International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 24-10-2020; Accepted: 09-11-2020: Published: 26-11-2020
www.botanyjournals.com
Volume 5; Issue 6; 2020; Page No. 277-283

In vivo evaluation of the antioxidant activity of seaweed *Sargassum wightii* in selected tissue on high fructose diet fed rats

K Suganthi[1], T Malarvili[2*], V Sivaranjani[3], A Dominic Amalraj[4]

[1, 3] Department of Biochemistry, Government Arts and Science College for Women (Affiliated to Bharathidasan University) Orathandu, Thanjavur, Tamil Nadu, India

[2] Dr. T. Malarvili, Principal, Bharathidasan University Constituents Arts and Science College Navalur, Kuttapattu, Srirangam, Tamil Nadu, India.

[4] Principal, Aadhavan College of Arts and Science, Alathur, Manapparai, Trichy, Tamil Nadu, India

Abstract
The objective of this work was to assess on antioxidant status of rat plasma, liver and adipose tissue the influence of ethanolic extract of *Sargassum wightii* (SWEE) under conditions of Obesity induced by dietary fructose. SWEE at 500 mg kg−1 of body weight was administered for 8 weeks to Wistar rats that were fed with a High fructose diet (HFD). The Body weight of the rats was determined and Plasma, Liver and Adipose tissue was isolated to determine oxidative stress markers [malondialdehyde (MDA), reduced glutathione (GSH)], antioxidant enzymes [superoxidedismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] activity and vitamin-C and vitamin-E activities. The concentration of MDA was significantly higher in HFD fed rats, as compared to control animals. Conversely, SOD, CAT and GPx, GSH, vitamin-C and vitamin-E content in HFD fed animals showed a significant decline when compared with control. The administration of SWEE to the rats reduced the body weight and the activities of these antioxidant factors attained a near-normalcy. Different compounds found in the SWEE explain the regulating effect over different antioxidant status that protects the animals fed a high fructose diet (HFD) against the production of free radicals.

Keywords: High fructose diet fed rats, *Sargassum wightii*, Antioxidant, Adipose tissue, vitamin-C and vitamin-E

1. Introduction

Obesity or increased fat accumulation is one of the most common chronic conditions worldwide and is associated not only with metabolic dysfunction, but also with increased levels of oxidative stress *in vivo* Salmon (2016) [1]. Several studies found that the administration of fodders enriched with fructose to rats induced oxidative stress leading to hypertriglyceridemia, insulin resistance and obesity (Tappy *et al* 2010) [2]. Fructose, commonly added as HFCS or sucrose in processed food, is believed to promote less satiety than other sugars, thus increasing caloric intake, mainly through sweetened beverages [3]. Increased use of high-fructose corn syrup (HFCS) and sucrose, together with the rising incidence of MetS in our society during last year's, lead scientific community to postulate that high fructose consumption is related to the development of the pathology (Martins Pereira *et al* 2017; Aydin *et al* 2014) [3, 4].

Oxidative stress (OS) is caused by free radicals (Chang *et al* 2007) [5]. OS is a state of unbalance between prooxidants and antioxidants that results in oxidative damage (Guo *et al* 2007) [6]. Oxidative damage has been implicated in several pathological conditions such as cell, tissue, and organ damage, including the liver, kidney, and heart (Valko *et al* 2007) [7]. Antioxidants are compounds that stop or interrupt the oxidation process in cells by scavenging free radicals and hence prevent or mitigate diseases (Kokabi *et al* 2013) [8]. There are many artificial antioxidant substances yet with unsafe effects. Thus, exploring new natural antioxidants have been a challenge target. In this concern, marine seaweeds have been known as traditional sources of natural antioxidants (Samaraweera *et al* 2012) [9]. Edible seaweeds are rich in bioactive antioxidants, soluble dietary fibers, proteins, minerals, vitamins, phytochemicals, and polyunsaturated fatty acids. Although previously the seaweeds were only used as gelling and thickening agents in the food or pharmaceutical industries, recent researches have revealed their potential as complementary medicine (Suhaila Mohamed *et al* 2012) [10].

Sargassum, one of the marine macro algae belonging to the class Phaeophyceae, family Sargassaceae and order Fucales. It is a large, cost effectively important and ecologically dominant brown algae present in much of the tropics. It is found to be the most diverse genus among Phaeophyta in India and is represented by 38 species. The bioactive compounds of *Sargassum* species possess pharmacological properties, such as anti-oxidant, anti-inflammatory, anti-hypertensive, anti-bacterial, and anti-viral activities. These pharmacological properties are a result of the biological activity of metabolites such as alkaloids, steroids, terpenoids, saponins, polyphenols, phlorotannin, and fucoidan (Liu *et al* 2012) [11]. *Sargassum wightii* (*S. wightii*) is one of the important species belonging to the genus *Sargassum* and wide range of bioactive properties has been reported from this species (Antonisamy *et al* 2012) [12]. *Sargassum wightii* one of the important species demonstrates a good number of flavonoids in support of its antioxidant activity (Meenakshi *et al* 2009) [13].

In recent years the determination of oxidative status in plasma and selected tissues has become one of common

laboratory methods in experimental models, as it can yield valuable information with respect to the hierarchy of different enzymatic and nonenzymatic responses in tissues, as well as to various pro- or antioxidative nutritional factors. Therefore the present study was designed to investigate the modulatory role of *Sargassum wightii* on oxidative stress markers in the plasma, liver and adipose tissue in HFD induced obese rats to shed the light on the effect of obesity on these organs.

2. Materials and Methods

Seaweed collection

Fresh edible brown seaweed *Sargassum wightii* was collected from the intertidal region of Mandapam area (09º7.4170N; 079º08.5580E) of south Tamilnadu, India. The collected Seaweeds were cleaned well with sea water to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The collected seaweeds were then thoroughly washed with tap water followed by distilled water.

For drying, washed seaweeds were blotted on the blotting paper and spread out at room temperature in the shade. The shade dried seaweeds were ground to a fine powder using tissue blender. The powdered sample was then stored in refrigerator for further use.

Preparation of extract

The powdered sample was soaked with 70% ethanol for 48 hours. A semisolid extract was obtained after complete elimination of alcohol under reduced pressure. The extract of the sample was stored in desiccator until used.

Animals

Healthy male adult albino rats (Wistar strain) 6-7 weeks old, weighing 160-180g was procured from "Sri Venkateswara Enterprises", Bangalore, India. They have been housed in clean sterile polypropylene cages with proper aeration and lighting (12 ± 1 hr day / night rhythm) and the temperature was maintained between 27ºC ± 2ºC throughout the experimental period. The animals were fed with commercially available energy rich pelleted rat feed (Gold-Mohur, M/S Hindustan Lever Ltd, Mumbai, India) during the acclimatization period and water *ad libitum*. The usage and handling of experimental rats were done by following the rules and regulations given by the Institutional Animal Ethics Committee (BDU/IAEC/2017/NE/34/Dt.21.03.2017).

Chemicals

Ethylenediaminetetra acetic acid (EDTA), Trichloro acetic acid (TCA), Thiobarbituric acid (TBA), Casein, Fructose, 1-chloro-2,4-dintiro benzene (CDNB), 5,5'-dithio-bis (2-nitrobenzoic acid), glutathione (reduced), glutathione (oxidized), Nicotinamide adenine dinucleotide phosphate (NADP+/NADPH) and L-ascorbic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade and were obtained from Glaxo Laboratories, Mumbai, India, and Sisco Research Laboratories, Mumbai, India.

Experimental design

Body weight of the animals was recorded and they were divided into 4 groups of 6 animals each as follows. Table 1shows the composition of diets fed to Experimental rats.

Group 1: Normal rats fed with control diet
Group 2: High fructose diet fed animals received fructose enriched diet for a period of 8 Weeks.
Group 3: High fructose diet fed animals co-administrated with Ethanolic Extract of *Sargassum wightii* by oral gavage daily at a dose of 500 mg/kg body weight (based on effective dosage fixation studies) for 8 weeks.
Group 4: High fructose diet fed animals treated with standard drug Orilstat at a dose of 9 mg/kg body weight for 8 weeks.

Collection of samples

On completion of the experimental period, animals were anesthetized with thiopentone sodium (50mg/kg). The blood was collected with and without EDTA as an anticoagulant. Blood, plasma and serum were separated for the estimation of various biochemical parameters. The liver and adipose tissues were dissected out, washed with ice-cold saline to remove blood and weighed. The tissues were sliced and homogenized in 0.1 M Tris-HCl buffer (pH 7.0). The homogenates were centrifuged at 1000 rpm for 10 min at 4ºC in a cold centrifuge and used for various biochemical analyses.

Table 1: Composition of diets fed to Experimental rats (Nandhini *et al* 2002) [14].

Ingredient	Control diet (%)	High-fructose diet (%)
Corn starch	61	-
Fructose	-	61
Casein	20	20
Methionine	0.7	0.7
Groundnut oil	5	5
Wheat bran	9.6	9.6
Mineral mixture	3.5	3.5
Vitamin mixture	0.2ml	0.2ml

Analytical Method

The clear supernatant thus obtained was used for the assay of MDA (Beuge and Aust 1978) [15], SOD (Kakkar 1984) [16], CAT (Beers and Sizer 1952) [17], GPx (Rotruck *et al* 1973) [18], GSH (Moron *et al* 1979) [19], Vit-C (Omaye *et al* 1979) [20] and Vit-E (Baker *et al* 1980) [21].

Statistical analysis

Values were expressed as Mean SD for six rats in each group and statistically significant differences between Mean values were determined by Oneway analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons (Harvey and Paige 1998) [22]. The results were statistically analyzed by Graphpad Instat Software (Graphpad Software, San Diego, CA, USA) version 3 and $p < 0.05$ was considered to be significant.

3. Results

Effect of SWEE on body weight

The initial and final body weights of the rats during the experimental period of 8 weeks are given in Table 2. The body weight of the animals increased progressively during the experimental period. There was a trend for the HFD fed animals to gain more weight than other rats, which was remarkable as compared with those of the control rats. Administration of SWEE displays a gradual increase in the body weight of animals.

Plasma, Liver and Adipose Tissue oxidative stress markers and antioxidants (Table 3)

Effects of SWE on MDA and GSH

An increase ($p<0.01$) in the MDA level and significant drop off ($p<0.01$) (relative to normal rats) in GSH levels was observed in the plasma, liver and adipose tissue of Group II (HFD fed rats) rats relative to Group I (normal) rats. Treatment with SWEE to group III rats extensively decreased MDA and increased GSH levels when compared to group-II rats. In Group IV orilstat treated rat no notable changes were observed.

Effects of SWEE on antioxidant enzymes

Significantly ($p<0.01$) lower activities of CAT, SOD and GPx were observed in the plasma, liver and adipose tissue of HFD fed rats when compared to the normal rats. In Group III rats that had been administered with SWEE, the activities of these enzymes were maintained at near normal levels. In Group IV standard treated rats remarkable increase was observed.

Effects of SWEE on vitamins C and E

Substantially ($p < 0.01$) lower levels of vitamin C were observed in the plasma, liver and adipose tissue of HFD fed rats, when compared to normal rats. Similarly, considerably ($p < 0.01$) lower concentration of vitamin E were noted in plasma of Group II rats compared to Group I rats. We also observed a significant increase in the levels of vitamins C and E in plasma of rats fed SWEE with fructose. Group IV standard treated rats showed no significant changes as compared to group I.

Table 2: Effect of *Sargassum wightii* in animal body weight (gm)

Days (gm)	G I (Normal)	G II HFD	G III SWEE	G IV Orilstat
1st	184.83±3.18^a	184.16±2.48^a	186.16±2.85^a	184.66±3.14^a
14th	191.83±2.13^a	212.33±2.94^b	192.83±2.99^a	192.33±2.50^a
28th	196.66±2.87^a	223.16±2.63^b	202.83±2.31^{as}	202.16±2.56^a
42nd	210.16±2.63^a	234.16±3.18^b	214.50±3.27^a	215.66±3.55^a
56th	222.66±3.14^a	248.16±4.02^b	223.66±2.94^a	223.16±2.92^a

Values are expressed as Mean ± SD for six rats

Mean values within the Row followed by different letters (Superscript) are statistically significant ($P<0.05$) from each other group and same letter was statistically non-significant ($P>0.05$) were comparison by ANOVA, Duncan's multiple range test (DMRT), significant level alpha 0.05.

4. Discussion

Oxidative stress is highly correlated with a wide variety of inflammatory and metabolic disease states, including obesity (Sonta *et al* 2004; Furukawa *et al* 2004; Reaven *et al* 2004) [23, 24, 25]. Evidence suggests that a clustering of sources of oxidative stress exists in obesity; hyperglycemia, increased tissue lipid levels, inadequate antioxidant defenses, increased rates of free radical formation and chronic inflammation (Vincent and Taylor 2006) [26]. It was demonstrated that in rats fed with high-fructose diet, the concentration of free radicals was three times higher than in the control group. A number of oxygenated compounds are produced during the attack of free radicals against membrane lipoproteins, proteins and polyunsaturated fatty acids (PUFA). One of them is malondialdehyde (MDA) which can be used as an indicator of oxidative stress, as its concentration in plasma increases as the result of free-radical processes.

The data presented in this study showed that obesity increased lipid peroxidation in plasma, hepatic and adipose tissues as expressed by increased tissue levels of MDA. Our results are in basic agreement with the results of (Vincent *et al* 2001; Olusi 2002; Amirkhizi *et al* 2007) [27, 28, 29] who showed that, obesity is an independent risk factor for increasing lipid peroxidation and decreased activity of cytoprotective enzymes. There are studies that link metabolic syndrome with elevated levels of oxidative stress; free radicals can be generated by several cellular mechanisms that include the uncoupling of mitochondrial oxidative phosphorylation, abnormal changes in the antioxidant system by changes in the SOD, CAT, and GPx enzymes, uncoupling of NO synthesis, and activation of NADPH oxidase. The generation of free radicals by these mechanisms produces various components of the metabolic syndrome.

In the present study, decline in the activities of plasma, hepatic and adipose tissue GSH, SOD, CAT, GPx, Vit-C and Vit-E in HFD fed rats as compared to control rats were observed. Antioxidant enzyme activity is lower in rats with the HFD because there is a permanent condition of oxidative stress, which surpasses the defense levels of antioxidant enzymes. This decreased activity agrees with other reports (Chen *et al* 2010) [30]. Obesity can cause increased oxidation in vivo by cell injury and lipid peroxidation. All these mechanisms lead to stimulation of antioxidant enzymes but over a period of time the stores of antioxidant enzymes are depleted and cannot cope with increasing OS (Alexander *et al* 2000) [31].

Table 3: Effect of *Sargassum wightii* in plasma Liver and tissues of control and Experimental rats

Parameters	G I (Normol)	G II HFD	G III SWEE	G IV Orilstat
Plasma				
MDA (nmol of MDA formed/L)	10.22±0.71^a	17.09±1.19^b	10.66±0.74^a	10.26±0.71^a
GSH (.mg/dl)	8.60±0.60^a	6.78±0.47^b	8.42±0.58^a	8.24±0.57^a
SOD (U/ml)	4.37±0.30^a	2.09±0.14^b	4.18±0.29^a	4.06±0.28^a
Cat (U/ml)	8.97±0.62^a	5.53±0.38^b	8.21±0.57^a	8.14±0.57^a
GPx (U/ml)	8.68±0.60^a	5.58±0.39^b	8.54±0.59^a	8.30±0.58^a
Vit-C (mg/dl)	7.82±0.54^a	4.84±0.33^b	7.73±0.54^a	7.42±0.51^a
Liver				
MDA	4.58±0.32^a	6.25±0.43^b	4.27±0.29^a	4.18±0.29^a
GSH	6.60±0.46^a	4.06±0.28^b	6.12±0.42^a	6.48±0.45^a
SOD	9.74±0.68^a	6.43±0.45^b	9.13±0.63^a	9.08±0.63^a
Cat	5.52±0.38^a	4.16±0.29^b	5.43±0.38^a	5.33±0.37^a
GPx	8.89±0.62^a	4.89±0.34^b	8.17±0.57^a	8.30±0.58^a
Vit-C	5.86±0.41^a	3.17±0.22^b	5.71±0.40^a	5.80±0.40^a
Vit-E	4.53±0.31^a	2.89±0.20^b	4.35±0.30^a	4.29±0.30^a
Adipose tissue				
MDA	5.55±0.38^a	9.33±0.65^b	5.41±0.37^a	5.33±0.37^a
GSH	5.63±0.39^a	2.96±0.20^b	5.51±0.38^a	5.39±0.37^a
SOD	10.47±0.73^a	6.76±0.47^b	10.30±0.72^a	10.41±0.72^a
Cat	5.61±0.39^a	3.65±0.25^b	5.14±0.36^a	5.26±0.36^a
GPx	9.34±0.65^a	5.48±0.38^b	9.26±0.64^a	9.22±0.64^a
Vit-C	6.80±0.47^a	4.37±0.30^b	6.74±0.47^a	6.70±0.46^a
Vit-E	3.52±0.24^a	2.08±0.14^b	3.47±0.24^a	3.50±0.24^a

Values are expressed as Mean ± SD for six rats

Mean values within the Row followed by different letters (Superscript) are statistically significant ($P<0.05$) from each other group and same letter was statistically non-significant ($P>0.05$) were comparison by ANOVA, Duncan's multiple range test (DMRT), significant level alpha 0.05.

Furthermore, oxidative damage is aggravated by the decrease in antioxidant enzymes activities such as superoxide dismutase, catalase (CAT), glutathione S-transferase (GST), and glutathione peroxidase (GPx) which acts as a free radical scavenger in conditions associated with oxidative stress (Blokhina *et al* 2002) [32]. It was reported that an obesogenic diet causes a decreased production of glutathione in the liver and other tissues such as the heart (Gosh *et al* 2012) [33]; thereby, GPx activity de-creases as the substrate is decreased. GPx is the most important enzyme involved in the neutralization of peroxidized or nitroperoxidized molecules.

Decline of SOD, CAT and GPx activity in obese rat was brought back to a normal level with administration of SWEE. Supplementation of SWEE to HFD fed rats improved the GSH, vitamin C and E levels as compared to control rats, Seaweed consumption generally increases the endogenous antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and sometimes catalase activities *in vivo* (Matanjun *et al* 2010; Yuan and Walsh 2006; Zhang *et al* 2004) [34, 35, 36]. Various studies have shown a direct relationship between the consumption of seaweeds and the prevention and/or treatment of conditions associated with oxidative stress (Mohamed *et al* 2012) [37]. Our previous report confirmed the *in vitro* antioxidant activity of *Sargassum wightii* through various models (Suganthi *et al* 2020) [38].

Seaweeds contain several compounds with antioxidant capacity, such as chlorophyll derivatives, phlorotannins, terpenes, halogenated volatile compounds and carotenoids such as fucoxanthin, the main carotenoid in brown algae (Gupta *et al* 2010) [39] in addition to flavonoids and phenolics and cinnamic acid (Meenakshi *et al* 2009) [13]. The presence of these compounds explains the different mechanisms of action of seaweeds, including the free radical scavenging ability, redox-active metal chelation, mechanisms of electron donation and acceptance, the interrupting capability of lipid peroxidation and increased antioxidant enzyme activity Thomas and Kim (2011) [40]. Our study suggested that, the antioxidant mechanism of this extract having free radical scavenging ability. Previous similar studies are reported that the highest antioxidant properties present in brown alga (Balboa *et al* 2013) [41]. It has been reported that Seaweed contains vitamins, poly unsaturated fatty acids and hydrocarbons high quantities of iodine in several chemical forms (I_, I2, IO2_), which can serve as an antioxidant, anti-goiter and anticancer agent (Eskin *et al* 1995) [42].

The interests in phenolic compounds, particularly flavonoids and tannins, have considerably increased in recent years because of their broad spectrum of chemical and diverse biological properties, which include the antioxidant effects Larson (1988) [43] and radical scavenging properties Agrawal (1989) [44]. Our study demonstrates that the SWEE is rich in naturally occurring polyphenolic compounds such as Quercetin, Catechin, Epicatechin, Ellagic acid and Gallic acid (3, 4, 5-trihydroxybenzoic acid). The interest in these compounds is due to its pharmacological activity as radical scavengers. It has been proved to have potential preventive and therapeutic effects in free radical mediated diseases (Nikolic 2006; Karamaæ *et al* 2005; Kaur *et al* 2005) [45, 46, 47]. Quercetin is considered to be a strong antioxidant due to its ability to scavenge free radicals and bind transition metal ions. These properties of quercetin allow it to inhibit lipid peroxidation (Hollman and Katan 1997; Sakanashi *et al* 2008) [48, 49]. Lipid peroxidation can create deleterious effects throughout the body, such as cardiovascular and neurodegenerative diseases; however, it can be terminated by antioxidants, like quercetin, which interfere by reacting with the radicals formed (Kahl and Hildebrandt 1986; Hollman and Katan 1997; Balazs and Leon 1994) [50, 48, 51]. Quercetin also has protection against oxidant damage to the heart, brain, liver, aorta and kidney for mid-term or long-term diabetic rat (Elik *et al* 2007) [52].

In addition, Catechin and epicatechin are two flavan-3-ols stereoisomers with similar radical scavenging and antioxidant activity due to their structure. It is possible that (–)-epicatechin acts as an antioxidant both directly as a scavenger of free radicals and indirectly as a modulator of superoxide dismutase and glutathione peroxidase (Bernatoniene and Kopustinskiene 2018) [53]. Prince *et al*. (2011) [54] have reported the antihyperglycaemic, antilipidperoxidative and antioxidant effects of gallic acid on streptozotocin induced diabetic male wister rats. Further In vitro study also revealed the potent antioxidant effect of gallic acid (Punithavathi *et al* 2011) [55]. It is implicated to that Ellagic acid (EA) is reported to have a number of biological activities, including antioxidant and antiproliferative properties as observed in some of the in vitro and animal models (Vattem and Shetty 2005; Seeram *et al* 2005; Emanuele *et al* 2017; Narayanan *et al* 1999) [56, 57, 58, 59].

Flavonoids have been associated with possible role in the prevention of several chronic diseases involving oxidative stress (Lee *et al* 2003) [60], as well as their capacity to inhibit low-density lipoprotein (LDL) oxidation (Silva *et al* 2000; Kondo *et al* 1996; Mazur *et al* 1999) [61, 62, 63]. The protective effects of flavonoids in biological systems are ascribed to their capacity to transfer electrons free radicals, chelate metal catalysts (Ferrali *et al* 1997) [64], activate antioxidant enzymes (Elliott *et al* 1995) [65], reduce alpha-tocopherol radicals (Hirano *et al* 2001) [66], and inhibit oxidases (Cos *et al* 1998) [67]. Flavonoids are well known for their superoxide and hydroxyl radical scavenging activities both in hydrophilic and lipophilic systems (Torel *et al* 1986; Afanas'ev *et al* 1989; Arora *et al* 1998) [68, 69, 70]. These compounds can donate hydrogen radicals to peroxyl, hydroxyl, and peroxynitrite radicals, stabilizing them and producing a stable semiquinone radical that undergoes resonance stabilization (Seyoum *et al* 2006; Heim *et al* 2002) [71, 72]. Termination of further reactions proceeds through the flavonoid radical that reacts with available free radicals. In general, the radical scavenging activities are mainly governed by their molecular structure and the substitution pattern of hydroxy moieties (Gil and Cout 2013) [73].

5. Conclusion

Our observation indicates that supplementation of SWEE to HFD fed rats decreased body weight and reversed fructose induced oxidative stress associated changes in antioxidant defence in plasma, liver and adipose tissue and decline lipid peroxidation and thus evidencing protective capacity of SWEE on plasma, liver and adipose tissue against oxidative stress. The administration of SWEE reduced peroxidation of lipids and improved the activity of antioxidative enzymes in plasma and selected organs. In conclusion, SWEE are able to reduce the oxidative stress, and develop antioxidative enzymatic protection system, which may help to alleviate

the free radicals' generation during several pathological states. Further research on the in vivo antioxidant properties of these extracts and the identification of active compounds is in progress. These studies should reveal the possible therapeutic uses of these algal species.

6. References

1. Salmon AB. Beyond Diabetes: does obesity induced oxidative stress drive the aging process? Antioxidants. 2016; 5:1-14.
2. Tappy L, Le KA, Tran Ch, Paquot N. Fructose and metabolic diseases: New findings, new questions. Nutrition, 2010.
3. Martins Pereira R, Botezelli JD, da Cruz Rodriges KC, Mekary RA, Cintra DE, Pauli JR, *et al*. Fructose consumption in the development of obesity and effects of different protocols of physical exercise on the hepatic metabolism. Nutrients, 2017, 9:405.
4. Aydin S, Aksoy A, Aydin S, Kalayci M, Yilmaz M, Kuloglu T, *et al*. Today's and yesterdays of pathophysiology: Biochemistry of metabolic syndrome and animal models. Nutrition. 2014; 30:1-9.
5. Chang CY, Wu KC, Chiang SH. Antioxidant properties and pro-tein compositions of porcine haemoglobin hydrolysates. Food Chem. 2007; 100:1537-1543.
6. Guo TT, Xu HL, Zhang LX. *In vivo* protective effect of Porphyra yezoensis polysaccharide against carbon tetrachloride induced hepatotoxicity in mice. Regulatory Toxicology and Pharmacology. 2007; 49:101-106.
7. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J, *et al*. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007; 39:44-84.
8. Kokabi M, Yousefzadi M, Ali ahmadi, Feghhi A, Amin KM. Antioxidant activity of extracts of selected algae from the Persian Gulf, Oran. Journal of the Persian Gulf. 2013; 4:45-50.
9. Samaraweera AM, Vidanarachchi JK, Kurukulasuriya MS. Industrial applications of macroalgae. West Sussex. UK.I: John Wiley & Sons Ltd west success UK, 2012, 506.
10. Suhaila Mohamed, Siti Nadia Hashim, Hafeedza Abdul Rahman I. Seaweeds: A sustainable functional food for complementary and alternative therapy Trends in Food Science & Technology February, 2012, 83-96.
11. Liu L, Heinrich M, Myers S, Dworjanyn SA. Towards a better understanding of medicinal uses of the brown seaweed Sargassum in Traditional Chinese Medicine: a phytochemical and pharmacological review. J Ethnopharmacol. 2012; 142:591-619.
12. Antonisamy JM, Essakimuthu P, Narayanan J, Anantham B, Tharmaraj RJJM, Arumugam S, *et al*. Phytochemical characterization of brown seaweed Sargassum wightii. Asian Pac J Trop Dis. 2012; 2(1):109-113.
13. Meenakshi S, Manicka GD, Tamilmozhi S, Arumugam M and Balasubramanian T. Total flavonoid and *in vitro* antioxidant activity of two seaweeds of Rameshwaram Coast. Global J Pharmacol. 2009; 3:59-62.
14. Nandhini AT, Balakrishnan SD, Anuradha CV. Response of liver antioxidant system to taurine in rats fed high fructose diet. Indian J Exp Biol. 2002; 40:1016-1019.
15. Beuge JA, Aust SD. The thiobarbituric acid assay. Methods in Enzymology. 1978; 52:306-307.
16. Kakkar P, Das B, Viswanathan P.N. A Modified Spectrophotometric Assay of Superoxide Dismutase. Ind J Biochem Biophys. 1984; 21:130-2.
17. Beers R, Sizer I. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. Journal of Biological Chemistry. 1952; 195:133.
18. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG Selenium: Biochemical Role as a Component of Glutathione Peroxidase. Science. 1973; 179:588-90.
19. Moron M.S., Depierre J.W., Mannervik B. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver; Biochem. Biophys Acta. 1979; 582:67-78.
20. Omaye ST, Tumball JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods in Enzymology, 1979; 62:1-11.
21. Baker H, Frank O, De Angeles B, Feinglod S. Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. Nutrition Reports International. 1980; 21:531.
22. Harvey J, Paige SM. The instat guide to choosing and interpreting statistical tests: A manual for graph pad instat. Version 3. San Diego, CA USA, 1998.
23. Sonta T, Inoguchi T, Tsubouchi H, Sekiguchi N, Kobayashi K, Matsumoto S,Utsumi H, Nawata H: Evidence for contribution of vascular NAD(P)H oxidase to increased oxidative stress in animal models of diabetes and obesity. Free Radic Biol Med. 2004; 37:115-115.
24. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Nakajima Y, Nakayama O, *et al*. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Investig. 2004; 114:1752-1761.
25. Reaven G, Abbasi F, McLaughlin T. Obesity, insulin resistance, and cardiovascular disease. Recent Prog Horm Res. 2004; 59:207-207.
26. Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity induced oxidant stress in humans. Int J Obes. 2006; 30:400-400.
27. Vincent HK, Powers SK, Dirks AJ, Scarpace P. Mechanism for obesity induced increase in myocardial lipid per-oxidation. Int J Obes. 2001; 25:378-378.
28. Olusi SO. Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte enzymes in humans. Int J Obes. 2002; 26:1159-1159.
29. Amirkhizi F, Siassi F, Minaie S, Djalali M, Rahimi A, Chamari M. Is obesity associated with increased plasma lipid peroxidation and oxidative stress in women? ARYA Atherosclerosis Journal. 2007; 2(4):189-192.
30. Chen H, Li JL, Jian JZ, Xu B, Li R. Effect of soybean oligosaccharides on blood lipid, glucose levels and antioxidant enzymes activity in high fat rats. Food Chem. 2010; 119:1633-1636.
31. Alexander CM, Landsman PB, Teutsch SM. Diabetes melliteus impairing fasting glucose, atherosclerotic risk factors & prevelance of coronary heart disease. Am J Cardiol. 2000; 86:897-902.
32. Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, Oxidative Damage and Oxygen

Deprivation Stress a Review. Ann Bot. 2002; 91:179-179.
33. Gosh S, Sulistyioningrum DC, Glier MB. Altered glutathione homeostasis in heart augments cardiac lipotoxicity associated with diet-induced obesity in mice. J Biol Chem. 2012; 286:42483-42493.
34. Matanjun P, Mohamed S, Kharidah M, Noordin MM. Comparison of cardiovascular protective effects of tropical seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera*, and *Sargassum polycystum*, on high-cholesterol/high-fat diet in rats.Journal of Medicinal Food. 2010; 13(4):792-800.
35. Yuan YV, Walsh NA. Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. Food and Chemical Toxicology. 2006; 44:1144-1150.
36. Zhang Q, Ning L, Xiguang L, Zengqin Z, Zhien L, Zuhong X, *et al*. The structure of a sulfated galactan from *Porphyra haitanensis* and its in vivo antioxidant activity. Carbohydrate Research. 2004; 339:105-111.
37. Mohamed S, Hashim SN, Rahman HA. Seaweeds: A sustainable functional food for complementary and alternative therapy. Trends Food Sci Tech. 2012; 23: 83-96.
38. Suganthi K, Malarvili T, Sivaranjani V, Dominic Amalraj A. *In vitro* antioxidant activity and scavenging effect of ethanolic extract of *Sargassum wightii*. Journal of Natural Remedies. 2020; 21:107-114.
39. Gupta S, Rajauria G, Abu-Ghannam N. Study of the microbial diversity and antimicrobial properties of Irish edible brown seaweeds. IntJ Food Sci Techno, 2010; 45(3):482-489.
40. Thomas NV, Kim SK. Potential pharmacological applications of polyphenolic derivatives from marine brown algae. Environ Toxicol Pharmacol. 2011; 32:325-335.
41. Balboa EM, Conde E, Moure A, Falque E, Domınguez H. *In vitro* antioxidant properties of crude extracts and com-pounds from brown algae. Food Chemistry. 2013; 138:1764-1785.
42. Eskin BA, Grotkowski CE, Connolly CP. Different tissue responses for iodine and iodide in rat thyroid and mammary glands. Biological Trace Element Research. 1995; 49:9-19.
43. Larson RA. The antioxidants of higher plants. Phytochem. 1988; 27:969-978.
44. Agrawal PK. Carbon-13 NMR of Flavonoids. New York: El-sevier Press, 1989, 5-31.
45. Nikolic KM. Theoretical study of phenolic antioxidants properties in reaction with oxygen-centered radicals. J Mol Struc. 2006; 774:95-105.
46. Karamaæ MA, Kosiñska, Pegg RB. Comparison of radical-scavenging activities of selected phenolic acids. Pol J Food Nutr Sci. 2005; 14:165-170.
47. Kaur S, Michael H, Arora S, Harkonen PL, Kumar S. The *in vitro* cytotoxic and apoptotic activity of Triphala-an Indian herbal drug. J Ethnopharm. 2005; 97:15-20.
48. Hollman PCH, Katan MB. Absorption, metabolism and health effects of dietary flavonoids in man. Biomed. & Pharmacother. 1997; 51:305-310.
49. Sakanashi Y, *et al*. Possible use of quercetin, an antioxidant, for protection of cells suffering from overload of intracellular Ca2+: a model experiment. Life Sciences. 2008; 83:164-169.
50. Kahl R, Hildebrandt AG. Methodology for studying antioxidant activity and mechanisms of action of antioxidants. Food and Chemical Toxicology. 1986; 24:1007-1014.
51. Balazs L, Leon M. Evidence of an oxidative challenge in the Alzheimer's brain. Neurochemical Research. 1994; 19:1131-1137.
52. Elik M, Serdaroğlu G, Özkan R. The Investigation of Antioxidant Activities of Myricetin and Quercetin with Dft Methods, C.Ü. Fen-Edebiyat Fakültesi Fen Bilimleri Dergisi. 2007; 28: 2.
53. Bernatoniene J**,** Kopustinskiene DM. The Role of Catechins in Cellular Responses to Oxidative Stress Molecules. 2018; 23(4):965.
54. Prince PSM, Dhanasekar K, Rajakumar S, Preventive effects of vanillic acid on lipids, bax, bcl-2 and myocardial infarct size on isoproterenol-induced myocardial infarcted rats: a biochemical and in vitro study. Cardiovasc Toxicol. 2011; 11(1):58-66.
55. Punithavathi VR, Prince PSM, Ramesh K. Antihyperglycaemic, antilipid peroxidative and antioxidant effects of gallic acid on streptozotocin induced diabetic Wistar rats. European Journal of Pharmacology 2011; 650:465-471.
56. Vattem DA, Shetty K. Biological function of ellagic acid: A Review. J Food Biochem. 2005; 29: 234-266.
57. Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Nair MG, Heber D. *In vitro* antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other poly-phenols as found in pomegranate juice. J Nutr Biochem. 2005; 16:360-367.
58. Emanuele S, Lauricella M, Calvaruso G, D'Anneo A, Giuliano M. Litchi chinensis as a functional food and a source of antitumor compounds: An overview and a description of biochemical pathways. Nutrients, 2017, 99.
59. Narayanan BA, Geoffroy O, Willingham MC, Re GG, Nixon DW. p53/p21 (WAF1/CIP1) expression and its possible role in G1 arrest and apoptosis in ellagic acid treated cancer cells. Cancer Lett. 1999; 136:215-221.
60. Lee KW, Kim YJ, Lee HJ, *et al*. Cocoa has more phenolic phy-tochemicals and a higher antioxidant capacity than teas and red wine. J Agric Food Chem. 2003; 51:7292-7295.
61. Silva FAM, Borges F, Guimaraes C, *et al*. Phenolic acids and derivatives: Studies on the relationship among structure, radical activity, and physicochemical parameters. J Agric Food Chem. 2000; 48:2122-2126.
62. Kondo K, Hirano R, Matsumoto A, Igarashi O. Itakura H. Inhi-bition of LDL oxidation by cocoa, Lancet. 1996; 348:1514-1518.
63. Mazur A, Bayle DC, Lab E, Rock Y. Rayssiguier. Inhibitory effect of procyanidin-rich extracts on LDL oxidation in vitro, Atherosclerosis. 1999; 145:421-422.
64. Ferrali M, Signorini C, Caciotti B, Sugherini L, Ciccoli L, Giachetti D, Comporti M. Protection against oxidative damage of erythrocyte membranes by the flavonoid quercetin and its relation to iron chelating activity, FEBS Lett. 1997; 416:123-129.
65. Elliott AJ, Scheiber SA, Thomas C, Pardini RS. Inhibition of glutathione reductase by flavonoids, Biochem Pharmacol. 1992; 44:1603-1608.

66. Hirano R, Sasamoto W, Matsumoto A, Itakura H, Igarashi O, Kondo K. Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation, J Nutr Sci Vitaminol (Tokyo). 2001; 47:357-362.
67. Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Van Poel P, *et al*. Structure-activity relationship and classification of flavonoids as inhibitors of xanthineoxidase and superoxide scavengers, J Nat Prod. 1998; 61:71-76.
68. Torel J, Cillard J, Pierre Cillard P. Antioxidant activity of flavonoids and reactivity with peroxy radical. Phytochemistry. 1986; 25:383-385.
69. Afanas'ev IB, Dcrozhko AI, Brodskii AV, Kostyuk VA, Pota-povitch AI. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochem Pharmacol. 1989; 38:1763-1769.
70. Arora A, Nair MG, Strasburg GM. Structure-activity relation-ships for antioxidant activities of a series of flavonoids in aliposomal system. Free Radic Biol Med. 1998; 24:1355-1363.
71. Seyoum A, Asres K, El-Fiky FK, Structure-radical scavengingactivity relationships of flavonoids. Phytochemistry. 2006; 67:2058-2070.
72. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. JNutr Biochem. 2002; 13:572-584.
73. Gil ES, Cout RO. Flavonoid electrochemistry: A review on the electro analytical applications. Rev Bras Farmacogn. 2013; 23:542-558.