

Biological Activities of Brown Algae (*Sargassum subrepandum*) and Their Effects on Biochemical, Molecular and Histological Liver Damage Induced by Benzo[a]Pyrene in Rats

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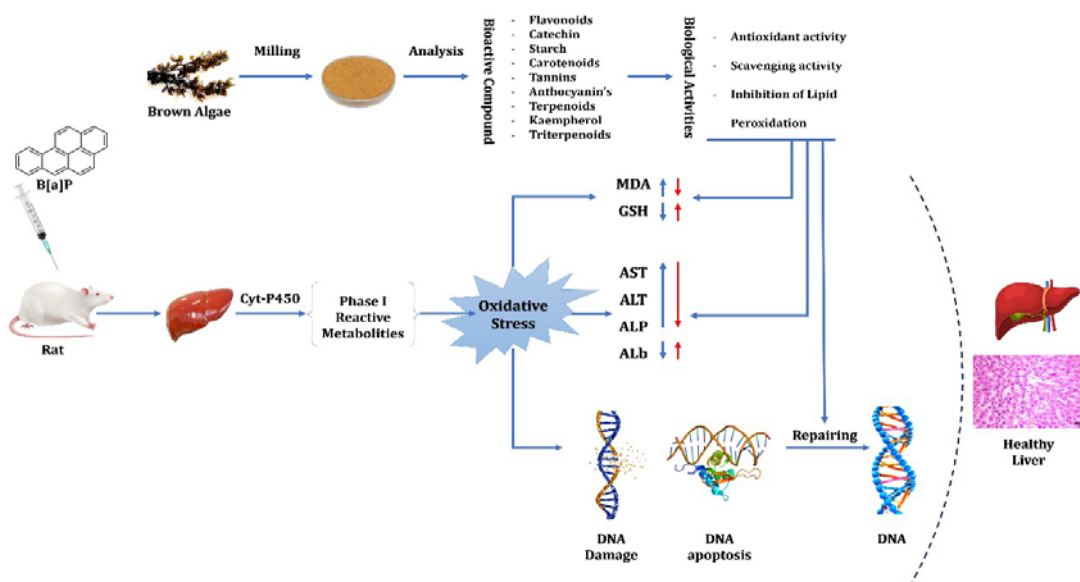
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Abstract The present study aims to investigate the effect of *Sargassum subrepandum* powder feeding intervention on biochemical, molecular and histological liver damage induced by benzo[a]pyrene in rats. *Sargassum subrepandum* powder showed a high nutritional composition through its high of essential nutrients (carbohydrates, fiber, protein and ash). It also contains many of the following bioactive constituents: polyphenols, Anthocyanin's, Carotenoids, Kaempferol, polysaccharides, terpenoids, triterpenoids, flavonoids, carotenoids, tannins, and dietary fiber, which resulted in high biological activates including antioxidant and peroxy radical (ROO⁻)-scavenging activities and inhibition of lipid peroxidation. For biological experiments, rats (n=36), were randomly assigned to six groups of 6 rats per each. Group 1 served as normal control. Groups 2 to 6 were injected intraperitoneally with B[a]P for 14 days and group (2) acted as a model control, while groups (3-6) received *Sargassum subrepandum* powder at concentrations of 1, 2, 3, and 4 g/100g diet for 28 days each, respectively. B[a]P-injected rats exhibiting significantly ($p \leq 0.05$) increased levels of AST (117.02%), ALT (120.24%) and ALP (115.75%) as well as decreased levels of serum albumin (45.67%) compared to the normal group. Also, data indicated that B[a]P induced significantly ($p \leq 0.05$) decreasing in liver GSH level by the ratio of -40.42% whereas, the liver MDA concentration was exhibited the opposite direction, increased by the rate of 195.54% compared to the normal group. Intervention with brown algae (*Sargassum subrepandum* powder (1- 4.0 g/100g diet) for 28 days significantly ($p \leq 0.05$) decreased the levels of liver function enzymes activities (AAST, ALT and ALP) and liver level of MDA, and increased serum albumin and liver level of GSH by different rates compared to the model group. Also, improvement in both molecular and histological parameters was recorded. The rate of improving in all of these parameters exhibited a dose-dependent manner with the *Sargassum subrepandum* powder intervention. The results of this study suggest that treatment with *Sargassum subrepandum* powder in the tested concentrations proved beneficial on manipulation of the liver biochemical, molecular and histological injuries induced by B[a]P.



Keywords: chemical composition, bioactive compounds, liver functions, glutathione, malonaldehyde, electrophoretic pattern of nucleic acid, apoptosis

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1. Introduction

The liver is the largest organ of the body, constituting 2-5% of the adult body weight. It receives blood supply from two major blood vessels. The hepatic artery supplies oxygenated blood, whereas the portal vein, which provides 80% of the total blood supply, supplies nutrient-rich deoxygenated blood. The liver thus acts as a guard between the digestive tract and the rest of the body, transforming, detoxifying, and accumulating metabolites. The liver also plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis and hormone production [1]. Furthermore, liver produces bile juice, an alkaline compound which aids in digestion via the emulsification of lipids. The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions [2]. For that, the liver is necessary for survival and its disease creates a huge economic, humanistic, and clinical burden all over the world.

Benzo[a]pyrene (B[a]P) is a member of the family polycyclic aromatic hydrocarbons (PAHs) that is a by-product of incomplete combustion or burning of organic (carbon-containing) items, e.g., cigarettes, gasoline, and wood [3]. The possible sources of B[a]P contamination of the foodstuffs in the modern human environment are numerous and varied. A review of the literature reveals that curing smokes, contaminated soils, polluted air and water, modes of cooking, food processing and endogenous sources have been considered [4]. Cooking processes have been found to be a major source of such compound in foods and can also be formed in curing and processing of raw food prior to cooking [4,5,6,7,8]. With this context B[a]P from incomplete combustion occur in several foods such as charcoal broiled and smoked foods [9,10,11]. Thus, the World Health Organization [12] stated that 99% of the oral intake of PAH contributed by food, 0.9% by inhalation and 0.1- 0.3% by drinking water.

B[a]P have been shown to be toxic, mutagenic and/or carcinogenic by extensive *in vivo* and *in vitro* experiments [3,6,13,14] [15,16,17,18,19]. Also, B[a]P exposure is associated with the development of liver cancer in all vertebrata [3,6,14,16,20,21,22]. It is known that the toxic and carcinogenic effects of B[a]P correlate with the cellular biotransformation of this compound to arene oxides, phenols, quinones, semiquinones, dihydrodiols and epoxides and with their subsequent inducing cytotoxicity and genotoxicity. Cytotoxicity comes from the ability of these reactive metabolites to cause oxidative stress in various cellular membranes such mitochondria,

lysosomes and cell wall membrane, causing major damage to their vital functions. While, genotoxicity/carcinogenicity induced by the interaction of the reactive intermediates covalently with DNA to form adducts [3,19,22,23,24,25].

Brown algae 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. Worldwide, over 1500–2000 species of brown algae are known and some of them are important in commercial use because they have become subjects of extensive research in their own right [26]. Members of *Sargassum* genus represent valuable sources of a several nutrients including proteins, lipids, vitamins, minerals, essential fatty and amino acids, and bioactive constituents [27,28,29,30]. In this context, polysaccharides are major components and comprise alginates, cellulose, and sulfated polysaccharides such as fucoidans and laminarins. Also, bioactive components include free mannitol, polyphenols, alkaloids, peptides, fatty compounds, and various pigments [28,31,32]. Thus, from a nutritional and therapeutic point of view, brown algae 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 [33,34]. Also, it is apparent that the unique levels of seaweed including brown algae intake contribute to the variance in the levels of breast cancer [35]. 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. The relative longevity and health of Okinawan Japanese populations has been attributed in part to dietary algae in studies [36]. In Brazil, a dietary intervention for 10 weeks study, 3g of decosahexaenoic acid, 5g of seaweed 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, several studies indicated that consuming of brown algae (*Sargassum subrepandum* L.) powder of the diet was effective in protecting of some diseases including obesity, diabetes, bone disorders and liver diseases [28,29,37,38,39]. Despite this, there is still a dearth of information related to the brown algae and liver diseases. Therefore, the current study aims to estimate the chemical composition, bioactive compounds and biological activities of brown algae genus *Sargassum* collecting from the Egyptian Mediterranean beaches. Also, the potential effects of intervention with brown algae powder on biochemical, molecular and histological liver damage induced by benzo[a]pyrene in rats will be in the scope of this investigation.

2. Materials and Methods

2.1. Material

2.1.1. Brown Algae Samples

Brown algae (*Sargassum subrepandum*) samples were collected from the Mediterranean Sea beaches, Alexandria, Egypt. The samples were drained from water and verified by the staff in Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

2.1.2. Reagents and Chemicals

Benzo[a]pyrene (B[a]P) was purchased from Sigma Chemical Co., St. Louis, MO, USA. Basal diet constituents (casein, vitamin and minerals mixture etc.) supplied by Morgan Chemical Company, Cairo, Egypt. Bioactive compounds standard [gallic acid (GA), catechine (CA), α -tocopherol and Butylated hydroxytoluene (BHT)], AAPH [2,2'-Azobis(2-methylpropionamidine) dihydrochloride], CuSO₄ and dimethyl sulfoxide (DMSO) 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.

2.1.3. Kits and Machines

Kit's assays for Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin (Alb), malondialdehyde (MDA), were purchased from BIODIAGNOSTIC, Dokki, Giza, Egypt. Reduced glutathione (GSH) was assayed by the kits provided by MyBioSource, Inc., San Diego, CA, USA). UV-visible-light spectrophotometer (UV-160A; Shimadzu Corporation, Kyoto, Japan) was used for all biochemical analysis.

2.1.4. Biological Experimental

2.1.4.1. Ethical Approval

Biological experiments for this study were ethically approved by the Scientific Research Ethics Committee (Animal Care and Use), Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval no. 11-SREC-09-2022).

2.1.4.2. Animals and Housing

Animals used in this study, adult male albino Sprague-Dawley rats (155±6.8 g per each) were obtained from the Laboratory Animal Unit, College of Veterinary Medicine, Cairo University, Egypt. Rats were kept in stainless steel cages under typical laboratory conditions (24±3 °C temperature, 56±2.8% relative humidity, and a 12/12 hour light-dark cycle and kept under normal healthy conditions. All rats were fed on basal diet (BD) for two-week before starting the experiment for acclimatization.

2.1.4.3. Standard/Basal Diet (BD)

The BD prepared according to the following formula as mentioned by Reeves et al., [40] 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 used vitamin and minerals mixtures component were formulated according to Reeves et al., [40].

2.2. Methods

2.2.1. Preparation of *Sargassum subrepandum* Powder

Sargassum subrepandum samples were cleaning and sorting manually and then dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at 70 °C until arriving by the moisture in the final product to about 8%. 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 polyethylene bags, stored in refrigerator at 4°C and used for different experiments later.

2.2.2. Preparation of *Sargassum subrepandum* Extracts

Sargassum subrepandum powder was used for their different types extracts as follow: A 20 g from dried *Sargassum subrepandum* powder plus 180 ml water were homogenized and transferred to a beaker and stirred at 250 rpm in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for 660 min at room temperature. The extract was then separated from the residue by filtration through Whatman No. 1 filter paper. The remaining residue was re-extracted twice, and then the two extracts were combined. The residual water of was removed under reduced pressure at 60°C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). The same protocol was followed as before with changing the extraction medium with metanol (80%, v/v), ethanol (80%, v/v) and hexane (98%, v/v) respectively. The residual solvent of was removed under reduced pressure at 45°C using a rotary evaporator.

2.2.3. Chemical Composition Analysis of *Sargassum subrepandum* Powder

Sargassum subrepandum powder samples were analyzed for proximate chemical composition including moisture, protein (T.N. $\times$ 6.25, micro - kjeldahl method using semiautomatic apparatus, Velp company, Italy), fat (soxhelt semiautomatic apparatus Velp company, Italy, petroleum ether solvent), ash, fiber and dietary fiber contents were determined using the methods described in the A.O.A.C. [41]. Carbohydrates calculated by differences: Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber).

2.2.4. Determination of Bioactive Compounds

Total phenolics in brown algae powder were determined using Folin-Ciocalteu reagent according to Singleton and Rossi, [42] and Wolfe et al., [43] and were expressed as gallic acid equivalents (GAE). Total flavonoids contents were estimated using colorimetric assay described by Zhisen et al., [44] and expressed as catechin equivalent, CAE. Total polysaccharides were

measured according to the method of Vazirian et al., [45] and were expressed as mg of starch equivalents. The total carotenoids were determined by using the method reported by Lichtenthaler, [46]. Tannins expressed as catechine equivalents. Anthocyanin's (mg Cyanidin 3-glucoside, CCy3G equivalent.100g⁻¹) was determined according to the method of Sharif et al., [47]. Total terpenoids were extracted and measured according to the method of Ghorai et al., [48]. Triterpenoids were extracted and measured according to the method of Schneider et al., [49] and was expressed as mg ursolic acid/100 g of dry extract. Kaempferol was measured according to the method mentioned in Fouda et al, [50].

2.2.5. Biological Activities

2.2.5.1. Antioxidant Activity

Antioxidant activity (AA) of plant extracts and standards (α -tocopherol and BHT) was determined according to the β -carotene bleaching (BCB) assay following a modification of the procedure described by Marco, [51].

2.2.5.2. Peroxyl Radical (ROO[•])-scavenging Activity

Peroxyl radical-scavenging activity was determined in brown algae (*Sargassum subrepandum*) by an improved oxygen radical absorbance capacity (ORAC) assay such as described by Ou et al., [52]. The sample (200 μ l), phosphate buffer (3.5 ml, 75 mM, pH 7.4), and FL (100 μ l, 35 nM) were mixed in a test tube and incubated at 37 °C for 5 min before the initial fluorescence intensity was recorded. The 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH) (200 μ l, 75 g/l) was added to initiate the reaction. Fluorescence readings were taken every three minutes until zero fluorescence intensity was reached. The excitation and emission wavelengths were 493 and 515 nm, respectively. Trolox was also used as a standard to calibrate the final results.

2.2.5.3. Inhibition of Low Density Lipoprotein (LDL) Oxidation

Inhibition of LDL oxidation was determined in *Sargassum subrepandum* according to the method of Princen et al., [53]. Adult male white albino rat, Sprague Dawley strain, serum was collected and diluted by phosphate buffer (50 mM, pH 7.4) to the concentration of 0.6%. Quantities of 5.0 ml diluted serum were mixed with 10 μ l DMSO or 10 μ l DMSO containing various concentrations of the all tested algae extracts. A 20 μ l of CuSO₄ solution (2.5 mM) was added to initiate the reaction and the absorbance at 234 nm was recorded then was taken every 20 min thereafter for 140 min at room temperature. The final result was expressed by calculation the net area under the curve.

2.2.6. Biological Experiment

2.2.6.1. Experimental Design

All biological experiments performed a complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council [54]. After the acclimatization period (7 days), rats (n=36), were randomly assigned to six groups

of 6 rats per each. Group 1: served as normal control, was fed on the BD and given corn oil intraperitoneally at a dosage of (10 ml/kg/b.wt.) on the seventh day only; Groups 2–6: were injected intraperitoneally with a single dose of B[a]P (125 mg/kg/b.wt. in corn oil) on the seventh day, and were fed on the BD. After 14 days of treatment with B[a]P, group (2) acted as a model control, while groups (3-6) received *Sargassum subrepandum* powder at concentrations of (1, 2, 3, and 4 g/100g diet) for 28 days each respectively.

2.2.6.2. Blood and Liver Tissues Sampling

At the end of the experiments, the rats were slaughtered under diethyl ether anesthesia after 12 hours of fasting. Blood samples were obtained through the abdominal aorta and placed in centrifuge tubes then serum was carefully separated after centrifugation at 3000 rpm for 10 minutes to assess the biochemical parameters such as described by Stroeve and Makarova [55]. Specimens of the liver organ were taken immediately after sacrificing rats and immersed in 10% neutral buffered formalin for the histological examination. Another specimen of the liver samples was promptly snap-frozen in liquid nitrogen before being kept at -70 °C for biochemical and molecular analysis.

2.2.6.3. Biochemical Analysis

Different tested parameters in serum were determination using the specific methods as follow: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities according to Yound, [56] and Tietz [57], respectively. Albumin was determined by a colorimetric method described by Vanessa et al., [58]. Reduced glutathione (GSH) was measured colorimetrically in serum samples such as described by Ellman, [59]. Serum malonaldehyde (MDA) content was measured by the thiobarbituric acid (TBA) method according to the methods of Buege and Aust, [60].

2.2.6.4. Molecular Studies

Electrophoretic pattern of nucleic acids (DNA and RNA)

Electrophoresis of lysate tissues

Electrophoresis of lysate tissues used to evaluate RNA pattern according to the method described by Hassab El-Nabi et al., [61]. A piece of 10 mg of liver, intestine and spleen from each mouse was excised immediately after perfusion. The hepatic, intestine and splenic tissues were squeezed gently in eppendorf tubes by yellow tips and lysed in 200 μ l lysing buffer (50mMNaCl, 1mMNa₂EDTA and 0.5%SDS, pH 8.3). Gel Preparation: Gel was prepared using 1.8% electrophoretic grade agarose. The agarose was boiled with tris borate EDTA (TBE) buffer (1xTBE buffer; 89 mMTris, 89mM boric acid, 2mM EDTA, pH 8.3). Then 0.5 microgram/ml ethidium bromide was added to agarose mixture at 40°C. Gel was poured and allowed to solidify at room temperature for 1 hour before samples were loaded. For detecting electrophoretic pattern of nucleic acids of tissue lysate, 20 μ l of lysate tissue was loaded in gel wells in addition to 5 μ l loading buffer. Electrophoresis was performed on (Biomtra Standard Power Pack P25) for 2h. at 50V in gel

buffer where DNA and RNA were visualized using a 312 nm UV transilluminator (Cole-Parmer; Cole Parmer instrument Co. Chicago, USA). The intensity of RNA was measured by gel pro analyzer program as maximal optical density.

Deoxyribonucleic acid (DNA) extraction and apoptosis detection

DNA extraction and apoptosis detection in liver tissue was done according to "salting out extraction method" of Aljanabib and Martinez [62] and modification introduced by Hassab El-Nabi, [63], protein was precipitated by saturated solution of NaCl (5M). Ten mg of tissues (0.01 gm) in eppendorf tubes were lysed with 600 microlitre of lysing buffer (50 mM NaCl, 1mM Na₂EDTA, 0.5% SDS, PH 8.3) and gently shaken. The mixture was incubated overnight at 37°C then, 200 microlitre of saturated NaCl was added to the samples, shaken and centrifuged at 12,000 rpm for 10 min. the supernatant was transferred to new eppendorf tubes and then DNA precipitated by 600 microlitre cold Isopropanol. The mix was inverted several times till fine fibers appear, and then centrifuged for 5 min at 12,000 r.p.m. The supernatant is removed and the pellets were washed with 500 microlitre 70% ethyl alcohol centrifuged at 12,000 r.p.m for 5 min. After centrifugation the alcohol was decanted or tipped out and the tubes blotted on whatman paper to be dry. The pellets were resuspended in 50 microlitres or appropriate volume of TE buffer (10 mM Tris, 1 mM EDTA, pH8) supplemented with 5% glycerol. The resuspended DNA was incubated for 30-60 min with loading mix (RNase + loading buffer) and then loaded into the gell wells for detection of apoptosis. Apoptotic bands appeared and located at 200 and its multiplies like 400bp and 600bp ect. The intensity of apoptotic bands were measured by software gel pro program as maximum optical density values comparing by 100bp ladder. Gel were photographed using digital camera while the DNA was visualized using a 312 nm UV light under a transilluminator.

3. Results and Discussions

3.1. Proximate Composition of Brown Algae (*Sargassum subrepandum*) % DW)

Data in Table 1 shows the proximate composition of brown algae (*Sargassum subrepandum*). Such data indicated that carbohydrates is the largest compound (59.36 ± 4.06 g/100g) followed by ash (19.80 ± 0.28 g/100g), crude fiber (8.15 ± 3.91 g/100g), total protein (5.14 ± 0.57 g/100g) and crude fat (0.93 ± 0.21 g/100g). Such data are in a partially accordance with that reported by several authors analyzed samples of brown algae collected from different Egyptian beaches [28,30,37,39,64,65]. Although *Sargassum subrepandum* represents low-calorie foods however its carbohydrate content is generally high and can represent up to 75% of dry weight [66]. Also, Holdt and Kraan [67] reported that *Sargassum subrepandum* contains a large amount of carbohydrate as structural, storage, and functional polysaccharides, and the total carbohydrate content may ranges from 20% to 76% of dry weight depending on the

species. Regarding the fat content, Dawczynski et al., [68] noticed that brown algae including *Sargassum subrepandum* contain fat between 0.9% and 4% of dry weight. Whereas, Antonopoulou et al., [69] reported that fats contain up to 1–3% of dry weight. Also, [70,71] found that brown algae has a very little lipid content, ranging from 1% to 5% of dry matter. All the above data with the others declared that the proximate composition of brown algae including *Sargassum* genus could be affected and vary by the following factors: species, geographical location, season, water temperature, depth, light intensity, nutrients, pH, salinity or combination of these factors [28,30,39,64,65,69,72].

Table 1. Proximate composition of brown algae (*Sargassum subrepandum*)

Component (g/100g)	Range	Mean ±SD
Moisture	7.98-10.11	9.03±1.03
Dry matter	89.65-93.65	91.97±1.76
Total protein	4.45- 6.84	5.14 ±0.57
Crude fat	0.86-1.01	0.93 ± 0.21
Ash	17.54-20.98	19.80 ± 0.28
Crude Fiber	6.09-11.65	8.15 ± 3.91
Carbohydrate	57.43-66.62	59.36 ± 4.06

Each value represents the mean of three replicates ±SD.

3.2. Bioactive Constituents

Table 2. Bioactive compounds of *Sargassum subrepandum* on dry weight basis

Component	Amount range	Mean ±SD
Polyphenols (mg gallic acid equivalent. 100 g ⁻¹)	161.45-186.67	178.45 ± 5.38
Anthocyanin's (mg Cyanidin 3-glucoside, CCy3G equivalent.100g ⁻¹).	7.20-10.94	9.17± 1.11
Carotenoids (mg.100g ⁻¹)	15.56-22.05	19.11 ± 3.27
Kaempferol (mg.100g ⁻¹)	12.09-14.07	14.31± 2.15
Polysaccharides (mg starch. g ⁻¹)	148.44-164.53	151.21 ± 7.98
Terpenoids (mg linalol.100 g ⁻¹)	799.34-845.23	815.24± 21.43
Triterpenoids (mg ursolic acid.100 g-1)	157.34-176.54	169.60 ± 9.54
Flavonoids (mg catechin equivalent.100 g ⁻¹)	20.43-28.05	24.41 ± 3.17
Tannins (mg catechine equivalent.100g ⁻¹)	22.54-33.99	26.96 ± 4.21
Dietary fiber (g/100g)	41.45-46.90	45.21± 2.89

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

Data in Table 2 shows the bioactive compounds in *Sargassum subrepandum*. Such data indicated that polysaccharides (151.21 ± 7.98 mg starch. g⁻¹) were the largest compound followed by terpenoids (815.24± 21.43 mg linalol.100 g⁻¹), polyphenols (178.45 ± 5.38 mg gallic acid equivalent.100 g⁻¹), triterpenoids (169.60 ± 9.54 mg ursolic acid.100 g-1), tannins (26.96 ± 4.21 mg catechine equivalent.100g⁻¹), flavonoids (24.41 ± 3.17 mg catechin equivalent.100 g⁻¹), carotenoids (19.11 ± 3.27 mg.100g⁻¹), kaempferol (14.31± 2.15 mg.100g⁻¹) and anthocyanin's (9.17± 1.11 mg Cyanidin 3-glucoside, CCy3G equivalent.100g⁻¹). Such data are in a partially accordance with that reported by several authors analyzed samples of brown algae collected from different Egyptian beaches [28,30,37,39,64,65]. Also, Chapman and Chapman, [31]

and Helen, [32] found that polysaccharides are major bioactive components in brown algae and comprise alginates, cellulose, and sulfated polysaccharides such as fucoidans and laminarins. Furthermore, Abd Elalal, [30] determined several bioactive constituents in *Sargassum subrepandum* powder including phenolics, tannins, carotenoids and flavonoids in quantities were close to what was recorded in this study. All of such bioactive compounds play significant roles in food processing, human nutrition and pharmaceutical applications. Polysaccharides are using as thickening and gelling agents, and emulsion stabilizers [32,73]. They also exhibited several biological activities including anticoagulant, antithrombotic, anti-inflammatory, anti-obesity, antiviral, anti-osteoporosis, antioxidant and antimicrobial activities [28,29,30,37,39,64,74,75]. Furthermore, polysaccharides absorb substances like cholesterol, which are then eliminated from the digestive system i.e. hypocholesterolemic and hypolipidemic responses [76,77]. Other bioactive compounds reported in *Sargassum subrepandum* including phenolics, flavonoids, carotenoids and anthocyanin's, are exhibiting different important biological roles i.e. antioxidant and scavenging activities and inhibiting the low density lipoprotein oxidation [21,30,39,78,79,80]. Triterpenoids can serve as a starting material for synthesis of more potent bioactive derivatives, such as experimental antitumor agents [81]. Also, several studies indicated that polysaccharides and triterpenoids exhibited protective activities against liver injuries induced xenobiotics [82,83]. The bioactive compound, 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 [84]. Finally, tannins have been reported to be good antimicrobial, antiviral, antiinflammatory, antidiabetic, cardioprotective, antitumor, antioxidant, biopesticide, as well as nutraceutical agent [85]. It is utilized as a food preservative and packaging material in the food industry. Tannins also have been reported in some other advanced applications such food supplement, and medicine-like tannin-containing legumes, which are used to treat gastrointestinal nematodes (GINs) [86,87]. On the other side, the present data indicated that *Sargassum subrepandum* is a rich source of dietary fiber (45.21 ± 2.89 g/100g). Such data are go along with several authors [30,67,88,89]. Dietary fibers are good for human health as they make an excellent intestinal environment by favoring the growth of intestinal microflora, including probiotic species so they can be considered as prebiotic [90]. In several cases, *Sargassum subrepandum* carbohydrates possessing a fiber level greater than those recorded for many vegetables or fruits [91,92]. Among the fibers, hydrocolloids such as alginate, agar, carrageenans, fucoidan, and laminaran are present in large proportions in

brown algae including *Sargassum subrepandum*. Many of these hydrocolloids (polysaccharides) play significant roles in food processing and human therapeutic nutrition applications [29,39,93].

3.3. Antioxidant Activities of *Sargassum subrepandum*

3.3.1. β -Carotene Bleaching (BCB)

Antioxidant activity of *Sargassum subrepandum* extracts assayed by the BCB method was illustrated in Table 3 and Figure 1. BCB assay based on measured the ability of *Sargassum subrepandum* extracts (and well-known antioxidants used as standards) to inhibit lipid peroxidation in an *in vitro* model system made of β -carotene and linoleic acid (51). From such data it could be noticed that ethanol extract (EtOHE) recorded the lowest decreasing in antioxidant activity followed by methanol extract (MtOHE), hexane extract (HE) and water extract (AqE). The values of EtOHE extract absorbance's through 120 min are coming well i.e. higher than 50 ml per gram and close to both BHT (50 mg/L) and closing to the lines of both 50 mg/L BHT, 50 mg/L α -tocopherol. Also, the values for the MtOHE extract absorbance's were also close to the EtOHE extract line. The values for the HE and AqE were recorded weak absorbance's values when compared to the rest of the tested extracts and standard antioxidants. Such data proved the high stability of the alcoholic *Sargassum subrepandum* extracts when comparing with that most common antioxidant standards α -tocopherol. Such data confirmed that the *Sargassum subrepandum* bioactive components were found in both lipohylic and hydrophilic phases i.e. accordance with the known rule "like dissolve like". Thus, the variation in the antioxidant activity values of different studied *Sargassum subrepandum* extracts may be possible due to the presence of specific compounds according to the solubility i.e. extraction media [28]. The present data are in accordance with that obtained by El-Gamal, [28] and Elhassaneen et al., [29] who found that different extracts of brown algae have highly significant ($p \leq 0.05$) differences in antioxidant activity (AA= 30.00 - 84.41%). The highest antioxidant activity was recorded for the ethanol extract (EtOHE) while the lowest one was for the Hexane extract (HE). Also, such attention was recorded by Elhassaneen et al., [94], Sayed Ahmed, [95] and Aly et al., [96] who studied the antioxidant/BCB stability of many plant parts extracts by different media/solvents commonly distributed in the Egyptian local markets. Therefore, through technical and economical point of view, data of the present study recommended the using of ethanol extract amongst the other extracts of the *Sargassum subrepandum* in different kinds of food technology and nutritional therapy applications.

Table 3. Antioxidant activity of brown algae extracts assayed by the BCB method

Extracts	Time (min)												
	0	10	20	30	40	50	60	70	80	90	100	110	120
Ethanol extract (EtOHE)	0.901	0.893	0.869	0.856	0.843	0.801	0.797	0.771	0.758	0.756	0.743	0.740	0.737 ^a
Hexan extract (HE)	0.901	0.791	0.776	0.728	0.674	0.650	0.640	0.620	0.613	0.598	0.590	0.598	0.595 ^b

Water extract (AqE)	0.901	0.766	0.712	0.684	0.600	0.592	0.582	0.573	0.567	0.553	0.551	0.533	0.522 ^b
Methanol extract (MeOHE)	0.901	0.884	0.841	0.840	0.831	0.792	0.781	0.775	0.742	0.731	0.721	0.719	0.716 ^a
Control	0.901	0.539	0.525	0.468	0.437	0.395	0.376	0.329	0.294	0.237	0.201	0.162	0.142
BHT, 50 mg/L	0.901	0.879	0.861	0.832	0.818	0.778	0.774	0.749	0.736	0.734	0.722	0.719	0.716 ^a
α -tocopherol, 50 mg/L	0.901	0.900	0.889	0.878	0.872	0.860	0.848	0.830	0.821	0.800	0.791	0.781	0.780 ^a
BHT, 200 mg/L	0.901	0.900	0.882	0.872	0.870	0.850	0.841	0.821	0.801	0.799	0.786	0.765	0.763 ^a

Each value represents the mean value of three replicates. Values under the same column with different superscripts letters are significant difference ($P \leq 0.05$). BCB, β -carotene bleaching, BHT, Butylated hydroxytoluene, (α -tocopherol at 50 mg/L and BHT at 50 and 200 mg/L concentrations were used as references).

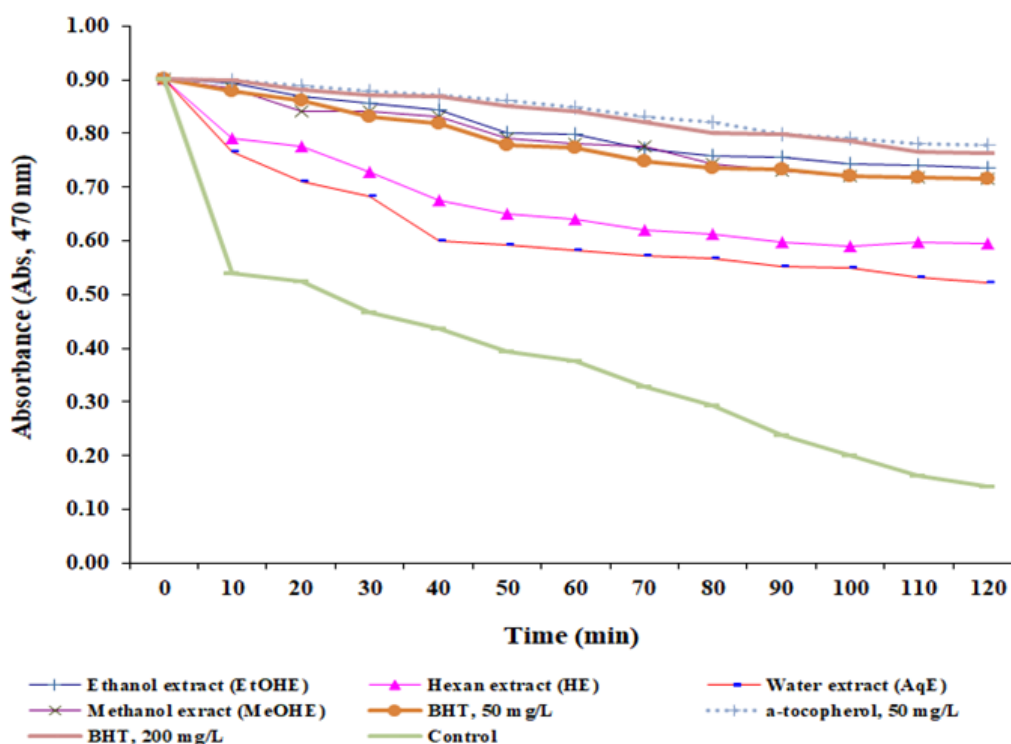


Figure 1. Antioxidant activity of different brown algae extracts assayed by the BCB method. Each value represents the mean value of three replicates. BCB, β -carotene bleaching, BHT, Butylated hydroxytoluene, (α -tocopherol at 50 mg/L and BHT at 50 and 200 mg/L concentrations were used as references)

3.3.2. Inhibition of Low Density Lipoprotein (LDL) Oxidation

Figure 2 shows the dose-dependent inhibition of CuSO_4 -induced LDL oxidation *in vitro* by *Sargassum subrepandum* extracts. From such data it could be noticed that the inhibitive action of the all *Sargassum subrepandum* extracts against CuSO_4 -induced LDL oxidation, as evidenced by decreased conjugated dienes production in a dose-dependent fashion. A comparative study amongst the *Sargassum subrepandum* extracts explained that the EtOHE, MeOHE, HE and AqE acted more dramatically in protecting LDL against oxidation. The protecting LDL against oxidation activity of different *Sargassum subrepandum* extracts was in the following order: EtOHE > MeOHE > HE > AqE. The present data proved that the protecting LDL against oxidation induced by *Sargassum subrepandum* extracts could be attributed to their content of different bioactive constituents (polysaccharides, phenolics, flavonoids, carotenoids,

anthocyanin's, Terpenoids, Triterpenoids, tannins, etc.) as antioxidants [28,38,39,97,98]. The same data was recorded by Elhassaneen et al., [37] with the extracts of *Sargassum subrepandum* samples collected from the Egyptian sea beaches. Also, [96] found that some plant parts extracts contained the same bioactive constituents including onion skin, tomato pomace and eggplant peels protect LDL against oxidation *in vitro*. Furthermore, Aviram et al., [99] and Li et al., [100] reported the same effect when applied with pomegranate peel extract, which was attributed to the high levels of phenolic compounds (such found in *Sargassum subrepandum* extracts). Such mechanisms of actions, protecting LDL against oxidation by phenolic compounds, could be attributed to increase the levels of reduced glutathione (GSH) and glutathione reductase (GSH-Rd) in liver and lungs as well as increase in inhibition of NADPH-dependent lipid peroxidation [28,37,101,102,103]. Also, phenolics, carotenoids and tannins exhibited a complex reaction with peroxy radicals

(ROO[•]) and inhibition of the LDL oxidation [85,104,105]. In this way, "oxidative modification of lipoproteins" hypothesis proposed that LDL oxidation plays a key role in early atherosclerosis [99,106]. The oxidized LDL is atherogenic due to its cytotoxic effects toward arterial cells which stimulates the monocytes to be adhesive to the

endothelium and leads to the development of atheromatous plaques [107]. Data of the present study proved that the *Sargassum subrepandum* extracts could be used as a promising contrivance in the prevention of atherosclerosis through inhibiting LDL oxidation process.

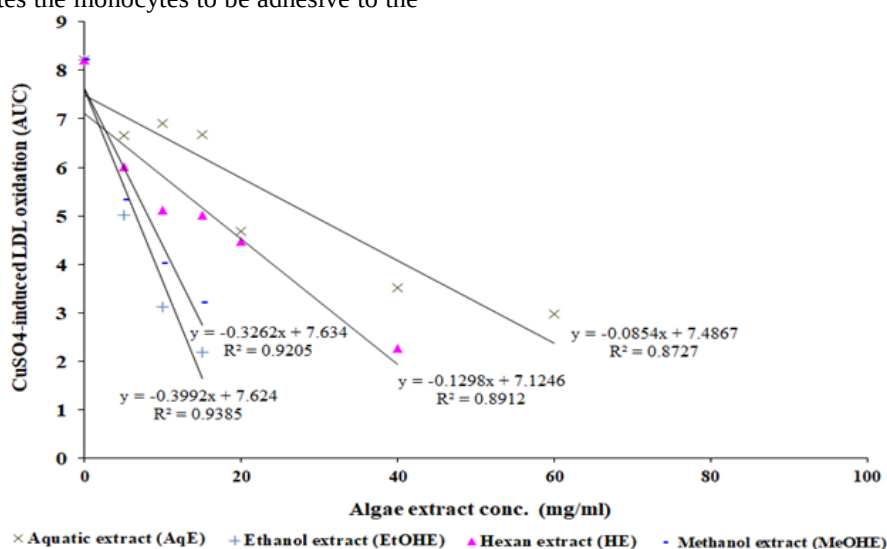


Figure 2. Dose-dependent inhibition of CuSO₄-induced LDL oxidation *in vitro* by brown algae extracts. The conjugated diene formation was measured as the absorbance at 234 nm and the result is expressed as the area under the curve (AUC)

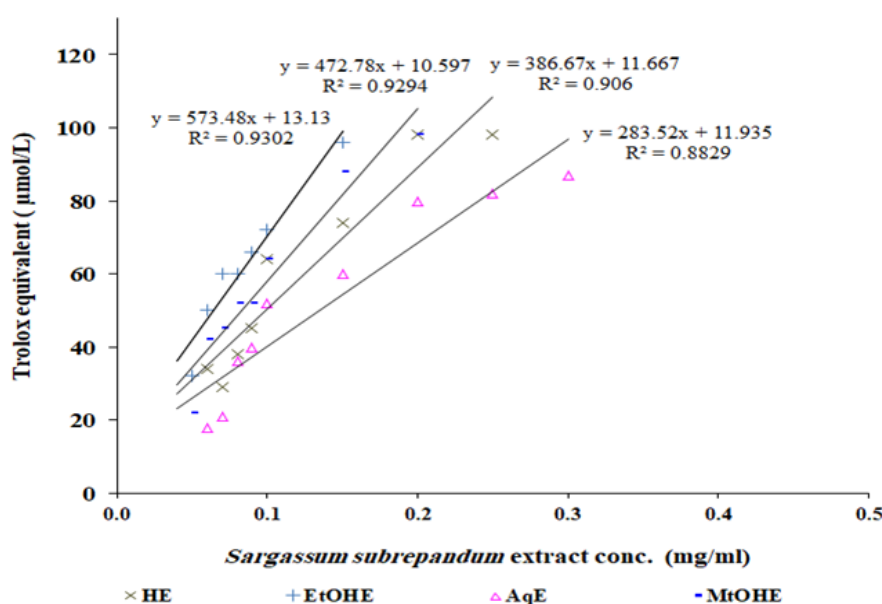


Figure 3. Dose-dependent ROO[•] scavenging capacity of *Sargassum subrepandum* as determined by improved oxygen radical absorbance capacity (ORAC) assay. EtOHE, ethanol extract, HE, hexan extract, AqE, water extract, and MeOHE, methanol extract

3.3.3. Peroxyl Radical (ROO[•])-scavenging Activity of *Sargassum subrepandum*

The peroxyl radicals (ROO[•]) prevention capacity assay was used to compare the preventive capacity of *Sargassum subrepandum* against ROO[•]. The assay is based on the preventive capacity against ROO[•] related to the metal-chelating capability of the extract(s) tested. The reagent, 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH), is used to generate ROO[•] and fluorescein is employed as the sensitive probe for free radical attack in this technique [100]. Data of the present study indicated that as compared to the *Sargassum subrepandum* extracts, the EtOHE, MeOHE, AqE, and HE acted more

dramatically in preventive capacity against ROO[•] formation/scavenging activity (Figure 3). The EtOHE and MeOHE appeared to be more effective than the rest of extracts in formation/scavenging the ROO[•]. The ROO[•] formation occurs during the oxidation of lipids in oxidative stress in living cells. ROO[•] may diffuse a considerable distance and can react with sulfhydryl groups, (-SH). Such data are in accordance with that reported by several authors with algae and different plant parts [30,38,64,96]. Thus, data of the present study indicated that *Sargassum subrepandum* extracts could be used successfully as a promising tool in the treatment or prevention of some diseases whose mechanisms of

occurrence include oxidative stress through scavenging some of the free radicals form during that process. These diseases include diabetes, cardiovascular disease, obesity, liver disease, and aging disease [19,37,103,108,109].

3.4. Biological Experimental

3.4.1. Effect of Feeding Intervention with Brown Algae (*Sargassum subrepandum*) Powder on Liver Function Disorders Induced by B[a]P in Rats

Effect of feeding intervention with brown algae (*Sargassum subrepandum*) powder on liver function disorders induced by B[a]P in rats were shown in Table 4. From such data it could be noticed that B[a]P-injected rats exhibiting significantly ($p \leq 0.05$) increased levels of AST (117.02%), ALT (120.24%) and ALP (115.75%) compared to the normal group. Intervention with brown algae (*Sargassum subrepandum* powder (1.0, 2.0, 3.0 and 4.0 g/100g diet) for 28 days significantly ($p \leq 0.05$) decreased the levels of these enzymes activities by the rate of 108.34, 94.16, 41.80 and 34.05% (for AST); 106.43, 100.35, 85.72 and 45.49% (for ALT), and 110.31, 101.93, 85.92 and 55.14% (for ALP), compared to the normal controls, respectively. The rate of decreasing in all of these parameters exhibited a dose-dependent manner with the *Sargassum subrepandum* powder intervention. The opposite direction was recorded with the serum albumen levels.

B[a]P-induced liver damage is a commonly used model in many experimental studies regarding the protective effects of drugs, natural extracts, and functional foods related to liver disease [17,19,110,111,112]. Such liver damage came from the reaction covalently between reactive intermediates/radicals of B[a]P with nucleic acids to form adducts and the effect of reactive intermediates/radicals to cause lipid peroxidation of membrane-bound fatty acids [3,10,11,14,23,113]. As a result, the structure and function of the liver cells membrane and intracellular organelles (mitochondria and lysosomes) become throwing into confusion [3,21,114,115]. Data of the present study indicated that B[a]P injection induced severe damage to liver cells through increasing the serum AST, ALT and ALP level activities. Aminotransferases (ALT and AST) plus ALP are normally intracellular enzymes. Thus, the presence of elevated levels of aminotransferase and ALP in the plasma

indicates damage to cells rich in these enzymes [115,116].

The present data also indicated that intervention feeding with the *Sargassum subrepandum* powder significantly ($p \leq 0.05$) reduced AST, ALT and ALP levels which demonstrating that it could prevent the live cells damage. Such preventive effects could be attributed to the high content of some important bioactive constituents that have been measured in *Sargassum subrepandum* powder with their biological activities exhibited including antioxidant activity and peroxy radical (ROO $\cdot$)-scavenging activities and inhibition of lipid peroxidation. Such data are partially confirmed by the study that found that consumption of *Sargassum subrepandum* powder is thought to ameliorate some inflammatory disorders including liver diseases. Also, Shannon and Abu-Ghannam [117] explain the hepatoprotective effect of *Sargassum subrepandum* powder through its a marked antioxidant effect which raising the antioxidative defense system in hepatic cells i.e. reducing malonaldehyde (MDA) levels and increasing total antioxidant capacity, catalase (CAT) activity, and hepatic glutathione (GSH) levels *Sargassum subrepandum* extract has been shown to markedly reduce plasma lipid peroxidation levels in diabetic mice. Finally, several authors reported that the effect of different plant parts containing the same bioactive constituents determined in *Sargassum subrepandum* on decreasing the serum liver function enzymes activity induced by different toxicants could be attributed to their high level content of those compounds [29,37,64,95,112,118].

For serum albumin level, the present data are in agreement showed that B[a]P induced significant ($p \leq 0.05$) decrease in the serum albumin content. Also, it was reported that treatment with onion skin significantly decreased the reduced levels of serum albumin induced by B[a]P [21]. Albumin is an important metal binding protein. It is a sacrificial antioxidant that can bind copper tightly and iron weakly to its surface serving as a target for their related free radical reactions. Thus, it inhibits copper ion dependent lipid peroxidation [119]. The possible mode of action of liver serum enzymes-lowering activity and albumin lowering level of the *Sargassum subrepandum* could be explained by one or more of the following process: 1) increasing the antioxidative defense system in hepatic cells, 2) decrease the hepatic lipid droplet accumulation, and 3) improve the level of albumin i.e. sacrificial antioxidant in serum.

Table 4. Effect of feeding intervention with brown algae (*Sargassum subrepandum*) powder on liver function disorders induced by B[a]P in rats

Groups	Aspartate aminotransferase (AST)		Alanine aminotransferase (ALT)		Alkaline phosphatase (ALP)		Albumin (Alb)	
	Value (IU/L)	% of change	Value (IU/L)	% of change	Value (U/L)	% of change	Value (g/dL)	% of change
Normal control	35.74 $\pm$ 4.72	0.00	31.25 $\pm$ 2.53	0.00	92.03 $\pm$ 9.77	0.00	5.35 $\pm$ 0.12	0.00
Model control	77.57 $\pm$ 7.89	117.02	68.82 $\pm$ 5.47	120.24	198.56 $\pm$ 14.17	115.75	2.91 $\pm$ 0.17	-45.67
T1	74.46 $\pm$ 4.76	108.34	64.51 $\pm$ 3.13	106.43	193.56 $\pm$ 10.82	110.31	2.94 $\pm$ 0.09	-45.04
T2	69.39 $\pm$ 5.09	94.16	62.61 $\pm$ 4.10	100.35	185.84 $\pm$ 17.05	101.93	3.07 $\pm$ 0.18	-42.65
T3	50.68 $\pm$ 3.98	41.80	58.03 $\pm$ 3.58	85.72	171.10 $\pm$ 10.34	85.92	3.72 $\pm$ 0.16	-30.48
T4	47.91 $\pm$ 2.90	34.05	45.46 $\pm$ 3.49	45.49	142.78 $\pm$ 14.79	55.14	4.03 $\pm$ 0.10	-24.61

Each value represents mean $\pm$ SD (n=6). Values under the same column with different superscripts letters are significant difference ($P \leq 0.05$). Normal control, healthy rats without intervention; Model control, B[a]P (Benzo[a]pyrene) induced hepatic disorders in rats without intervention; T1, T2, T3 and T4, B[a]P induced hepatic disorders in rats with brown algae (*Sargassum subrepandum*) powder feeding intervention by 1.0, 2.0, 3.0 and 4.0 g/100g diet, respectively.

3.4.2. Effect of Feeding Intervention with Brown Algae (*Sargassum subrepandum*) Powder on Liver Oxidative stress Parameters Induced by B[a]P in Rats

Effect of intervention with *Sargassum subrepandum* powder on liver oxidative stress parameters induced by B[a]P in rats were shown in Table 5. Such data indicated that B[a]P induced significantly ($p \leq 0.05$) decreasing in liver GSH level by the ratio of -40.42% whereas, the liver MDA concentration was exhibited the opposite direction, increased by the rate of 195.54%. As the result of *Sargassum subrepandum* powder intervention, liver GSH content significantly ($p \leq 0.05$) increased and MDA was significantly ($p \leq 0.05$) decreased. The rate of increasing in GSH and decreasing in MDA exhibited a dose-dependent manner with the *Sargassum subrepandum* intervention. Such data are in agreement with that obtained by Fayez, [111] as the result of treatment of liver injuries inducing by B[a]P with turmeric and curcumin powders.

GSH has received considerable attention related to its biosynthesis, regulation, and different intracellular functions [3,9]. Its role in detoxifications process represent the central position through conjugate of xenobiotics electrophilic intermediates including B[a]P and as an important antioxidant. The antioxidant functions of GSH include its role in the activities of the antioxidant enzymes system such glutathione peroxidase and glutathione reductase. Additionally, GSH can apparently serve as a nonenzymatic scavenger of oxyradicals i.e. reactive oxygen species (ROS) [3,120,121]. Data of the present study indicated that exposing rats to B[a]P generated ROS, as demonstrated by decreased GSH levels. These data are in accordance with that reported by Elhassaneen et al., [122], who found that treatment of rats with B[a]P resulted in a significant ($p \leq 0.05$) reduction in GSH by the rate of -38.35% as compared to normal rats. Also, Elhassaneen, [11], Elhassaneen and El-Badawy, [113] found that GSH levels in human erythrocytes were reduced significantly ($p \leq 0.05$) after B[a]P ingestion. Several studies reported that the GSH level may fall due to decrease the glutathione reductase activity which increases the antioxidant activities of glutathione-S-transferase and glutathione peroxidase, both of which employ GSH as a cofactor in their processes [19] [123]. On the other side, human survey study for B[a]P-associated oxidative stress have been provided by assay of either biomarkers or end-products of free radical-mediated oxidative processes [11]. For example, lipid peroxidation markers such as MDA represent one of the most significant compound and major product of the oxidation of polyunsaturated fatty acids. MDA found to be elevated in plasma patients subjected to ingest B[a]P [11]. Additionally, the possible significance of MDA on human health has been documented several decades ago by reports that are mutagenic and carcinogenic compound [124]. Several studies have reported that the antioxidant activities of bioactive compounds such as measured in *Sargassum subrepandum* powder where by decreased of lipid peroxidation and oxidative stress in several tissues were demonstrated [21,112,115]. Therefore, data of the present study prove that *Sargassum subrepandum* powder is a promising successfully tool in the prevention of oxidative stress

caused by exposure of cells/hepatocytes to B[a]P.

Table 5. Effect of feeding intervention with brown algae (*Sargassum subrepandum*) powder on liver oxidative stress parameters induced by B[a]P in rats

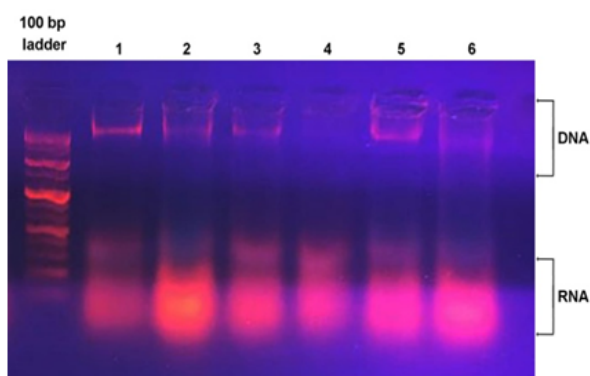
Groups	Reduced glutathione concentration (GSH)		Malondialdehyde concentration (MDA)	
	Value ($\mu\text{mol/mg}$ tissue protein)	% of change	Value (nmol/mg tissue protein)	% of change
Normal control	8.64 $\pm$ 0.38	0.00	2.85 $\pm$ 0.07	0.00
Model control	5.15 $\pm$ 0.11	-40.42	8.42 $\pm$ 0.11	195.54
T1	5.27 $\pm$ 0.52	-39.01	8.22 $\pm$ 0.03	188.52
T2	5.59 $\pm$ 0.28	-35.31	7.19 $\pm$ 0.10	152.48
T3	6.22 $\pm$ 0.58	-28.07	6.11 $\pm$ 0.06	114.46
T4	7.23 $\pm$ 0.30	-16.36	4.02 $\pm$ 0.06	41.10

Each value represents mean $\pm$ SD (n=6). Values under the same column with different superscripts letters are significant difference ($P \leq 0.05$). Normal control, healthy rats without intervention; Model control, B[a]P (Benzo[a]pyrene) induced hepatic disorders in rats without intervention; T1, T2, T3 and T4, B[a]P induced hepatic disorders in rats with brown algae (*Sargassum subrepandum*) powder feeding intervention by 1.0, 2.0, 3.0 and 4.0 g/100g diet, respectively.

3.5. Electrophoretic Pattern of Nucleic Acids (RNA and DNA)

As illustrated in Table 6 and Figure 4 and Figure 5, the mean value of optical density of RNA in liver rats injected by B[a]P showed significant increase ($P < 0.05$) with mean value 156 ± 2.6 as compared with normal control group (124 ± 2.7). However, rats were injected with B[a]P and treated with brown algae (*Sargassum subrepandum*) powder feeding (1.0, 2.0, 3.0 and 4.0 g/100g diet) showed a significant decrease in mean value of maximal optical density of RNA (153 ± 1.9 , 140 ± 1.3 , 147 ± 2.2 and 155 ± 2.5 respectively) as compared with B[a]P injected rats. As shown in Table 7 and Figure 6 and Figure 7, results showed a significantly decreased in mean of maximal optical density of intact DNA in rats liver injected by B[a]P with value 108 ± 2.5 as compared with normal rats. The mean values of maximal optical density of intact DNA of rats injected by B[a]P and treated with brown algae (*Sargassum subrepandum*) powder feeding (1.0, 2.0, 3.0 and 4.0 g/100g diet) illustrated significant increase (121 ± 1.9 ; 125 ± 1.4 ; 124.5 ± 1.3 and 112 ± 2.1 ; respectively) as compared with B[a]P injected rats. While, significantly increased in mean values of maximal optical density of fragmented DNA of B[a]P rats as compared with normal rats. fragmented DNA of rats injected by B[a]P and treated with brown algae (*Sargassum subrepandum*) powder feeding (1.0, 2.0, 3.0 and 4.0 g/100g diet) illustrated significant decrease (96 ± 1.5 ; 84 ± 0.9 ; 87 ± 2.3 and 90 ± 2.9 ; respectively) as compared with B[a]P injected rats. This result agree with Salazar et al., [125] who mention that When B[a]P was administered intraperitoneally to rats 48 hr before they were killed, the DNA-synthesizing capability of isolated rat liver nuclei was decreased as compared with control animals. B[a]P inhibited *in vitro* DNA synthesis in nuclei purified from control animals. Also, Kim et al., [126] indicated that BPDE –DNA adduct levels were significantly increased in

liver, kidney and stomach of rats following B[a]P exposure. B[a]P is known to be the most potent carcinogen. B[a]P can be metabolized to its ultimate carcinogen, BaP-7,8-diol-9,10-epoxide (BPDE), by cytochrome P450 (CYPs) enzymes. BPDE then can either be detoxified by Phase II enzymes or covalently bound to DNA and cause DNA damage, which can further lead to genetic mutation [127]. On the other hand, Azqueta and Collins, [128] indicated that polyphenols are a very broad group of chemicals, widely distributed in plant foods, with low concentration decrease DNA damage in experimental animals. Also, Ansar et al., [129] reported that quercetin (QR) is a polyphenolic flavonoid compound which is found in large amounts in certain foods protects against DNA damage and indicating that QR possesses DNA-protective properties. In addition, Pastor, et al., [130] and Tieppo et al., [131] used quercetin or other antioxidant substances, such as rutin, n-acetylcysteine, and vitamins E and C, all of which decreased the severity of hepatic fibrosis.



Lane 1: Normal control; Lane 2: B[a]P (Benzo[a]pyrene) injected rats; Lane 3: B[a]P induced hepatic disorders in rats with brown algae (*Sargassum subrepandum*) powder feeding (1.0 g/100g diet); lane 4: B[a]P induced hepatic disorders in rats with brown algae powder feeding, (2.0 g/100g diet); lane 5: B[a]P induced hepatic disorders in rats with brown algae powder feeding (3.0 g/100g diet) and lane 6: B[a]P induced hepatic disorders in rats with brown algae (*Sargassum subrepandum*) powder feeding (4.0 g/100g diet)

Figure 4. Electrophoretic pattern of nucleic acid in the tissue lysate of hepatic rats treated by feeding with brown algae (*Sargassum subrepandum*) after being injected with B[a]P

Table 6. Mean of maximum optical density of total RNA in tissue lysate in hepatic rats treated by feeding with brown algae (*Sargassum subrepandum*) after being injected with B[a]P

Groups	Normal control	Model	T1	T2	T3	T4
MOD of RNA	124±2.7	#156±2.6	*153±1.9	*140±1.3	*147±2.2	*155±2.5

Data are expressed as Mean ± SD. * Significant difference compared to in model control group at ($P \leq 0.05$). # Significant difference compared to normal control. Normal control, healthy rats without intervention; Model control, B[a]P (Benzo[a]pyrene) induced hepatic disorders in rats without intervention; T1, T2, T3 and T4, B[a]P induced hepatic disorders in rats with brown algae (*Sargassum subrepandum*) powder feeding intervention by 1.0, 2.0, 3.0 and 4.0 g/100g diet, respectively.

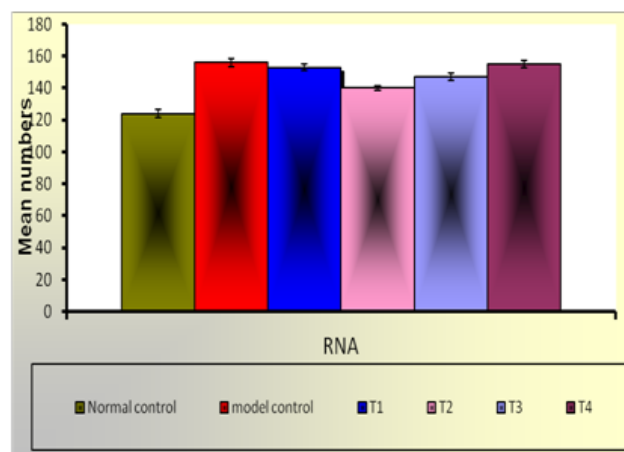
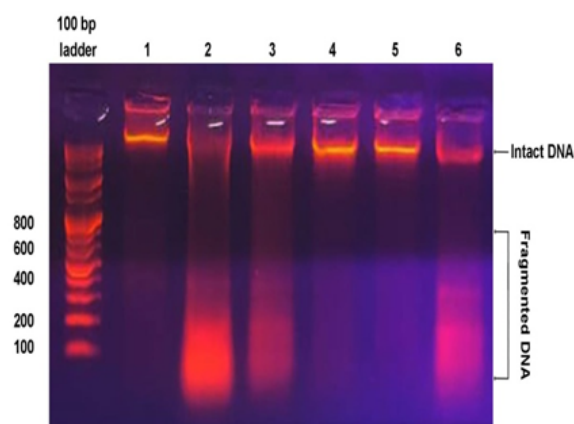


Figure 5. Mean of maximal optical density of total RNA in tissue lysate of B[a]P injected rats liver feeding with brown algae (*Sargassum subrepandum*) powder



Lane 1: Normal control; Lane 2: B[a]P (Benzo[a]pyrene) injected rats; Lane 3: B[a]P induced hepatic disorders in rats with brown algae (*Sargassum subrepandum*) powder feeding (1.0 g/100g diet); lane 4: B[a]P induced hepatic disorders in rats with brown algae powder feeding, (2.0 g/100g diet); lane 5: B[a]P induced hepatic disorders in rats with brown algae powder feeding (3.0 g/100g diet) and lane 6: B[a]P induced hepatic disorders in rats with brown algae (*Sargassum subrepandum*) powder feeding (4.0 g/100g diet).

Figure 6. Mean of maximum optical density of apoptotic fragments of DNA in hepatic rats treated by feeding with brown algae (*Sargassum subrepandum*) after being injected with B[a]P.

Table 7. Mean of maximum optical density of apoptotic fragments of DNA in hepatic rats treated by feeding with brown algae (*Sargassum subrepandum*) after being injected with B[a]P

Experimental Groups	Normal control	Model	T1	T2	T3	T4
Intact DNA	125±1	108#±2.5	121*±1.9	125*±1.4	124.5*±1.3	112*±2.1
Fragmented DNA	79±1.2	98#±1.8	96*±1.5	84*±0.9	87*±2.3	90*±2.9

Data are expressed as Mean ± SD. * Significant difference compared to in model control group at ($P \leq 0.05$). # Significant difference compared to normal control. Normal control, healthy rats without intervention; Model control, B[a]P (Benzo[a]pyrene) induced hepatic disorders in rats without intervention; T1, T2, T3 and T4, B[a]P induced hepatic disorders in rats with brown algae (*Sargassum subrepandum*) powder feeding intervention by 1.0, 2.0, 3.0 and 4.0 g/100g diet, respectively.

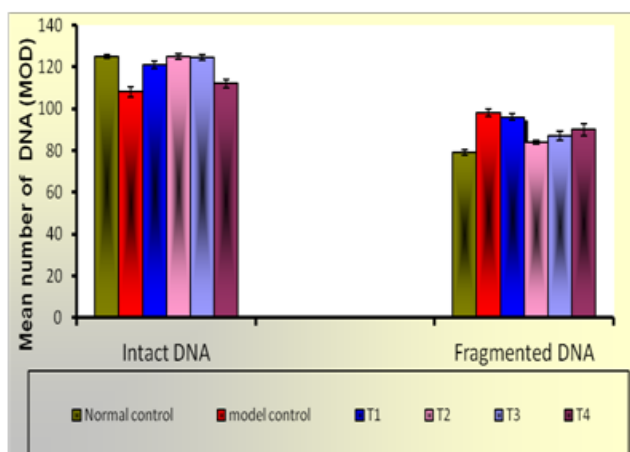


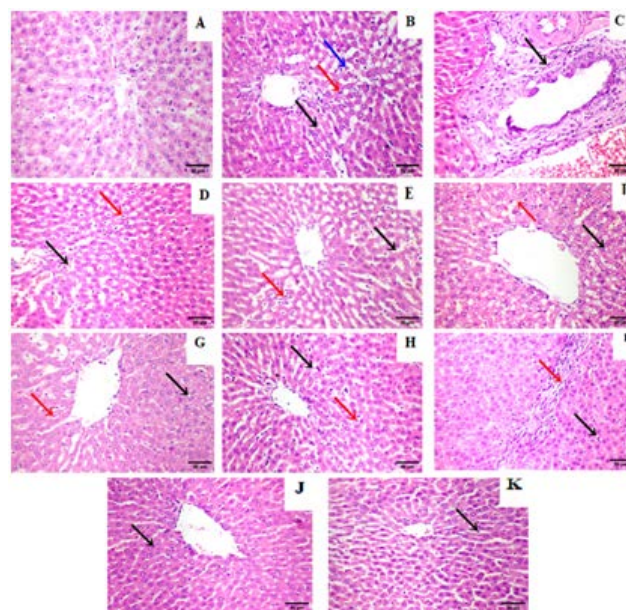
Figure 7. Mean of maximum optical density of apoptotic fragments of DNA in hepatic rats treated by feeding with brown algae (*Sargassum subrepandum*) after being injected with B[a]P

3.6. Histopathological Studies

Effect of intervention with *Sargassum subrepandum* ethanol extract (SSEE) on B[a]P-induced liver histopathological alterations in liver tissue

Effect of intervention with *Sargassum subrepandum* ethanol extract (SSEE) on B[a]P-induced liver histopathological alterations in liver tissue of rats was shown in Figure 8. Microscopically, liver of rats from group 1 revealed the normal histoarchitecture of hepatic tissue (Photo 1). On contrary, liver of rats from group 2 showed hepatocellular vacuolar degeneration, Kupffer cells activation, apoptosis of hepatocytes (Photo 2), and hyperplasia of biliary epithelium (Photo 3). On the other hand, liver of rats from group 3 revealed slight congestion of hepatic sinusoids Kupffer cells activation (Photo 4), and small focal hepatocellular necrosis associated with inflammatory cells infiltration (Photo 5). Meanwhile, liver of rats from group 4 exhibited hepatocellular steatosis, slight congestion of hepatic sinusoids (Photos 6 and 7) and Kupffer cells proliferation (Photo 7). Otherwise, sections from group 5 showed slight vacuolization of some hepatocytes, Kupffer cells proliferation (Photo 8), vacuolar degeneration of some hepatocytes and slight fibroplasia in the portal triad (Photo 9). Furthermore, liver of rats from group 6 exhibited no histopathological changes except Kupffer cells proliferation (Photos 10 and 11). Our results are in line with these previous publications and show that B[a]P induced histological changes in liver. For example, Elhassaneen et al., [132] found that B[a]P treatment resulted in some pathological changes in liver such significant expansion of hepatic sinusoid between hepatic cords and inflammatory cell infiltration. Also, Kyeong e al., [133] reported that B[a]P induced hepatic tissues including cell infiltration, mononuclear cells, and multifocal cells in rats. On the other side, several previous studies indicated that plant parts contain the bioactive constituents such as found in *Sargassum subrepandum* exhibited the protective role against BaP-induced hepatic tissue damage of rats. For example, Elhassaneen et al., [64] observed that *Silybum marianum* seeds feeding ameliorate the histopathological

changes in liver of rats induced by carbontetrachloride which due to the antioxidant activities exhibited by such plant part. Also, Kyeong e al., [133] reported that treatment of B[a]P induced toxic effects with curcumin helped to restore the normal histological architecture of the liver tissues. Plant parts have been found to manifest its protective benefits against severe histopathological damage via good antioxidant activities, ROS scavenging abilities, lipid inhibiting oxidation, inhibiting B[a]P metabolism enzymes lead to B[a]P-DNA formation [19,111,112,115,134].



A) group 1 showing the normal histoarchitecture of hepatic tissue, **B) group 2** showing hepatocellular vacuolar degeneration (black arrow), Kupffer cells activation (red arrow) and apoptosis of hepatocytes (blue arrow), **C) group 2** showing fibroplasia in the wall of bile duct (black arrow), **D) group 3** showing slight congestion of hepatic sinusoids (black arrow) and Kupffer cells activation (red arrow), **E) group 3** showing slight congestion of hepatic sinusoids (black arrow) and small focal hepatocellular necrosis (red arrow), **F) group 4** showing hepatocellular steatosis (black arrow) and slight congestion of hepatic sinusoids (red arrow), **G) group 4** showing hepatocellular steatosis (black arrow) and Kupffer cells proliferation (red arrow), **H) group 5** showing slight vacuolization of some hepatocytes (black arrow) and Kupffer cells proliferation (red arrow), **I) group 5** showing vacuolar degeneration of some hepatocytes (black arrow) and slight fibroplasia in the portal triad (red arrow), **J) group 6** Kupffer cells proliferation (arrow), **K), group 6** Kupffer cells proliferation (arrow).

Figure 8. Effect of intervention with *Sargassum subrepandum* ethanol extract (SSEE) on B[a]P-induced liver histopathological alterations in rats liver tissue (H & E X 400, scale bar 50µm)

4. Conclusion

B[a]P is considered as a ubiquitous environmental and food contaminants as well as a top risk factor in the development of liver diseases. Data of the present study supported our hypothesis that *Sargassum subrepandum* powder contains several classes of bioactive compounds which exhibited to several biologically activities (antioxidant activity and peroxyl radical (ROO)-scavenging activities and inhibition of lipid peroxidation) that are able to prevent or inhibit B[a]P hepatotoxicity

through improving the liver functions enzymes activities, oxidative stress parameters (GSH and MDA), and molecular and histological changes thereby adversely affecting the injuries process to the benefit of the biological system. We recommended that *Sargassum subrepandum* powder by the tested concentrations (up to 4%) to be included in our daily dishes, drinks and pharmaceutical preparations/formulae

Conflict of Interest

The authors declare that they have no conflict of interest in publishing this paper.

Authors' Contribution

Yousif Elhassaneen participated in proposing and developing the study protocol, following up on the laboratory experimental part, retrieving conceptual information, reviewing and validating the results and statistical analyses, preparing a draft of the manuscript, conducting a critical review to Basma Helmy conducted laboratory experiments, collected, tabulated, analyzed and interpreted the results. She was also involved in retrieving conceptual information and preparing the draft of the manuscript. Amal Zaki participated in proposing the study protocol, retrieving conceptual information, validating the study results, and preparing the draft manuscript. Asmaa Bayomi participated in proposing the study protocol, participated in conducting the molecular part, retrieving conceptual information, and providing contributions to the concept and design of the work.

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Abbreviations

AA, antioxidant activity; AAPH, 2-amidinopropane dihydrochloride; Alb, albumin; ALT, Alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; B[a]P, benzo[a]pyrene; BCB, β - Carotene Bleaching; BD, basal diet; BHT, butylated hydroxyltoluene; CA, catechine; DMSO, dimethylsulfoxide; DNA, deoxyribonucleic acid; GA, gallic acid; GSH, reduced glutathione; LDL, low density lipoprotein; MDA, malondialdehyde; ORAC, oxygen radical absorbance capacity; RNA, ribonucleic acid; ROS: reactive oxygen species.

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