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Effects of brown algae (*Sargassum subrepandum*) consumption on obesity induced changes in oxidative stress and bone indices

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Abstract: Brown algae are a large group of multicellular algae including many seaweeds located in many countries including Egypt. Most brown algae live in marine environments, where they play an important role both as food and as habitat. The present study aims to investigate the effect of brown algae (*Sargassumsubrepandum*) feeding on oxidative stress (OS), antioxidant defense status and bone healthy indices in obese rats. Thirty albino male rats (140±10g per each) were divided into two main groups, the first group (Group 1, 5 rats) still fed on basal diet and the other main group (30 rats) was feed with diet-induced obesity (DIO) for 8 weeks which classified into five sub groups as follow: group (2), fed on DIO as a positive control; groups (3-6), fed on DIO containing 1, 2, 3 and 4% of brown algae powder (BAP), respectively. At the end of the experiment (8 weeks), rats of the obese group recorded 155.07% of the control (normal) group for the body weight. Replacement of corn starch with 1, 2, 3 and 4% BAP induced significant decreasing on body weight of the obese rats which recorded 137.75, 123.87, 107.69 and 94.92% as a percent of control, respectively. Biochemical analysis data indicated that obesity induced a significant increased ($p \leq 0.05$) in plasma oxidants concentration (MDA, 59.92%) and significant decreased ($p \leq 0.05$) in plasma non-enzymes antioxidant (GSH, -43.16 % and GSSG, -26.22 %), as a percent of normal group. Feeding on 1-4% of BAP induced significant exhibited a significant improvement ($p \leq 0.05$) in all of these parameters by different rates. The same behavior was recorded for the bone healthy indices. The amelioration effects were increased with the elevation of the BAP feeding rate. In conclusion,the present data support the possibility of adding brown algae powder by up to 4% to our daily diets as it contains biologically active compounds that

reduce oxidative stress and improve bone health markers associated obesity.
Keywords: Brown algae, bioactive compounds, body weight, malonaldehyde, glutathione, bone mineral density.

Introduction

Brown algae (BA) belong to the group Heterokontophyta, a large group of eukaryoticorganisms distinguished most prominently by having chloroplasts surrounded by four membranes, suggesting an origin from a symbiotic relationship between a basal eukaryote and another eukaryotic organism (Chapman and Chapman,1980). Most BAcontain the pigment fucoxanthin, which is responsible for the distinctive greenish-brown color that gives them their name. Brown algae are unique among heterokonts in developing into multicellular forms with differentiated tissues, but they reproduce by meansof flagellated spores and gametes that closely resemble cells of other heterokonts. Genetic studies show their closest relatives to be the yellow-green algae(Bold and Wynne, 1985). . Worldwide, over 1500–2000 species of BA are known. Some species are important in commercial use because they have become subjects of extensive research in their own right. They have environmental importance too through Carbon fixation (Mann and Martin, 2002).

In Egypt, BAare also frequently encountered as the major vegetation in shallow water tropical and subtropical habitats, even though herbivorous predators are plentiful. Hence, the correlation between secondary metabolite synthesis within this family and predator avoidance seems to be pronounced (Gerwick*et al.,* 1981). In the littoral zone of the Egyptian coast, BA are currently the most dominant group. Members of *Sargassum*genus represent valuable sources of a wide spectrum of complex lipids, essential fatty acids and amino acids (Hossain*et al.*, 2003). *Sargassumsubrepandum*(Forsk) C.Ag.is quite common in the Egyptian Red Sea coast (El-Naggar*et al.*, 1995), Brown algae consist mainly of water (90 percent) in the native state. Polysaccharides are major components and comprise alginates, 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 (Chapman and Chapman, 1980 and Helen, 2003). Alginates, 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 (Helen, 2003).

Obesity is defined as an excessive accumulation of body fat mass to the extent that individual's health will be negatively affected. Indeed, obesity is considered as a top risk factor to develop deleterious associated pathologies as liver and coronary heart diseases, osteoarthritis and asthma, and a combination of medical disorders which includes: type 2 diabetes, high blood pressure, high blood cholesterol, and high triglyceride levels (Esra*et al.,* 2012; Le Lay *et al.,* 2014 and Sayed Ahmed, 2016).The prevalence of obesity over the past years has been in constant progression leading the World Health Organization (WHO) to consider it as an epidemic pathology and one of the leading preventable causes of death worldwide (Mokdad*et al.*, 2004).

Oxidative stress (OS) was initially defined by Sies (1985) as a serious imbalance between oxidation and antioxidants, "a disturbance in the prooxidant–antioxidant balance in favor of the former, leading to potential damage". So, it reflects an imbalance between the systemic manifestation of reactive oxygen species(ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Oxidative stress from oxidative metabolism causes base damage, as well as strand breaks in DNA (Rahman*et al.,* 2012).In humans, OSis thought to be involved in the development ofin several diseases including cancer, atherosclerosis, malaria, chronic fatigue syndrome, rheumatoid arthritis and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and Huntington's disease (Halliwell, 1991 and Chaitanya*et al.,* 2010). Furthermore, associations between obesity and markers of oxidative stress and the susceptibility of lipid to oxidative modification have been observed in humans (Van Gaal*et al.*, 1998).The association between oxidative stress and obesity are discussing by many authors (Chaitanya*et al.,* 2010 and Le Lay *et al.*, 2014). OS appears as a major contributor in the development of many metabolic complications associated obesity.

Degradation in bone indices i.e. Osteoporosis is one of the most widespread metabolic bone disorders affecting one in three women and one in twelve men at some point in their lives (Ligett and Reid, 2000 and Henry, 2001). Such bone indices include bone mineral density (BMD), bone g- protein (BG-P) and Bone mineral content (BMC). According to the WHO "Osteoporosis is a disease characterized by low bone mass and micro-architectural deterioration of bone tissues, leading to enhanced fragility and consequent increase in fracture risk that results in fractures with minimal trauma" (Meryl, 1997). Several factors such as genetic, nutritional and lack of exercise etc., along with aging have been shown

to be risk factors in the aetiology of osteoporosis (Malhotra andMithal, 2008). The degradation in bone indices (BMD, BG-P and BMC) in obese animals is reported and similar to the symptoms of osteoporosis (Gali, 2001; Henry, 2001 and Délérise*t al.*, 2016).

Therapeutics designed to lower ROS production and degradation in bone indices may have beneficial effects on health. Therefore, one of the aims of the present study is to observe the effect of BA(*Sargassumsubrepandum*) feeding on OS and antioxidant defense status in obese rats. Also, the effect of such algae feeding on the bone healthy indices of the obese rats will be in the scope of this investigation.

Materials and methods

Materials

Dried BA (*Sargassumsubrepandum*) were obtained and verified as a donation from the Faculty of Agriculture, Alexandria university, Alexandria, Egypt Ministry of Agriculture, Egypt. Casein was obtained from Morgan Chemical Co., Cairo, Egypt.Thiols compounds, GSH and GSSG, were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals, reagents and solvents were of analytical or HPLC grade were purchased from El-Ghomhorya Company for Trading Drug, Chemicals and Medical Instruments, Cairo, Egypt.

Brown algae powder (BAP)

Dried parts of BAwere ground into a fine powder in high mixer speed (Moulinex Egypt, Al-Araby Co., Egypt). The material that passed through an 80 mesh sieve was retained for use.

Equipment's

A SP Thermo Separation Products Liquid Chromatograph (Thermo Separation products, San Jose, CA) was used throughout this study with a ConstaMetvic 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) were a Spherosorb ODC-2 (5 µm, 150 x 4.6 mm I.d.) for glutathione fractions ; a reversed-phase water Adsorbosil C_{18} (5 µM, 100 mm × 4.6 mm I.d.) for vitamin C; and normal Ultrasphere Si (5 µM, 250 mm × 4.6 mm I.d.) for analysis of vitamins A and E. Also, absorbance and fluorescence for different assays were measured using Labo-med. Inc., spectrophotometer, CA and Schematzu fluorescence apparatus, Japan, respectively.

Biological Experiments

Animals

Animals used in this study, adult male albino rats (140-150 g per each) were obtained from Helwan Station, Ministry of Health and Population, Helwan, Cairo, Egypt.

Basal Diet

The basal diet prepared according to the following formula as mentioned by (AIN, 1993) 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 diet induced obesity (DIO) prepared according to Research Diets, Inc. NJ, as follow:casein, 80 mesh (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 H_2O (1.92%), vitamin mixture (1.17%), choline bitartrate (0.23%). The used vitamins and salt mixtures components were formulated according to Campbell, (1963) and Hegsted, (1941), respectively.

Experimental design

All biological experiments performed in Biology lab, Faculty of Home Economics, Minoufiya University, Egypt. All the experiments were a complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council (NRC, 1996). Rats (n=36 rats), 140-150g per each, were housed individually in wire cages in a room maintained at 27 ± 5^0C, relative humidity (55±5%), a 12-hr lighting cycle and kept under normal healthy conditions. All rats were fed on basal diet for one-week before starting the experiment for acclimatization. After one week period, the rats were divided into two main groups, the first group (Group 1, 6 rats) still fed on basal diet and the other main group (25 rats) was with diet-induced obesity (DIO) for 8 weeks which classified into sex sub groups as follow: group (2), fed on DIO as a positive control; group (3), fed on DIO containing 1.0 % BAP; group (4), fed on DIOcontaining 2.0 % BAP; group (5), fed on DIO containing 3.0 % BAP and group (6), fed on DIO containing 4.0 % BAP.Body weight gain (as percent of initial weight) was assayed every week in rats.

Blood and bone sampling

Blood and bone samples were collected at the end of experiment period, 8 weeks, after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized. Blood samples were

received into glass centrifuge tubes, containing oxalate solution (1.34 %) as anticoagulant. After centrifugation at 3000 rpm for 10 min., plasma was with drown and used for the analysis of blood lipid parameters and vitamins. The erythrocyte residue was washed with three successive portions of sodium chloride solution (0.9 %) and then haemolysed with deionised water for 30 min. Haemolysate was then centrifuged at 3000 rpm for 30 min. and the supernatant fractions was transferred to a clean test tube and analyzed of antioxidant enzymes (Stroev and Makarova, 1989). Rat's femora samples were collected for bone indices examination.

Hematological analysis

Glutathione fractions

Glutathione fractions (GSH and GSSG) were determined by HPLC according to the method of McFarris and Reed (1987). In brief, 100µl of aliquot were placed in 2 ml of 10% perchloric acid containing 1 mMbathophenanthrolinedisulfonic acid and homogenized. The homogenate was cold centrifuged at 10000 rpm for 5 min and the internal standard (γ-glutamyl glutamate) was added to the supernatant. A 250 µl aliquot of acidic extract was mixed with 100 µl of 100 mMiodoacetic acid in 0.2 mM cresol purple solution. The acid solution was brought to pH 8.9 by the addition of 0.4 ml of KOH (2 M) – $KHCO_3$ (2.4 M) and allowed to incubate in the dark at room temperature for 1 hr to obtain S-carbooxymethyl derivatives. The N-nitrophenol derivatization of the samples were taken overnight at 4 ^{0}C in the presence of 0.2 ml of 1% 1-fluoro-2,4-dinitrobenzene and injected onto the HPLC system.

Malonaldehyde (MDA) determination

MDA was measured as described by Buege and Aust, (1978). Half milliliter of plasma were added to 1.0 ml of thiobarbituric acid reagent, consisting of 15% TCA, 0.375% thiobarbituric acid (TBA) and 0.01% butylatedhydroxytoluene in 0.25 N HCl. Twenty-five microliters of 0.1 M $FeSO_4.7H_2O$ was added and the mixture was heated for 20 min in boiling water. The samples were centrifuged at 1000 xg for 10 min and the absorbance was read at 535 nm using Labo-med. Inc., spectrophotometer against a reagent blank. The absorbance of the samples was compared to a standard curve of known concentrations of MDA and expressed as nmol/mL.

Bone indices analysis

In rats, bone mineral density (BMD) was measured by DEXA scans or abdominal computed tomography (CT) scans via the Siemens method and presented as T score (normal >−1, osteopenia−1> to 2.5,

osteoporosis <−2.5) according to Andreas *et al.,* (2014). Bone mineralization, bone mineral content (BMC), of rat femora were determined by μCT scans (μCT40, Scanco Medical AG, Wangen-Brüttisellen, CH) such as mentioned in Andrea*set al.,* (2014). Bone G protein (BG-P) were determined such as mentioned by Linkhart*et al.,* (1996).

Statistical Analysis

All measurements were done in triplicate and recorded as mean±SD. Statistical analysis was performed with the Student *t*-test and MINITAB 12 computer program (Minitab Inc., State College, PA).

Results and discussion

The effect of BAPon body weight of obese rats

The effect of BAP on body weight gain (BWG, Percent of change from the base line)of obese rats was shown in Table (1) and Figure (1). From such data it could be noticed that feeding of rats on diet induced obesity (DIO) leads to increase the body weight than the control group. At the end of the experiment (8 weeks), rats of the obese group recorded 155.07% of the control (normal) group for the body weight. Also, replacement of corn starch with 1, 2, 3 and 4% BAP induced significant decreasing on body weight of the obese rats which recorded 137.75, 123.87, 107.69 and 94.92% as a percent of control, respectively. The effect of BAP on weigh decreasing was elevated with the increasing of its level in diet. Such date are in agreement with that observed by many authors who found that the countries of this part of the world have lower prevalence of metabolic syndrome than Western countries which may be because of their dietary intake of fish, soy, and seaweeds (Kolovou*et al.,* 2007; Hwang *et al.,* 2006 and Déléris*et al.,* 2016). The positive effects of BAP regarding the control of the obesity could be attributed to their high level content of different classes' of bioactive compounds including fiber, phenolics, flavonols, anthocyanins, polysaccharides, carotenoids etc (Helen, 2003). For example, fiber is the largest component in seaweeds including BAP and may therefore prevent obesity-related disorders. Besides fiber, BAP are also rich in antioxidants, minerals and omega -3- fatty acids, which may have their importance in preventing obesity and its associated complications (serum fat profile, serum glucose, cardiovascular diseases,

ateroscalorosesetc (Délérise*t al.,* 2016). Also, carotenoids such as fucoxanthin is represent a group of bioactive molecules found in BAP and of interest for human health. BAPsupplementation of rats diet with this fucoxanthin-rich fractions led to a significant reduction in white adipose tissue after 4 weeks (Maeda *et al.,* 2008). An explanation of this fucoxanthin effect may reside in an up regulation of mitochondrial uncoupling protein 1, which would result in an increase in resting energy expenditure. Other potential mechanisms include a suppression of adipocyte differentiation and lipid accumulation by inhibiting glycerol-3-phosphate dehydrogenase (G-3-PD) activity or down regulation of peroxisome proliferator-activated receptor-γ responsible for adipogenic gene expression (Kim and Lee, 2012). Furthermore, BAP bioactive compounds and their conversion products have been shown to induce/participate in several mechanisms which contribute to their action control of adipocyte function and adiposity subsequently obesity (Bonet*et al.,* 2015). Amongst of these mechanisms, such compounds could be interacted with several transcription factors of the nuclear receptor superfamily, interfered with the activity of other transcription factors, modulated signaling pathways which are associated with inflammatory and oxidative stress responses; and scavenged of reactive species such ROD and RNS (Bonet*et al.,* 2015 and Le Lay *et al.,* 2014)

Table (1): The effect of BAP on body weight gain (Percent of change from the base line)of obese rats*

Groups	Feeding period (weeks)								
	0	1	2	3	4	5	6	7	8
Control (-) Std diet	0.00	9.06	17.52	28.31	45.45	62.02	77.53	84.71	89.01^{f}
Control (+) Obese	0.00	33.27	50.91	68.08	84.89	117.36	132.69	144.71	155.07^{a}
1% BAP	0.00	29.25	43.94	50.56	75.56	103.86	123.54	129.59	137.75^{b}
2% BAP	0.00	25.48	33.91	44.72	66.95	99.32	102.84	116.05	123.87^{c}
3% BAP	0.00	19.93	31.90	40.85	66.17	73.75	93.95	100.89	107.69^{d}
4% BAP	0.00	13.63	24.45	35.30	52.20	67.11	83.03	90.43	94.92^{e}

* BAP, brown algae powder.Values in the same column with different superscript letters are significantly different at $p \leq 0.05$.

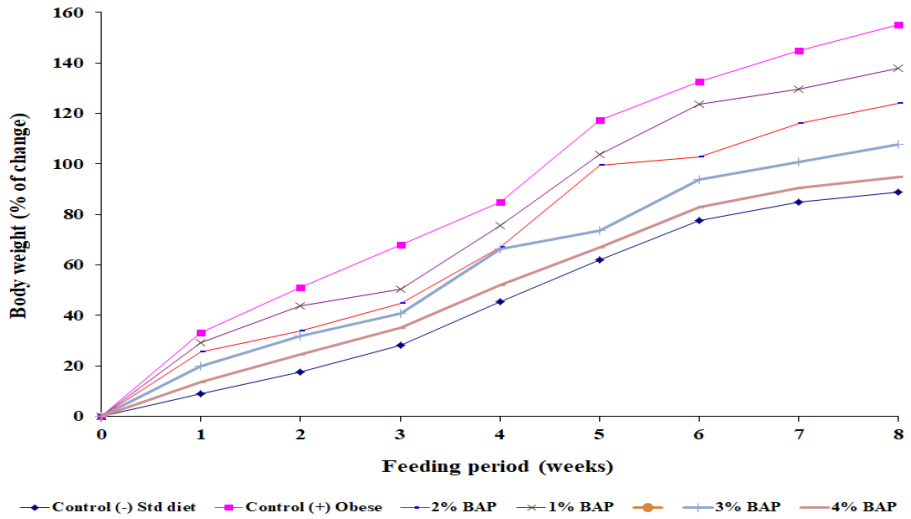

Figure (1): The effect of BAP on body weight gain (Percent of change from the base line)of obese rats

Plasma oxidants concentration in obese rats feeding BAP

OS status in obese rats feeding BAP was assessed by measuring some oxidants concentration in plasma including malonaldehyde (MDA) concentration (Table 2 and Figure 2). From such data it could be noticed that obesity induced a significant increased ($p \leq 0.05$) in MDA concentration in plasma by 59.92% compared to normal controls, respectively. Supplementation of the rat dietswith 1, 2, 3 and 4% w/w by BAP significant decreasing on this parameter concentration in plasma by the ratio of 40.43, 27.65, 24.99 and 19.63%, respectively. The amelioration effect in plasma MDA concentration rising in obesity rats was elevated with the increasing of BAP concentration.

In similar studies, clinical evidences for obesity-associated oxidative stress have been provided by measurement of either biomarkers or end-products of free radical-mediated oxidative processes (Elhassaneen and Salem, 2014 and Sayed Ahmed, 2016). For instance, lipid peroxidation markers such as malondialdehyde (MDA), one of the most important compounds in TBARS and major products of the oxidation of polyunsaturated fatty acids,, lipid hydroperoxides and conjugated dienes are found to be increased in plasma from obese subjects in many clinical studies (Vincent and Taylor, 2006). Systemic metabolic alterations associated with obesity contribute to the increase in

oxidative stress have been reported by many authors. For example, hyperglycemia as a hallmark of type II diabetes, a metabolic complication of obesity, induces oxidative stress through activation of the polyol and hexosamine pathways, production of advanced glycation end-products (AGE), and increase of diacylglycerols (DAG) synthesis (DCCTRG, 1993 and Le Lay *et al.,* 2014). Excess of circulating lipids induces ROS formation pathways, which contribute to the increase in lipid oxidation and protein carbonylation (Jensen *et al.,* 1989). Leptin and angiotensin II, secreted at high levels by adipocytes, are inducers of ROS generation and might therefore promote inflammation and lipid peroxidation (Bouloumie*et al.,* 1999). Altogether, dysregulation of metabolic parameters occurring with fat mass expansion will contribute to inducing oxidative-stress damages notably at the vascular level (Brandes and Kreuzer, 2005). Several decades ago, interest in the possible significance of MDA on human health has been stimulated by reports that are mutagenic and carcinogenic compound (Shambergere*t al.,* 1974).

The positive effects of BAP on oxidants formation/concentration of obese rats could be attributed to several mechanisms induced by their bioactive components content. In this context, Coskun*et al.,* (2005) found that flavonoids such as found in BAP, have anti-oxidative and anti-inflammatory activities. Such dietary phenolics found in BAP are metabolized in liver, inhibiting liver injury induced by diabetes i.e. enhancing lipid metabolism, reducing oxidative stress may be particularly effective, consequently.

Table 2. Plasma oxidants concentration (malonaldehyde, MDA, nmol/mL) in obese rats feeding brown algae powder (BAP)

Value	Control (-)	Control (+)	BAP (%, w/w)			
			1	2	3	4
Mean	3.66 [c]	5.85 [a]	5.14 [b]	4.67 [b]	4.58 [b]	4.38 [b]
SD	0.84	0.94	0.90	1.07	1.03	1.90
% of Change	0.00	59.92	40.43	27.65	24.99	19.63

[*]Means in the same row with different litters are significantly different at $p < 0.05$.

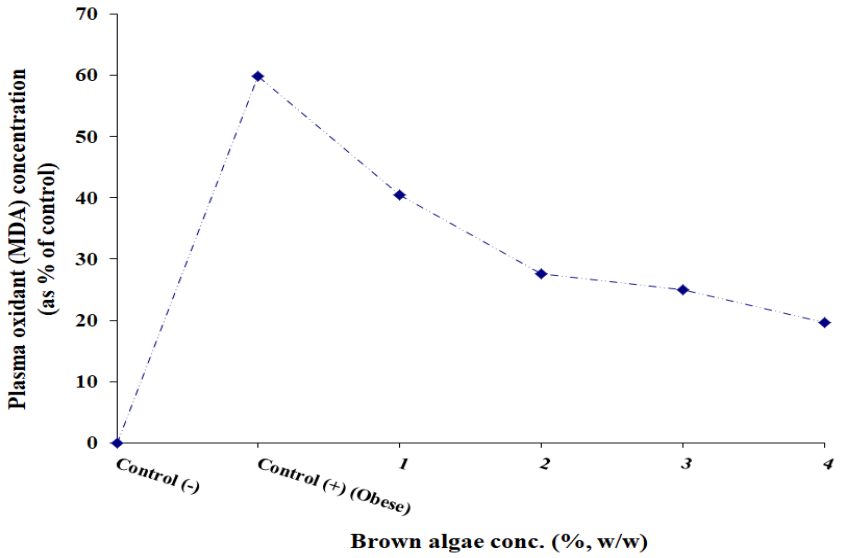

Figure 2. Plasma oxidants concentration in obese rats feeding brown algae powder (BAP)

Plasma antioxidants (GSH fractions concentration) in obese rats feeding BAP

Biological antioxidant macromolecules i.e. glutathione fractions concentration in plasma of obese rats consumed BAP were assessed (Table 2 and Figure 2). From such data it could be noticed that obesity induced a significant decreased ($p \leq 0.05$) in GSH and GSSG concentrations and GSH/GSSG ratio in plasma by -43.16, -26.22 and -22.96% compared to normal controls, respectively. Supplementation of the rat dietswith 1, 2, 3 and 4% w/w by BAP significant decreasing on these parameter (GSH and GSSG concentrations and GSH/GSSG ratio) in plasma by the ratio of -36.42, -25.69, -22.70 and -18.04%; -24.13, -11.62, -10.43 and -8.49%; and -16.20, -15.92, -13.70 and -10.43%, respectively. The amelioration effect in plasma glutathione fractions concentration rising in obesity rats were elevated with the increasing of BAP concentration.

Reduced glutathione (GSH) is a tripeptide-thiol (γ-glutamylcysteinyl-glycine) that has received considerable attention in

terms of its biosynthesis, regulation, and various intracellular functions (Larsson *et al*., 1983). These functions include its roles in detoxifications processes such a key conjugate of electrophilic intermediates, principally via glutathione-*s*-transferase activities in phase II metabolism, and an important antioxidant. The antioxidant functions of GSH include its role in the activities of GSH enzymes family i.e. glutathione peroxidase (GSH-Px) and peroxiredoxins (PRXs). In addition, GSH can apparently serve as a nonenzymatic scavenger of oxyradicals (Halliwell and Gutteridge, 1985 and Elhassaneen *et al.,* 2016).

A fall in glutathione fractions observed in obese rats group generally accompanied by a concomitant decreased in the ratio of GSH/GSSG. Di Giulio (1991) mentioned that a more fundamental effect of oxyradical-generating compounds as the obesity development, however, is their effect on what can be referred to as the redox status (GSH/GSSG) of cells or tissues. Few studies have been addressed directly the

Table 3. Plasma antioxidants (fractions concentration) in obese rats feeding brown algae powder (BAP)*

Value	Control (-)	Control (+)	BAP (%, w/w)			
			1	2	3	4
Reduced glutathione concentration (GSH, µmol /L)						
Mean	8.63 [a]	4.91 [d]	5.49 [c]	6.42 [ab]	6.67 [ab]	7.08 [ab]
SD	1.09	0.43	0.43	1.37	1.28	0.61
% of Change	0.00	-43.16	-36.42	-25.69	-22.70	-18.04
Oxidized glutathione concentration (GSSG, µmol /L)						
Mean	0.677 [a]	0.499 [c]	0.513 [abc]	0.598 [ab]	0.606 [ab]	0.619 [a]
SD	0.03	0.03	0.02	0.03	0.03	0.03
% of Change	0.00	-26.22	-24.13	-11.62	-10.43	-8.49
GSH/GSSG ratio						
Mean	12.76 [a]	9.83 [c]	10.69 [b]	10.73 [b]	11.01 [ab]	11.43 [a]
SD	2.67	0.87	1.01	1.22	1.00	0.98
% of Change	0.00	-22.96	-16.20	-15.92	-13.70	-10.43

* Means in the same row with different litters are significantly different at $p \leq 0.05$.

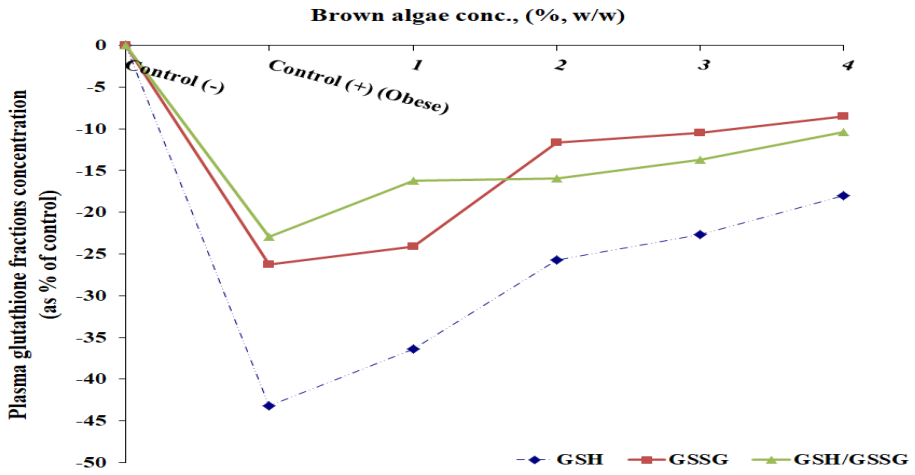

Figure 3. Plasma antioxidants (glutathione fractions concentration) in obese rats feeding BAP

issue of effects of pro-oxidants on redox status. Also, Elhassaneen *et al*., (2004) mentioned that increased fluxes of oxyradicals might be decreased in the GSH/GSSG ratio, due either to direct radical scavenging or to increased peroxidase activity. This effect could also occur indirectly due to reduced NADPH availability [necessary for glutathione reductase (GSH-Rd) activity] resulting, for example, from oxidations in the first step of the redox cycle (Bedard, and Krause, 2007). In this context, Bedard and Krause (2007) reported that various enzymes inside the cells including adipocytes can also produce ROS. Particularly, the family of NAPDH oxidases (NOX) is considered to be an important source of ROS generation. Such effect could be one of the most important reasons for reducing the GSH/GSSG ratio in obese rats. The BAP selected in the present study feeding are rich in bioactive compounds which exhibited antioxidant effects against ROS formation as the obesity development through several mechanism of action including the raising of redox status (GSH/GSSG ratio) in the body.

Effect of BAP on bone indices of obese rats

Bone indices of obese rats consumed brown algae powder (BAP) were shown in Table (4) and Figure (4). From such data it could be noticed that inducing obese in animals caused a significant decreased

($p \leq 0.05$) in Bone mineral density (BMD, -20.27%), Bone g- protein (BG-P, -38.01%) and Bone mineral content (BMC, -31.41%) compared to normal controls. Supplementation of the rat diets with BAP (1.0 to 4.0 g/100g) prevented the lower of mean bone BMD, BG-P and BMC indices. The rate of preventative was increased with the increasing of the BA) concentration. The rate of increasing in the bone indices were recorded -19.21, -11.20, -7.20 and -5.60% (For BMD); -39.14, -26.13, -22.08 and-17.56% (for BG-P) and -31.56, -21.68, -13.89 and-7.79 % (for BMC) with the rat diets supplemented by 1.0, 2.0, 3.0 and 4.0 g/100g of BAP, respectively.

In general, the degradation in bone indices (BMD, BG-P and BMC) in obese animals is inherent similarity of the symptoms of osteoporosis. It is one of the most widespread metabolic bone disorders affecting one in three women and one in twelve men at some point in their lives (Henry, 2001). According to the WHO "Osteoporosis is a disease characterized by low bone mass and micro-architectural deterioration of bone tissues, leading to enhanced fragility and consequent increase in fracture risk that results in fractures with minimal trauma" (Meryl, 1997). Several factors such as genetic, nutritional and lack of exercise etc., along with aging have been shown to be risk factors in the aetiology of osteoporosis (Malhotra andMithal, 2008). With aging, however, an erratic absorption of calcium from gut disturbs the calcium homeostasis leading to an imbalance in the calcium regulating hormones (parathyroid hormone and calcitonin) and thereby increase bone turnover (Shoback*et al.,* 1993). Osteoblastic activity and calcium absorption from the gut also suffers with the age (Tanna, 2005). In addition to menopause and aging, hereditary factors, lack of exercise or immobilization, lifestyle, prolonged steroid administration, excessive diet, alcohol intake, smoking, thyroxin therapy and geographical variations are the major causes of osteoporosis, among which lifestyle changes, diet and oestrogen deficiency are modifiable factors, whereas hereditary factors are non modifiable (Ferguson, 2004). Genetic factors responsible for the onset of osteoporosis can be related to family history, small body frame, skin type, low stature, early grey hair and white women (Kumar and Clark , 2002). In men, osteoporosis can be linked to decreased testosterone levels or loss of long term remodeling efficiency (Gali, 2001).

The earlier treatment regimens for postmenopausal osteoporosis suggested prevention by classical hormone replacement therapy (HRT) which has today become obsolete. Recently, a clinical trial with HRT in healthy postmenopausal women was stopped by the American National Institute of Health as the increase of cardiovascular and mammary cancer risks under HRT far outweighed the benefits, namely a reduction of hip fractures (antiosteoporotic effect) and of colon cancer (Rossouw*et al.,* 2002). Besides HRT, many pharmacological agents, used to manage the osteoporosis act by decreasing the rate of bone resorption, thereby slowing the rate of bone loss or by promoting bone formation. Synthetic agents like calcium carbonate (calcium consumption), vitamin D supplements, Raloxifene and Droloxifene (selective estrogen receptor modulators), calcitonin, bisphosphonates, sodium fluoride (increases trabecular bone mineralization), along with physical activity to strengthen muscles, stimulate osteoblast formation and prevent resorption (Gali, 2001). They are also however associated with side effects such as hypercalcemia, hypercalciuria, increased risk of endometrial and breast cancer, breast tenderness, menstruation, thromboembolic events, vaginal bleeding, hot flashes, dyspepsia and gastrointestinal ulcers (Genant*et al.,* 1989) further, the lack of direct head to head trials of treatments for osteoporosis, with reduction in fractures as an end point, makes it difficult to determine the relative efficacy of the different treatments (Malhotra and Mithal, 2008).

To overcome the wide range of side effects produced by these synthetic drugs, there is an increasing demand for 'green medicines' which are thought to be healthier and safer for the treatment of osteoporosis. The phytoestrogens, which are known to bind to the estrogen receptor sites of the cell and trigger the components and processes of estrogenic activity, have a promising role in the treatment of osteoporosis (Adams, 1989). The isoflavonoids are among the most active phytoestrogens in the flavonoid class. Ipriflavone, a synthetic flavonoid derivative (Agnusdei*et al.,* 1989) has been found to be effective in preserving bone mass in several models of experimental osteoporosis (Benvenut*et al.,* 1991). The isoflavones found in soybeans, such in BAP, were found to prevent bone loss in the ovariectomized rat model of osteoporosis (Fanti*et al.,* 1998). In addition, it was demonstrated that terrestrial algaeattenuated the progress of bone loss in rats with ovariectomy-induced osteoporosis.

Table 4. Effects of brown algae powder (BAP) on obesity induced changes in bone indices

Groups	Control (-)	Control (+)	Brown algae powder (BAP, %)			
			1	2	3	4
Bone mineral density (BMD, g/cm2)						
Mean	0.131 [a]	0.104 [d]	0.106 [d]	0.116 [c]	0.121 [b]	0.124 [b]
SD	0.008	0.009	0.005	0.007	0.008	0.008
% of Change	0.00	-20.27	-19.21	-11.20	-7.20	-5.60
Bone g- protein (BG-P, ng/ml)						
Mean	24.79 [a]	15.36 [c]	15.08 [c]	18.31 [b]	19.31 [b]	20.43 [b]
SD	2.15	1.44	0.96	1.83	1.24	0.99
% of Change	0.00	-38.01	-39.14	-26.13	-22.08	-17.56
Bone mineral content (BMC, g)						
Mean	0.37 [a]	0.25 [c]	0.25 [c]	0.29 [ab]	0.32 [ab]	0.34 [a]
SD	0.03	0.02	0.03	0.02	0.02	0.02
% of Change	0.00	-31.41	-31.56	-21.68	-13.89	-7.79

* Means in the same row with different litters are significantly different at $p \leq 0.05$.

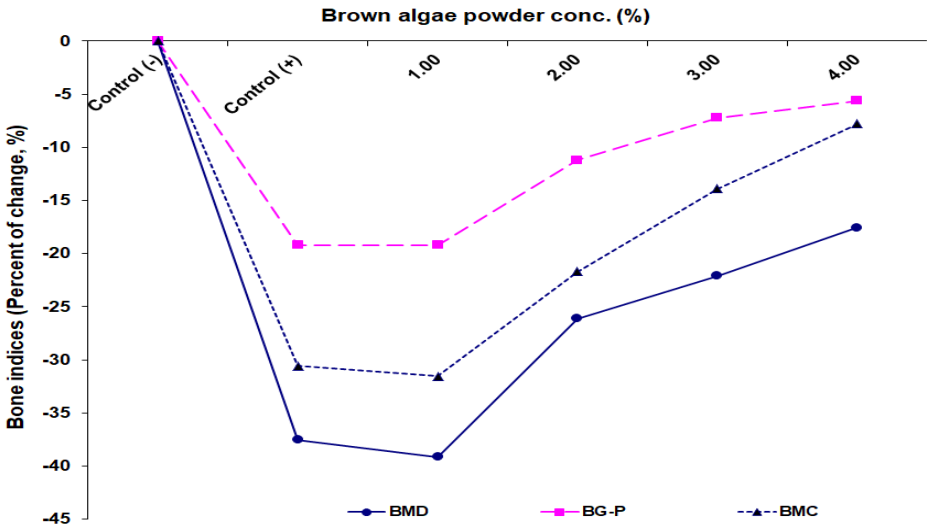

Figure 4. Effects of brown algae powder (BAP) on obesity induced changes in bone indices

* BMD,bone mineral density; BG-P, bone g- protein; BMC, bone mineral content

In conclusion, obesity is nowadays considered as a top risk factor in the development of several diseases and is causative of morbidity of patients suffering from metabolic syndrome. Oxidative stress and degradation of bone healthy indices appears as a major contributor in the development of many metabolic complications associated obesity. Lowering oxidative stress to prevent such metabolic disorders and complications therefore constitutes an interesting target. Feeding of BAP has been proven to be essential in the treatment and/or prevention of obesity but also beneficial for oxidative stress reduction and improvement the degradation of bone healthy indices. The present data support the possibility of adding brown algae powder by up to 4% to our daily diets as it contains biologically active compounds that reduce oxidative stress and improve bone healthy markers associated obesity.

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تأثر تناول الطحالب البنية (*Sargassum subrepandum*) على التغيرات التي تحدثها السمنة في الإجهاد التأكسدي ومؤشرات العظام

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الملخص العربى:

تمثل الطحالب البنية مجموعة كبيرة من الطحالب متعددة الخلايا، وهي تتضمن العديد من الطحالب الب4حرية التي تعيش في العديد من دول العلم بما فيها مصر. هذه الطحالب تلعب دوراً مهماً في البيئات المائية، لكونها تصلح كطعام وأيضا للمواطن التي تشكله. تهدف الدراسة الحالية إلى استكشاف تأثير الطحالب البنية (*Sargassumsubrepandum*) على جهد الأكسدة، وحالة الدفاع المضادة للأكسدة ، ومؤشرات صحة العظام في الفئران المصالة بالسمنة. لذلك تم تقسيم ستة وثلاثون فأر (140 ± 10جم) إلى مجموعتين رئيسيتين، المجموعة الأولى (مجموعة 1، 6 فئران) تم تغذيتها على الغذاء الأساسي، والمجموعة الرئيسية الأخرى (30 فار) تم تغذيتها علي نظام غذائي يسبب السمنة (DIO) لمدة 8 أسابيع، تم تقسيمها فيما بعد إلى خمسة مجموعات فرعية على النحو التالي: المجموعة (2) تم تغذيتها على غذاء DIO كمجموعة ضابطة موجبة، اما المجموعات (3 - 6) تم تغذيتها علي غذاء DIO يحتوي على 1، 2، 3، 4٪ من مسحوق الطحالب البنية. وفي نهاية فترة التجربة (8 اسابيع) سجلت أوزان الفئران المصابة بالسمنة زيادة في الوزن بنسبة 155,07٪ مقارنة بالفئران السليمة (الغير مصابة).ولقد أدى اضافة مسحوق الطحالب البنية بنسب 1 ، 2 ، 3 ، 4٪ إلى حدوث انخفاض كبير في وزن الجسم لدى الفئران البدينة وذلك بنسب 137.75 ، 123.87 ، 107.69 ، 94.92٪ كنسبة مئوية من العينة الضابطة ، على التوالي. كما أشارت النتائج الى أن الاصابة بالسمنة قد ادت إلي ارتفاع معنوي في تركيز المؤكسدات بالبلازما (تركيز المالونالدهيد بمعدل59,92 ٪)، وانخفاض معنوي في كل من مضادات الاكسدة الغير انزيمية (الجلوتاثيون في صورته المختزلة بمعدل 43,16 ٪ والجلوتاثيون فى صورته المؤكسدة بمعدل26,22٪ بالمقارنة بالمجموعة الضابطة السالبة. كما أظهرت التغذية على 1-4 ٪ منمسحوق الطحال البنية الى تحسن معنوى ($p\leq0.05$) في جميع المقييس السابقة وبمعدلات مختلفة . كما تم تسجيل سلوكا مماثلا للمؤشرات الخاصة بصحة العظام.كما كان هناك اتجاه لزيادة تأثيرات التحسين مع ارتفاع معدل التغذية على مسحوق الطحالب البنية. وقد خلصت الدراسة الى امكانية اضافة مسحوق الطحالب البنية بنسبة قد تصل الى 4% لما يحتوية من مركبات نشطة بيولوجيا تقوم بالحد من الاجهاد التأكسدي وتحسين دلالات صحية العظام المرتبط بمرض السمنة.

الكلمات المفتاحية: الطحالب البنية، المركبات النشطة حيويا، وزن الجسم، المالونالدهيد، الجلوتاثيون، كثافة معادن العظام