%), vitamin mixture (1.17%), choline bitartrate (0.23%). The used vitamins and salt mixtures components were formulated according to Reeves, et al. [47].

2.2.6.3. Experimental Design

All the experiments were a complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council NRC [48]. Rats (n=36 rats), were housed individually in wire cages in a room maintained at 25 ± 4 °C, relative humidity (53±4%), a 12-hr lighting cycle and kept under normal healthy conditions. All rats were fed on BD for two weeks before starting the experiment for acclimatization. After that, the rats were randomly divided into two main groups, the first group (Group 1, 6 rats) still fed on BD as negative control/normal group and the second main group (30 rats) was classified into five sub groups as follow: group (2), fed on HFD as a positive control/model; group (3), fed on HFD containing 1.5 % SSP; group (4), fed on HFD containing 3 % SSP; group (5), fed on HFD containing 4.5 % SSP and group (6), fed on HFD containing 6 % SSP. Each of the above group was kept in a single cage for eight weeks. During the experimental period (eight weeks), body weight gain (BWG, as a percent of initial weight) was recorded every week in rats.

2.2.6.4. Sampling

At the end of experiment period (eight weeks) blood samples were collected after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized. Blood samples were collected part in clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum according to Stroev and Makarova [49]. The clear, not hemolyzed serum was carefully aspirate, transferred into labeled Eppendorf tubes and stored frozen at -20°C for further biochemical analysis. The different samples of the heart and fatty tissues were separated and stored in neutral formalin (10%) for histopathological studies.

2.2.6.5. Biochemical Analysis

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities were measured in serum using the modified kinetic method of Tietz [50]. Alkaline Phosphatase (ALP) activity was determined using modified kinetic method of Vassault, et al. [51]. Triglycerides (TGs), total cholesterol (TC), HDL-Cholesterol and LDL-cholesterol were determined in serum according to the methods of Ahmadi, et al. [52], Fossati and Prencipe [53], Lopes-Virella, et al. [54], Richmond [55], respectively. Glutathione fractions (GSH and GSSG) were measured colorimetrically in serum samples such as described by Ellman [56]. Serum malonaldehyde (MDA) content was measured using the colorimetric method described by Buege and Aust [57] based on the reaction of thiobarbituric acid (TBA) with MDA, one of the aldehyde products of lipid peroxidation. Reactive oxygen species (ROS) was determined by a colorimetric method described by Erel [58].

2.2.6.6. Histopathological Examination

Heart and fatty tissues were prepared for histopathological studies such as described by Drury, et al. [59]. The tissues were accurately fixed in a neutral formalin solution (10%). They were dehydrated in an ascending series of ethanol, were cleared in xylene, embedded in paraffin wax, sectioned by microtome, stained with eosin and hematoxylin, and examined to detect the histopathological changes.

2.3. Statistical Analysis

All tests/measurements were done in triplicates and presented as mean± standard deviations (SD). Statistical analysis was performed using Student t-test and MINITAB 12 computer program (Minitab Inc., State College, PA).
3. Results and Discussion

3.1. Nutritional Composition, Minerals, Vitamins, Bioactive Constituent's Content and Antioxidant Activity of Sargassum Subrepandum Powder (SSP)

3.1.1. Nutritional Composition

Nutritional composition of SSP was shown in Table 1. From such data it could be noticed that fibers was the largest compound (46.34 ± 3.27 g.100g\(^{-1}\)) followed by ash (24.51 ± 2.01 g.100g\(^{-1}\)), carbohydrate (21.26 ± 1.92 g.100g\(^{-1}\)), total protein (5.03 ± 0.83 g.100g\(^{-1}\)) and crude fat (2.86 ± 0.17 g.100g\(^{-1}\)). Such data are in accordance with that reviewed by Elhassaneen, et al. [28], Percival [60], Mabeau and Fleurence [61], Holdt and Kraan [62], Abd Elalal, et al. [63], Fayez [64], Abdel [65], Also, Holdt and Kraan [62] found that genus Sargassum contains a large amount of carbohydrate as structural, storage, and functional polysaccharides, and the total carbohydrate content may range from 20% to 76% of dry weight depending on the species. Furthermore, mentioned that brown algae including Sargassum subrepandum carbohydrates possessing a fiber level greater than those recorded for several vegetables or fruits [66, 67]. Fibers abundant in Sargassum subrepandum include alginate, agar, carrageenans, fucoidan, and laminaran. In this way, Erniaty, et al. [68] found that the brown algae genus Sargassum contain carbohydrates, proteins and ash. For fat content, Rodrigues, et al. [69] found that genus Sargassum has a very little lipid content, ranging from 1% to 5% of dry matter. Also, Dawczynski, et al. [70] brown algae contain fats between 0.9% and 4% of dry weight whereas Antonopoulou, et al. [71] reported range 1 to 3% of dry weight. Therefore, Sargassum subrepandum represents low-calorie foods i.e. consumption of 100 g powder cover only about 9.20% of the daily requirement of the adult person for energy [28]. Such data confirm the possibility of successfully using brown algae genus Sargassum in nutritional applications for obese/overweight patients. On the other side, all the above studies and others could be reported that the cutnritional composition of Sargassum subrepandum vary with one or combination of the following factors including season, geographical location, light intensity and duration, water and air temperature, water depth, nutrients in media, water pH, water salinity and around residents' activities [6, 7, 63, 72].

3.1.2. Minerals Content

Data in Table 2 indicated the minerals content of SSP. Results showed that the SSP is rich in different estimated elements. K recorded higher contents followed by Ca, Na, Fe, Zn, Cu and Mn. Such data are in accordance with that reported by Erniaty, et al. [68] found that the brown algae genus Sargassum contain macro and microminerals comprising K, Na, Mg , P, I, and Fe. Also, Erbably and Junianto [73] reported that the mineral level consisted of 21.52, 0.50, 21.53 and 27.60 mg/g of Mg, Fe, Na, and K, respectively. All scientific studies indicate that there is a general public interest in the availability of essential and non-essential elements in the foods consumed daily. Minerals such as K, Ca and Mg are said to be major elements because they are in high concentrations of Sargassum subrepandum. However, Na is relatively less in SSP, thus, brown algae such Sargassum subrepandum, is said to be good for patients with hypertension. On the other side, trace metals are found in SPP in moderate amounts. As a source of trace elements, the situation of Sargassum subrepandum becomes quite practical when considered consumption of daily or several times a week by inserting it into different diet dishes. In general, such trace elements are biologically very vital to the human body through prevention and/or fighting several diseases including anemia, immunodeficiency, cancer, atherosclerosis and cardiovascular diseases (CVD) [74, 75]. For example, Mn has an important role in the metabolism of lipids and lipoproteins and it participates in the pathogenesis of atherosclerosis and CVD [76]. Also, Zn is important in different biological roles includes the cell growth, division and maturation, cell membrane stabilization, as well as in DNA and RNA synthesis [77, 78]. Furthermore, Cu has an important role in the process of erythropoiesis, maturation, signal-mediated activity of immune cells, contributes to iron resorption in the digestive tract, catalyzes hemoglobin biosynthesis, and helping to incorporate heme iron [79]. Finally, Fe exhibit he main role as an integral part of hemoglobin in red blood cells i.e. the transfer of oxygen from the lungs to the tissues of all organs in the body, necessary for DNA synthesis, and plays important roles in the human immune system [80].

3.1.3. Vitamins Content

The vitamins content of SSP is given in Table 3. Results showed that vitamin B2 was the most abundant vitamins, followed by vitamins B3, C, E, A and B9. Such data are accordance with that observed by Erniaty, et al. [68] who found that the brown algae genus Sargassum contain vitamins such vitamins B1, B2, B6, B16, C and niacin. Such limited studies indicated that the difference in vitamins contents of genus Sargassum could be due to a number of factors including the species, stage of development, the origin and the method determination. In nutritional point of view, vitamins are essential for life because we need them for good health and for growth. Data of the present study indicated that Sargassum subrepandum is a good source of some member of vitamin B including B2, B3 and B9. Vitamin B9 (folate) which provide methyl groups necessary for DNA methylation, play an important role in the pathogenesis of neurological diseases, involved in the metabolism of several amino acids [81]. Low intakes of vitamin B9 with vitamins (B6 and B12) are inversely associated with plasma homocysteine concentrations, and elevated plasma homocysteine concentrations (>15 μ) are associated with several dangerous diseases including premature coronary artery disease, premature occlusive vascular disease and cerebral or peripheral vascular disease [82-84]. Folate deficiency or poor folate status is also suspected in the development (initiation) of some cancers, especially colon and colorectal cancers [85, 86]. Vitamin A is important for normal vision, the immune system, reproduction, and growth, development and helps to keep the heart, lungs, and other
organisms work properly [87]. Vitamin C is essential for the development and maintenance of connective tissues, and plays an important role in bone formation, wound healing and the maintenance of healthy gums [88]. Vitamin E is important as an essential dietary factor for reproductive health, central nervous system working properly and acts as an antioxidant during lipid peroxidation i.e. reacts with the peroxyl radical before it can attack the PUFA but generates a tocopheroxyl radical that must be reduced by other antioxidants, such as vitamin C [89, 90].

### 3.1.4. Bioactive Constituents

Bioactive constituents in SSP were shown in Table 4. Data indicated that polysaccharides were the largest compound followed by polyphenols, tannins, carotenoids, flavonoids, terpenoids, triterpenoids, anthocyanin's and kaempferol. Such data are in accordance with that reported by several authors who found that polysaccharides are major components and comprise alginates, cellulose, and sulfated polysaccharides such as fucoids and laminarins [9, 10, 28, 63]. Also, El-Gamal [6] found that the total carotenoids and total phenolics content in *Sargassum subrepandum* ethanol extract were 358 – 511 mg.100g⁻¹ and 593 - 4278 mg GAE.100 g⁻¹, respectively. Furthermore, Abd Elalal, et al. [63] determined several bioactive constituents in SSP including phenolics, tannins, carotenoids and flavonoids in quantities were close to what was recorded in this study. Some of such compounds i.e. polysaccharides play significant roles in food processing, human nutrition and pharmaceutical applications through using as thickening and gelling agents, and emulsion stabilizers [10, 91]. Also, polysaccharides exhibited several biological activities including anticoagulant, antithrombotic, anti-inflammatory, anti-obesity, antiviral, anti-osteoporosis, antioxidant and antimicrobial activities [6, 7, 28, 63, 92, 93]. Furthermore, polysaccharides absorb substances like cholesterol, which are then eliminated from the digestive system i.e. hypocholesterolemic and hypolipidemic responses [94, 95]. For the other bioactive constituents determined in SSP i.e. Phenolics, flavonoids, carotenoids and Anthocyanin’s, they are playing important biological roles such antioxidant and scavenging activities and inhibiting the low density lipoprotein oxidation [25, 45, 63, 96-99]. Triterpenoids such as ursolic acid can serve as a starting material for synthesis of more potent bioactive derivatives, such as experimental antitumor agents [88]. Also, several studies indicated that polysaccharides and triterpenoids exhibit protective activities against liver injuries induced xenobiotes [89, 90]. Finally, Kaempferol reduces the risk of chronic diseases, especially cancer, augments human body's antioxidant defense against free radicals, and modulates apoptosis, angiogenesis, inflammation and metastasis agents [100]. Also, several studies indicated that polysaccharides and triterpenoids exhibit protective activities against liver injuries induced xenobiotes [101, 102]. Finally, Kaempferol reduces the risk of chronic diseases, especially cancer, augments human body's antioxidant defense against free radicals, and modulates apoptosis, angiogenesis, inflammation and metastasis [103].

#### 3.1.5. Antioxidant Activity

Antioxidant activity of SSP and references/standards antioxidants was assayed by β-carotene bleaching (BCB) such as shown in Table 5. Such data indicated that SSP recorded antioxidant activity equal 67.15 ± 2.21% which compares well with the references/standards antioxidants used i.e. BHT (50 mg/ml), BHT (50 mg/ml) and α-tocopherol (50 mg/ml) by 75.09 ± 1.97, 71.97 ± 1.39 and 69.96 ± 0.93%, respectively. Several previous studies reported that the BCB method have been used successfully to evaluate the antioxidant activity in various plant parts including algae *in vitro* [25, 28, 63, 99, 104-106]. All of these studies reported that polyphenols, flavonoids, carotenoids, lycopene, anthocyanin’s and polysaccharides content, such as found in a highly content *Sargassum subrepandum*, and antioxidant activity are highly correlated. In general, antioxidants may have a positive effect on human health. They can protect human body against damage by free radicals, which attack macromolecules including membrane lipids, proteins and DNA, lead to many health disorders/diseases including cancers, diabetes, heart vascular diseases, aging, inflammatory diseases, obesity, anemia, etc. [25, 30, 107-111].

At the end of this part, data made the *Sargassum subrepandum* a complete package of healthy/functional food through being an excellent source of nutritional and nutraceutical compounds. Many of such compounds exhibited important biological roles including antioxidant activity. All of these factors encourage the use of this alga in many applications of therapeutic nutrition, and in this study it is limited to obesity and its complications in experimental rats.

### 3.2. Biological Application

#### 3.2.1. The Effect of SSP on body Weight of Obese rats

The effect of SSP on body weight gain of obese rats was shown in and Table 6 and Fig-1. Such data indicated that feeding of rats on high fat diet (HFD) leads to increase the body weight than the control group. At the end of the experiment (8 weeks), rats of the normal group recorded 255.39g i.e. an increase in its amount by 64.36% when compared to the base line. Rats of the model obese group recorded 335.01 g i.e. increased by the rate of 115.60% when compared to the base line. The intervention with SSP by 1.5, 3.0, 4.5 and 6.0 of SSP lead to significant (p≤0.05) decreasing on body weight of the model obese rats which recorded 108.06, 94.46, 77.28 and 73.18 %, respectively when compared to the base line. Also, a positive relationship was noticed between the rate of weight loss and the increase in the concentration of SSP intervention. The same behavior was reported by Elhassaneen, et al. [27] who have used *Sargassum subrepandum* extract by 0.25 to 1.0% w/w in the intervention process. With the context, Maeda, et al. [112] reported that brown algae supplementation of rats diet with led to a significant (p≤0.05) reduction in white adipose tissue after 4 weeks of feeding. Also, Elhassaneen, et al. [113] reported that addition of *Sargassum subrepandum* powder by the rate of 1 to 4% to the diet induced significant (p≤0.05) decreasing on body
weight of the model obese rats. Furthermore, several studies confirmed that the Eastern countries of part of the world have lower prevalence of metabolic syndrome than Western countries which could be attributed to high dietary intake of seaweeds beside fish and soy [114, 115]. The present data with the others proved that positive roles of SSP in the control of the obesity could be attributed to its high level content of several classes of bioactive compounds including polyphenols, flavonoids, terpenoid, triterpenoids, polysaccharides, carotenoids, tannins, anthocyanin’s and kaempferol [113]. The potential mechanisms of such bioactive compounds in obesity treatment/prevention could be summarized in one or more of the following roles: 1) up-regulation of mitochondrial uncoupling protein 1 which may lead to an increase in resting energy expenditure, 2) suppression of adipocyte differentiation and lipid accumulation by inhibition of glycerol-3-phosphate dehydrogenase (G-3-PD) activity or down-regulation of the peroxisomes proliferator-activated receptor-γ that are responsible for adipogenesis gene expression, 3) ability of these compounds and their metabolites to induce several mechanisms that contribute to controlling their action in adipocyte function and subsequent obesity, and 4) potential for these compounds to interact with several transcription factors to the nuclear receptor superfamily, and interfere with the activity of other transcription factors, modulated signaling pathways that are associated with inflammatory and oxidative stress responses as well as scavenged the free radicals [25, 27, 63, 112, 113, 116-121]. Additionally, SSP are also rich in several antioxidants, vitamins, minerals and omega -3- fatty acids, which may have their importance in preventing obesity and its associated complications [113, 114]. On the other side, fiber is the largest component in SSP (around 50%) may therefore prevent obesity-related disorders. Fiber is indigestible, it plays significant nutritional role since, it helps to provide bulk to stool and aid in the movement through the digestive tract. Also, by promoting the growth of gut bacteria, dietary fiber could prevent obesity, metabolic syndrome, and unwanted changes in the intestine [122, 123].

3.2.2. Effects of SSP on Liver Functions of Obese Rats

Effects of SSP intervention on liver functions of obese rats liver functions of obese rats were shown in Table 7 and Fig 2. Such data indicated that obesity induced significant (p≤0.05) increased in ALT, AST and ALP by the rate of 41.34, 39.66 and 35.67, respectively compared to the normal control rats. The intervention of the rat’s diet with SSP by 1.5 to 6.0% induced significant (p≤0.05) improvements on liver functions through decreasing the AST, ALT and ALP activates by different rates. Also, a positive relationship was noticed between the liver function improvement and the increase in the concentration of SSP intervention.

Aminotransferases (ALT and AST) and ALP are normally intracellular enzymes and the presence of elevated levels of such enzymes in plasma indicates damage to cells rich in these enzymes. Data of the present study indicated that SSP improve the liver functions in obese rats induced by obesity through the activity of hepatic aminotransferases and ALP. Such data are in accordance with that observed by Elhassaneen, et al. [27] who have used Sargassum subrepandum extract by 0.25 to 1.0% w/w in the intervention process. Also, Fitton, et al. [92] reported that consumption of brown alga is thought to ameliorate some inflammatory disorders including liver diseases. Data of the present study indicated that SSP is a rich source of different classes of bioactive compounds including polyphenols, flavonoids, terpenoids, triterpenoids, polysaccharides, carotenoids, tannins, anthocyanin’s and kaempferol. All of these compounds exhibited several biological activities including antioxidant and scavenging activities and inhibition of lipid peroxidation [28, 63, 64]. A strong association between the increasing of the brown algae consumption and human diseases prevention and has been explained by their content of bioactive compounds [124]. Also, Shannon and Abu-Ghannam [125] explain the hepatoprotective effect of brown algae through its a marked antioxidant effect which increasing the antioxidative defense system in hepatic cells i.e. reducing malonaldehyde (MDA) levels and increasing total antioxidant capacity, and hepatic glutathione (GSH) levels.

3.2.3. Effect of SSP on Serum Lipids Profile Concentration of Obese rats

The effect of SSP on serum lipids profile concentration of obese rats was shown in Table 8 and Fig 3. Such data indicated that obesity induced a significant (p≤0.05) increased in TG, TC, LDL-c and VLDL-c by 61.38, 41.43, 101.85 and 61.38%, respectively compared to normal control group. The opposite direction was recorded for HDL which decreased by -25.25%. The intervention of the rat’s with SSP by 1.5, 3.0, 4.5 and 6.0% induced significant (p≤0.05) improvements on serum lipid profile through decreasing the TG, TC, TC, LDL-c, VLDL-c and HDL-c levels by different rates. The present data with the others proved that positive roles of SSP in improving of the serum lipid profile which resulted from obesity complications. Such role could be attributed to high level content of several classes of bioactive compounds including polyphenols, flavonoids, terpenoid, triterpenoids, polysaccharides, carotenoids, tannins, anthocyanin’s and kaempferol in SSP. Such data are in accordance with that observed by several authors who reported that feeding of obese rats with plant parts contains the same previous bioactive compounds lead to improve the serum lipid profile of obese rats [7, 25, 29, 116, 120, 126-128]. In the same context, modeling based on systematic reviews of RCTs suggests that modest and sustained weight loss (5-10 kg) in patients with overweight or obesity is associated with reductions in LDLp, TC and TG and with increased levels of HDL [129].

Generally, cardiovascular disease (CVD) is a major health problem in both industrial and developing countries including Egypt. Blood elevated concentrations of TC or LDL-c and slided in HDL in the blood are powerful risk factors for CHD, [130]. The composition of the human diet(s) plays an important role in the management of lipid profile in the blood. Reduction in saturated fat and cholesterol intake has traditionally been the first goal of dietary therapy in lowering the risk for CVD.
In recent years, however, the possible hypocholesterolemic effects of several dietary components/phytochemicals such as found in SSP have attracted much interest. Data of the present study with the others indicated that might improve serum lipid profile subsequently beneficial effects on cardiovascular health through the antioxidant, scavenging and anti-inflammatory activities as well as inhibition of LDL induced by the previous bioactive compounds [63, 120, 128, 131, 132]. On the other side, fiber, the largest component in SSP, ples an important roles in improving the obesity complications including te serum lipid profile. For example, Camire, et al. [133] and Elbasouny, et al. [116] reported that fibers are primarily insoluble, and can bind bile acids. It is believed that binding of bile acids is one of the mechanisms whereby certain sources of dietary fibers lower plasma cholesterol. Also, El-Saadany [134] reviewed that the hypocholesterolemic effect of dietary fiber and found that after four weeks of feeding on potato peels, rats showed 40% reduction in plasma cholesterol content and 30% of hepatic fat cholesterol levels were reduced as compared with animals fed only with cellulose supplemented diet.

3.2.4. Effects SSP Intervention on Serum Glutathione Fractions of Obese Rats

The effects SSP intervention on serum glutathione fractions (GSH and GSSG) of obese rats was shown in Table 9 and Fig. 4. From such data it could be noticed that obesity caused a significant (p≤0.05) decreased in GSH and GSSG by the ratio of -27.74 and -11.73%, respectively compared to normal controls. The intervention with SSP by the ratio of 1.5, 3.0, 4.5 and 6.0 % of SSP lead to significant (p≤0.05) increasing of the serum glutathione fractions concentrations induced by obesity. Also, a positive relationship was noticed between the glutathione fractions concentrations and the increase in the concentration of SSP intervention. The same behavior was reported for GSH by Elhassaneen, et al. [27] who have used Sargassum subrepandum extract by 0.25 to 1.0% w/w in the intervention process. With the same context, Abd-elnaby [135] noticed that tannins extract of brown algae increase hepatic glutathione and total antioxidant capacity diabetic treated groups compared with diabetic non-treated groups.

Generally, GSH is a tripeptide-thiol that has received considerable attention in terms of its various intracellular functions including serve as a nonenzymatic scavenger of oxyradicals and construct roles in detoxifications processes i.e. as a key conjugate of electrophilic intermediates, principally via glutathione-s-transferase activities in phase II metabolism, and as an important antioxidant [136-138]. Present data with the others suggested that secretion of glutathione fractions from the liver to blood might be blocked by different disease complications including obesity because of intracellular structural failure, elevation of the lipid peroxidation and/or the energy depletion suggested by the marked decrease in glycogen content [7, 64, 117, 139]. The present study indicated that SSP was described by its high content of different classes of bioactive compounds including polyphenols, flavonoids, terpenoid, triterpenoids, polysaccharides, carotenoids, tannins, anthocyanin’s and kaempferol which exhibited high antioxidant and scavenging, anti-inflammatory, and anticarcinogenic activities as well as inhibition of the lipid peroxidation [7, 140-143]. Several studies reported that a fall in glutathione fractions observed generally accompanied by a concomitant increased in the liver lipid peroxidation [117, 126, 139]. Therefore, all of those previous biological effects of SSP, reducing the lipid peroxidation rate subsequently elevate the glutathione fractions content in both liver and serum.

3.2.5. Effects of SSP on Biological Oxidants Levels of Obese Rats

The effects of SSP on biological oxidants levels ((Malondialdehyde, MDA and Reactive oxygen species, ROS) concentration of obese rats was shown in Table 10 and Fig 5. Obesity as the result of HFD feeding caused a significant (p≤0.05) increasing in both serum MDA and ROS concentration by the ratio 68.62 and 53.96%, respectively compared to normal controls. The intervention with SSP by the ratio of 1.5, 3.0, 4.5 and 6.0% lead to significant (p≤0.05) decreasing of the serum MDA and ROS concentrations induced by obesity. Also, a positive relationship was noticed between the serum MDA and ROS concentrations and the increase in the concentration of SSP intervention. The same behavior for MDA was reported by Elhassaneen, et al. [27] who have used Sargassum subrepandum extract by 0.25 to 1.0% w/w in the intervention process.

Generally, several previous studies indicated that that the level of MDA in the serum is a good marker of lipid peroxidation/oxidative stress, which is closely related to the pathogenesis of many diseases, including obesity [26, 109, 110, 128, 129, 139]. It is one of the most important compounds in lipid peroxidation products and major product of the oxidation of polyunsaturated fatty acids, lipid hydroperoxides and conjugated dienes which found to be increased in plasma from obese subjects in many clinical studies [129, 144]. Systemic metabolic alterations associated with obesity contribute to the increase in ROS subsequently MDA have been reported by several authors. For example, Jensen, et al. [145] reported that the excess of circulating lipids induces ROS formation pathways, which contribute to the increase in lipid oxidation and protein carbonylation. Also, Bouloumie, et al. [146] found that leptin and angiotensin II, secreted at high levels by adipocytes, are inducers of ROS formation/generation and might therefore promote inflammation and lipid peroxidation. Additionally, possible significance of MDA on human health has been stimulated by Shamberger, et al. [147] who reports that it is mutagenic and carcinogenic compound. Data of the present study with the others reported that SSP intervention normalized the oxidant stats in serum due to several mechanisms induced by its bioactive components content including antioxidant and scavenging activities and inhibition of the lipid peroxidation [28, 63]. With the same context, Liu, et al. [148] investigated the effects of bioactive compounds of brown algae on hepatocyte lipid peroxidation induced by ferric nitrotriacetate in vitro, and showed that significantly decreased intracellular reactive oxygen species (ROS) and DNA damage, and markedly decreased the level of MDA and protein carbonyl contents. Also, several previous studies reported that the plant parts are rich by different classes of bioactive compounds such as found in SSP, have the ability to decrease the...
oxidant status level i.e. the level of MDA and ROS in different biological fluids and organs [28, 30, 63, 118, 139, 149].

3.2.6. Effect of SSP Intervention on the Histopathological Alterations of Obese rats

Effect of SSP intervention on the heart histopathological alterations of obese rats was illustrated in Fig 6. Heart of rat from group 1 revealed the normal histological structure of cardiac myocytes (Photo 1 & 2). In contrast, heart of rats from group 2 showed vacuolation of the sarcoplasm of cardiac myocytes, inflammatory cells infiltration between the cardiac myocytes (Photo 3), intermuscular hemorrhage (Photo 4) and intermuscular edema (Photo 5). Meanwhile, heart of rats from group 3 exhibited only slight intermuscular edema (Photo 6 & 7). Furthermore, heart of rats from group 4 described slight intermuscular edema (Photo 8), slight congestion of myocardial blood vessel (Photo 9) and capillaries (Photo 10). Moreover, heart of rats from group 5 revealed slight congestion of myocardial blood vessels (Photo 11) in some sections, whereas, other sections showed no histopathological lesions (Photo 12). Likewise, some examined sections from group 6 showed slight intermuscular edema (Photo 13), whereas, other sections exhibited no histopathological lesions (Photo 14 & 15).

Effect of SSP intervention on the adipose tissue histopathological alterations of obese rats was illustrated in Fig 7. Adipose of rat from group 1 revealed normal unilocular adipocytes, polygonal in shape and having signet ring appearance (Photo 1 & 2). In contrast, adipose tissue of rats from group 2 showed notable histological changes characterized large size unilocular adipocytes (Photo 3 & 4), inflammatory cells infiltration and congestion of blood vessel (Photo 5). Meanwhile, adipose tissue of rats from group 3 described some large size unilocular adipocytes and some small size adipocytes (Photo 6 & 7). Otherwise, some examined sections from group 4 exhibited apparent histologically normal adipocytes (Photo 8), whereas, other sections revealed congestion of blood vessels (Photo 9). Furthermore, some sections from group 5 exhibited apparent histologically normal adipocytes (Photo 10), whereas, other sections revealed showing some large size unilocular adipocytes and some small size adipocytes (Photo 11) as well as congestion of blood vessels (Photo 12). Otherwise, sections from group 6 revealed apparent histologically normal adipocytes (Photo 13 & 14).

Such data are in accordance with El-Gamal [6] who found that obesity induced focal areas of inter-muscular hemorrhage, congested cardiac vessels and scattered necrotic muscle fibers for cardiac tissue. A good restoration of the cardiac muscles with only few scattered necrotic muscle fibers was observed in obese rats as the result of feeding brown algae. Also, Huimin, et al. [150] reported that significant changes in cardiac function, hypertrophy, fibrosis, and apoptosis were found in HFD rats as compared with the normal rats. Furthermore, Desoky [151] found that obesity can incidence cardiac abnormalities through oxidative stress, mitochondrial dysfunction, pressure or volume overload, cardiac fibrosis and apoptosis as well as light microscope study revealed significant increase in cardiac cell permeability and interstitial collagen. In another study, Gabbia, et al. [152] reported that a high-fat diet can lead to cardiac dysfunction, hypertrophy, and fibrosis. A nutraceutical formulation containing brown algae reduces hepatic lipid accumulation by modulating lipid metabolism and inflammation in experimental models of NAFLD and NASH which directly affects the functions of the heart and adipose tissue. Finally, data of the present study and others confirmed that a good restoration of the cardiac muscles and adipose tissues was observed in obese rats as the result of feeding intervention with SSP.

4. Conclusion

Data of the present study made the Sargassum subrepandum a complete package of healthy-functional food through being an excellent source of nutritional and nutraceutical compounds. Many of such compounds exhibited important biological roles including antioxidant activity. Biological experiments indicated that intervention with SSP lead to significant decreasing on body weight of the model obese rats. Also, SSP was effective in protecting against obese complications including inhibit liver disorders through liver serum enzymes-lowering activity, improvement of the serum antioxidant status (increase the glutathione fractions and decrease the formation of malonaldehyde and reactive oxygen species). Therefore, we recommended SSP by a concentration up to 6% to be included in daily diets, drinks and food supplementation of normal and obese people.

Abbreviations

ALP, serum alkaline phosphatase, ALT, serum alanine aminotransferase, AST, serum aspartate aminotransferase, BD, basic diet, FDA, food and drug administration, GSH, reduced glutathione, GSSG, oxidized glutathione, HDL-c, high density lipoprotein-cholesterol, HFD, high fat diet, LDL-c, low density lipoprotein-cholesterol, MDA, malonaldehyde, ROS, reactive oxygen species, SSP, Sargassum subrepandum powder, TC, total cholesterol, TG, Triglycerides, VLDL-c, Very low density lipoprotein-cholesterol.

Ethical Approval

Biological experiments of the study were ethically approved by the Scientific Research Ethics Committee (SREC), Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval # 25- SREC- 12-2021).
Acknowledgements

The present study was financed partially by the Research Support Unit (RSU), Minoufiya University, Shebin El-Kom, Egypt. The authors extend sincere thanks to Dr. Mohammed Zakaria Mahran, associate professor, and the postgraduate students, Areeg Nour El-Deen, Rawan Shawky and Reem Abi ElFatah, Nutrition and Food Science Department, Faculty of Home Economics, Minoufiya University for their assistance during conducting the biological experiments. Also, our thanks extend to the staff of the Experimental Animals Unit of the Faculty of Home Economics, Minoufiya University, for their assistance.

Conflict of Interests

Authors declared no competing of interest whatsoever.

References


### Table 1. Nutritional composition of SSP on dry weight basis

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (g.100g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>11.83 ± 1.37</td>
</tr>
<tr>
<td>Total protein</td>
<td>5.03 ± 0.83</td>
</tr>
<tr>
<td>Crude fat</td>
<td>2.86 ± 0.17</td>
</tr>
<tr>
<td>Ash</td>
<td>24.51 ± 2.01</td>
</tr>
<tr>
<td>Fibers</td>
<td>46.34 ± 3.27</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>21.26 ± 1.92</td>
</tr>
</tbody>
</table>

*Each value is the Mean ± SD of three replicates.

### Table 2. Minerals content of SSP on dry weight basis

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Amount (mg.100g⁻¹ dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>31.53 ± 2.41</td>
</tr>
<tr>
<td>Na</td>
<td>19.14 ± 1.59</td>
</tr>
<tr>
<td>Ca</td>
<td>28.78 ± 2.56</td>
</tr>
<tr>
<td>Zn</td>
<td>2.01 ± 0.08</td>
</tr>
<tr>
<td>Fe</td>
<td>6.39 ± 0.44</td>
</tr>
<tr>
<td>Cu</td>
<td>0.73 ± 0.03</td>
</tr>
<tr>
<td>Mn</td>
<td>0.68 ± 0.01</td>
</tr>
</tbody>
</table>

*Each value is the Mean ± SD of three replicates.

### Table 3. Vitamins content of SSP on dry weight basis

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (β-carotene, µg/100g)</td>
<td>198.37 ± 5.17</td>
</tr>
<tr>
<td>C (Ascorbic acid, mg/100g)</td>
<td>1.93 ± 0.14</td>
</tr>
<tr>
<td>E (Tocopherols, mg/100g)</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td>B2 (Riboflavin, mg/100g)</td>
<td>14.54 ± 1.14</td>
</tr>
<tr>
<td>B3 (Niacin, mg/100g)</td>
<td>13.96 ± 2.08</td>
</tr>
<tr>
<td>B9 (Folate, µg/100g)</td>
<td>98.03 ± 2.04</td>
</tr>
</tbody>
</table>

*Each value is the Mean ± SD of three replicates.

### Table 4. Bioactive constituents of SSP on dry weight basis

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenols (mg gallic acid equivalent. g⁻¹)</td>
<td>98.43 ± 2.59</td>
</tr>
<tr>
<td>Flavonoids (mg catechin equivalent. g⁻¹)</td>
<td>30.36 ± 1.87</td>
</tr>
<tr>
<td>Carotenoids (mg .g⁻¹)</td>
<td>28.04 ± 2.03</td>
</tr>
<tr>
<td>Tannins (mg catechine equivalent. g⁻¹)</td>
<td>29.97 ± 1.51</td>
</tr>
<tr>
<td>Terpenoids (mg linalol.100 g⁻¹)</td>
<td>784.59± 10.28</td>
</tr>
<tr>
<td>Triterpenoids (mg ursolic acid,100 g⁻¹)</td>
<td>159.66 ± 2.09</td>
</tr>
<tr>
<td>Polysaccharides (mg starch. g⁻¹)</td>
<td>140.33 ± 5.77</td>
</tr>
<tr>
<td>Anthocyanin’s (mg Cyanidin 3-glucoside, CCy3G equivalent.100g⁻¹)</td>
<td>4.02± 0.32</td>
</tr>
<tr>
<td>Kaempherol (mg.100g⁻¹)</td>
<td>11.58± 0.89</td>
</tr>
</tbody>
</table>

*Each value represents the mean of three replicates ±SD.

### Table 5. Antioxidant activity of SSP

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA (%)</td>
<td>67.15 ± 2.21</td>
</tr>
<tr>
<td>AA [% of butylhydroxy toluene (50 mg/ml)]</td>
<td>75.09 ± 1.97</td>
</tr>
<tr>
<td>AA [% of butylhydroxy toluene (100 mg/ml)]</td>
<td>71.97 ± 1.39</td>
</tr>
<tr>
<td>AA [% of α-tocopherol (50 mg/ml)]</td>
<td>69.96 ± 0.93</td>
</tr>
<tr>
<td>BHT (50 mg/ml)</td>
<td>89.43 ± 0.53</td>
</tr>
<tr>
<td>BHT (100 mg/ml)</td>
<td>93.3 ± 0.44</td>
</tr>
<tr>
<td>α-tocopherol (50 mg/ml)</td>
<td>95.98 ± 0.19</td>
</tr>
</tbody>
</table>

*Each value is the Mean ± SD of three replicates.
### Table 6. The effect of SSP on body weight (g) of obese rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Feeding period (weeks)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>155.39</td>
<td>165.74</td>
<td>175.21</td>
<td>183.06</td>
<td>200.51</td>
<td>220.88</td>
<td>231.16</td>
<td>243.82</td>
<td>255.39</td>
</tr>
<tr>
<td>Model control</td>
<td></td>
<td>155.39</td>
<td>180.65</td>
<td>211.00</td>
<td>234.23</td>
<td>258.21</td>
<td>286.38</td>
<td>308.69</td>
<td>322.42</td>
<td>335.01</td>
</tr>
<tr>
<td>1.5% SSP</td>
<td></td>
<td>155.39</td>
<td>177.09</td>
<td>207.68</td>
<td>219.32</td>
<td>251.74</td>
<td>277.21</td>
<td>303.98</td>
<td>312.20</td>
<td>323.29</td>
</tr>
<tr>
<td>3.0% SSP</td>
<td></td>
<td>155.39</td>
<td>175.68</td>
<td>200.70</td>
<td>216.20</td>
<td>246.85</td>
<td>275.97</td>
<td>287.60</td>
<td>294.87</td>
<td>302.16</td>
</tr>
<tr>
<td>4.5% SSP</td>
<td></td>
<td>155.39</td>
<td>174.12</td>
<td>186.45</td>
<td>199.44</td>
<td>235.33</td>
<td>240.94</td>
<td>259.03</td>
<td>267.29</td>
<td>275.47</td>
</tr>
<tr>
<td>6.0% SSP</td>
<td></td>
<td>155.39</td>
<td>174.28</td>
<td>185.97</td>
<td>196.92</td>
<td>220.98</td>
<td>230.83</td>
<td>252.81</td>
<td>261.03</td>
<td>269.10</td>
</tr>
</tbody>
</table>

*SSP, Sargassum subrepandum powder. Values with different superscript letters in the same column are significantly different at p≤ 0.05.

### Table 7. Effects of SSP intervention on liver functions of obese rats

<table>
<thead>
<tr>
<th>Value</th>
<th>Normal control</th>
<th>Model control</th>
<th>SSP (%)</th>
<th>1.50</th>
<th>3.00</th>
<th>4.50</th>
<th>6.00</th>
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</thead>
<tbody>
<tr>
<td>Serum aspartate aminotransferase (AST, U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>39.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.81&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>51.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>46.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>4.74</td>
<td>6.86</td>
<td>3.69</td>
<td>8.08</td>
<td>5.53</td>
<td>6.46</td>
<td></td>
</tr>
<tr>
<td>% of Change</td>
<td>0.00</td>
<td>41.34</td>
<td>34.90</td>
<td>28.05</td>
<td>19.72</td>
<td>15.96</td>
<td></td>
</tr>
<tr>
<td>Serum alanine aminotransferase (ALT, U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>22.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.07&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>SD</td>
<td>2.48</td>
<td>5.17</td>
<td>3.22</td>
<td>2.27</td>
<td>4.38</td>
<td>3.64</td>
<td></td>
</tr>
<tr>
<td>% of Change</td>
<td>0.00</td>
<td>39.66</td>
<td>35.44</td>
<td>34.94</td>
<td>21.29</td>
<td>17.79</td>
<td></td>
</tr>
<tr>
<td>Serum alkaline phosphatase (ALP, U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>96.70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>131.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>128.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>120.34&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>115.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>111.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>9.21</td>
<td>20.80</td>
<td>11.95</td>
<td>9.81</td>
<td>15.63</td>
<td>12.49</td>
<td></td>
</tr>
<tr>
<td>% of Change</td>
<td>0.00</td>
<td>35.67</td>
<td>32.49</td>
<td>24.45</td>
<td>19.72</td>
<td>15.76</td>
<td></td>
</tr>
</tbody>
</table>

Means in the same row with different letters are significantly different at p≤0.05.
Fig. 2. Effects of SSP powder intervention on liver functions (as a % of control) of obese rats

![Graph showing the effects of SSP powder intervention on liver functions](image)

Table 8. Effects SSP intervention on serum lipids profile concentration of obese rats*

<table>
<thead>
<tr>
<th>Value</th>
<th>Normal control</th>
<th>Model control</th>
<th>SSP (%)</th>
<th>1.50</th>
<th>3.00</th>
<th>4.50</th>
<th>6.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (TG, mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>92.56^a</td>
<td>149.37^a</td>
<td>142.65^ab</td>
<td>134.23^b</td>
<td>114.44^c</td>
<td>107.00^cd</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>4.11</td>
<td>6.22</td>
<td>7.93</td>
<td>13.10</td>
<td>5.87</td>
<td>8.49</td>
<td></td>
</tr>
<tr>
<td>% of Change</td>
<td>0.00</td>
<td>61.38</td>
<td>54.12</td>
<td>45.02</td>
<td>23.64</td>
<td>15.60</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (TC, mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>134.19^c</td>
<td>189.79^a</td>
<td>184.74^a</td>
<td>180.56^ab</td>
<td>169.76^b</td>
<td>161.72^b</td>
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</tr>
<tr>
<td>SD</td>
<td>10.03</td>
<td>5.50</td>
<td>15.31</td>
<td>8.92</td>
<td>9.44</td>
<td>11.03</td>
<td></td>
</tr>
<tr>
<td>% of Change</td>
<td>0.00</td>
<td>-25.25</td>
<td>-23.87</td>
<td>-20.69</td>
<td>-14.58</td>
<td>-10.36</td>
<td></td>
</tr>
<tr>
<td>High density lipoprotein-cholesterol (HDL-c, mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>57.89^a</td>
<td>43.27^c</td>
<td>44.07^c</td>
<td>45.91^bc</td>
<td>49.45^b</td>
<td>51.89^b</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>3.32</td>
<td>4.09</td>
<td>5.70</td>
<td>7.27</td>
<td>4.52</td>
<td>4.17</td>
<td></td>
</tr>
<tr>
<td>% of Change</td>
<td>0.00</td>
<td>-25.25</td>
<td>-23.87</td>
<td>-20.69</td>
<td>-14.58</td>
<td>-10.36</td>
<td></td>
</tr>
<tr>
<td>Low density lipoprotein-cholesterol (LDL-c, mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>57.79^a</td>
<td>116.65^a</td>
<td>112.14^a</td>
<td>107.80^ab</td>
<td>97.42^b</td>
<td>88.43^b</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>9.18</td>
<td>9.16</td>
<td>12.32</td>
<td>6.29</td>
<td>10.54</td>
<td>12.42</td>
<td></td>
</tr>
<tr>
<td>% of Change</td>
<td>0.00</td>
<td>101.85</td>
<td>94.05</td>
<td>86.55</td>
<td>68.59</td>
<td>53.02</td>
<td></td>
</tr>
<tr>
<td>Very low density lipoprotein-cholesterol (VLDL-c, mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>18.51^a</td>
<td>29.87^a</td>
<td>28.53^ab</td>
<td>26.85^b</td>
<td>22.89^c</td>
<td>21.40^cd</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>1.93</td>
<td>4.46</td>
<td>4.84</td>
<td>4.09</td>
<td>3.20</td>
<td>4.79</td>
<td></td>
</tr>
<tr>
<td>% of Change</td>
<td>0.00</td>
<td>61.38</td>
<td>54.12</td>
<td>45.02</td>
<td>23.64</td>
<td>15.60</td>
<td></td>
</tr>
</tbody>
</table>

*Means in the same row with different superscript letters are significantly different at p≤ 0.05.

Fig. 3. Effects SSP intervention on serum lipids profile concentration (as a % of control) of obese rats

![Graph showing the effects of SSP intervention on serum lipids profile concentration](image)
Table 9. Effects SSP intervention on serum glutathione fractions of obese rats

<table>
<thead>
<tr>
<th>Value</th>
<th>Normal control</th>
<th>Model control</th>
<th>SSP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.50</td>
</tr>
<tr>
<td>Reduced glutathione concentration (GSH, µmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.04&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>SD</td>
<td>1.05</td>
<td>0.94</td>
<td>1.38</td>
</tr>
<tr>
<td>% of Change</td>
<td>0.00</td>
<td>-27.74</td>
<td>-24.64</td>
</tr>
<tr>
<td>Oxidized glutathione concentration (GSSG, µmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.659&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.582&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.589&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SD</td>
<td>0.043</td>
<td>0.106</td>
<td>0.044</td>
</tr>
<tr>
<td>% of Change</td>
<td>0.00</td>
<td>-11.73</td>
<td>-10.62</td>
</tr>
</tbody>
</table>

*Means in the same row with different letters are significantly different at p≤0.05

Fig-4. Effects SSP intervention on serum glutathione fractions (as % of change) of obese rats

Table 10. Effects of SSP intervention on biological oxidants levels of obese rats

<table>
<thead>
<tr>
<th>Value</th>
<th>Normal control</th>
<th>Model control</th>
<th>SSP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.50</td>
</tr>
<tr>
<td>Malondialdehyde concentration (MDA, nmol/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>6.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SD</td>
<td>0.79</td>
<td>2.70</td>
<td>1.13</td>
</tr>
<tr>
<td>% of Change</td>
<td>0.00</td>
<td>68.62</td>
<td>56.81</td>
</tr>
<tr>
<td>Reactive oxygen species concentration (ROS, U/mL))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>57.44&lt;sup&gt;d&lt;/sup&gt;</td>
<td>88.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.77&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>SD</td>
<td>5.21</td>
<td>7.43</td>
<td>8.32</td>
</tr>
<tr>
<td>% of Change</td>
<td>0.00</td>
<td>53.96</td>
<td>49.33</td>
</tr>
</tbody>
</table>

Means in the same row with different letters are significantly different at p≤0.05

Fig-5. Effects of SSP intervention on biological oxidants levels (as a % of control) of obese rats
Fig 6. Effect of SSP intervention on the histopathological alterations in heart of obese rats (H & E X 400)

Photo (1): Photomicrograph of heart of rat from group 1 showing the normal histological structure of cardiac myocytes.

Photo (2): Photomicrograph of heart of rat from group 1 showing the normal histological structure of cardiac myocytes.

Photo (3): Photomicrograph of heart of rat from group 2 showing vacuolation of the sarcoplasm of cardiac myocytes (black arrow) and inflammatory cells infiltration between the cardiac myocytes (red arrow).

Photo (4): Photomicrograph of heart of rat from group 2 showing intermuscular hemorrhage (arrow).

Photo (5): Photomicrograph of heart of rat from group 2 showing intermuscular edema (arrow).

Photo (6): Photomicrograph of heart of rat from group 3 showing slight intermuscular edema (arrow).

Photo (7): Photomicrograph of heart of rat from group 3 showing slight intermuscular edema (arrow).

Photo (8): Photomicrograph of heart of rat from group 4 showing slight intermuscular edema (arrow).

Photo (9): Photomicrograph of heart of rat from group 4 showing slight congestion of myocardial blood vessel (arrow).

Photo (10): Photomicrograph of heart of rat from group 4 showing slight congestion of myocardial blood capillaries (arrow).

Photo (11): Photomicrograph of heart of rat from group 5 showing slight congestion of myocardial blood vessel (arrow).

Photo (12): Photomicrograph of heart of rat from group 5 showing no histopathological lesions.

Photo (13): Photomicrograph of heart of rat from group 6 showing slight intermuscular edema (arrow).

Photo (14): Photomicrograph of heart of rat from group 6 showing no histopathological lesions.

Photo (15): Photomicrograph of heart of rat from group 6 showing no histopathological lesions.
Fig. 7. Effect of SSP intervention on the histopathological alterations in adipose tissue of obese rats (H & E X 400).

Photo (1): Photomicrograph of adipose tissue of rat from group 1 showing normal unilocular adipocytes, polygonal in shape and having signet ring appearance.

Photo (2): Photomicrograph of adipose tissue of rat from group 1 showing normal unilocular adipocytes, polygonal in shape and having signet ring appearance.

Photo (3): Photomicrograph of adipose tissue of rat from group 2 showing large size unilocular adipocytes (black star).

Photo (4): Photomicrograph of adipose tissue of rat from group 2 showing large size unilocular adipocytes (black star) and inflammatory cells infiltration (arrow).

Photo (5): Photomicrograph of adipose tissue of rat from group 2 showing congestion of blood vessel (arrow).

Photo (6): Photomicrograph of adipose tissue of rat from group 2 showing some large size unilocular adipocytes (black star) and some small size adipocytes (red star).

Photo (7): Photomicrograph of adipose tissue of rat from group 3 showing some large size unilocular adipocytes (black star) and some small size adipocytes (red star).

Photo (8): Photomicrograph of adipose tissue of rat from group 3 showing some large size unilocular adipocytes (black star) and some small size adipocytes (red star).

Photo (9): Photomicrograph of adipose tissue of rat from group 4 showing apparent histologically normal adipocytes.

Photo (10): Photomicrograph of adipose tissue of rat from group 4 showing apparent histologically normal adipocytes.

Photo (11): Photomicrograph of adipose tissue of rat from group 4 showing apparent histologically normal adipocytes.

Photo (12): Photomicrograph of adipose tissue of rat from group 5 showing large size unilocular adipocytes (black star) and some small size adipocytes (red star).

Photo (13): Photomicrograph of adipose tissue of rat from group 5 showing large size unilocular adipocytes (black star) and some small size adipocytes (red star).

Photo (14): Photomicrograph of adipose tissue of rat from group 5 showing large size unilocular adipocytes (black star) and some small size adipocytes (red star).