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Fucus vesiculosus L. (Bladderwrack) modulates oxidative stress and inflammation on high-fat diet induced obesity in mice

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Abstract

High dietary levels of lipid consumption cause ectopic fat accumulation and trigger non-alcoholic fatty liver disease (NAFLD) which leads to liver injury. This study aims to investigate the hepatoprotective effect of *Fucus vesiculosus L. (Fv)* seaweed on the high fat diet (HFD) induced obesity in mice. Forty adult male C57BL/6J mice were divided into 4 groups. Group 1 (control) and group 2 (HFD); animals fed on standard and HFD respectively. Group 3 *(Fv)* and group 4 (HFD + *Fv*); animals supplemented with *Fv* (575 mg/kg body weight/day) orally by gavage along with standard and high fat diet respectively for 6 consecutive weeks. Body weights, liver enzymes, lipid profile, oxidative stress markers and inflammatory cytokines were evaluated. Liver histopathological fatty changes were investigated. The mRNA expression levels of sterol regulatory element binding protein (SREBP-1c), fatty acid synthase (FAS), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and heme oxygenase-1 (HO-1) genes were quantitated. The transcriptional activity of nuclear factor E2-related factor 2 (N2rf) and nuclear factor kappa beta (NF-κB) were assessed by DNA-binding-based ELISA. Results revealed that *Fv* supplementation along with HFD regulated body weight gain, improved all biochemical parameters, alleviated liver steatosis, reduced fatty vacuoles and downregulated the SREBP-1c, FAS, TNF-α and IL-6 genes expression. Conversely, Nrf2-inducing Heme oxygenase-1 (HO-1) was activated with concomitant increase in HO-1mRNA expression and enzymatic activity levels in liver tissue. Furthermore, *Fv* relieves inflammation by reducing the transcriptional activity of the NF-κB. In conclusion, *Fv* seaweed may be a promising candidate for liver injury induced by high-fat consumption.

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Introduction

Obesity, a global complex epidemic problem with rising prevalence, becomes an alarming warning to public health worldwide. It resulted in increasing health care burden and reducing life prospect. The liver is the most favorable part of ectopic fat accumulation in the body. High dietary levels of fat consumed cause excessive lipid accumulation in the liver. Accumulated-fat can induce oxidative stress which mediated inflammation. Consequently, hepatic steatosis and NAFLD led to liver injury (Byrne and Targher, 2014). Unfortunately, the histopathological fatty liver can progress to cirrhosis which may develop to hepatocellular carcinoma (HCC) (Marchesini *et al.,* 2008). Therefore, oxidative stress and inflammation induced by high-fat consumption should be managed in order to prevent obesity and its associated NAFLD.

In response to oxidative stress, numerous antioxidant and detoxifying genes such as heme oxygenase-1 (HO-1) were expressed to defend the tissues from injury. The expression of these genes was regulated by the transcription factor Nrf2. Similarly, NF-κB is another inducible transcription factor regulating a large array of genes involved in multiple processes of the inflammatory responses including IL-6, TNF-α (Liu *et al.,* 2017). Nevertheless, the NF-kB pathway merges the inflammatory and metabolic responses as well. In addition, the hepatic lipid metabolism and lipogenic-related genes expression are associated with both oxidative stress and inflammation in the NAFLD progression.

SREBP-1c and FAS are two fatty acid-synthesis involved genes. FAS is a soluble protein existing as a homodimer of 273 kDa subunits, is considered as a central enzyme in lipogenesis. It is essential for the *de novo* synthesis of long-chain saturated fatty acids from acetyl coenzyme A (CoA), malonyl CoA and NADPH. It is universally expressed in various human tissues, with the highest level in liver, adipose tissue, and lung (Postic and Girard, 2008). The gene encoding FAS enzyme was known as a candidate gene for body fat determination. Its expression is highly regulated by the transcription factor SREBP-1c, which makes it a vital factor for TG synthesis (Dorn *et al.,* 2010). Indeed, the crucial role of lipogenic-involved genes in fat accumulation and their effects on hepatic NF-κB and Nrf2 pathways represent a promising strategy toward the developing of novel natural product-based treatment.

Diverse classes of obesity and fat accumulation drugs are widely distributed. However, their serious side effects and long-term usage are among the drawbacks of their use. Until now, no agreement on effective therapy was established (Bonamichi *et al.,* 2018). Meanwhile, natural products still considered an excellent alternative strategy for developing new promising, safe and effective agents for treatment of obesity and its accompanied metabolic syndrome (Mayer *et al.,* 2009).

Fucus Vesiculosus L. sp.(Fv), one of the brown-algae seaweeds was known by its high nutritional value including carbohydrates, protein, polyunsaturated lipids, minerals as well as several other health-promoting agents efficient against various diseases. Furthermore, it was considered as virtuous sources of dietary fibers and minerals, especially iodine. In addition, it possesses antioxidant, anti-inflammatory, anti-tumor, anti-obesity, anti-coagulant and anti-diabetic activities (Cardoso *et al.,* 2015). Therefore, the present study was intended to investigate the hepatoprotective impact of *Fv seaweed* on the HFD-induced obesity in mice and accompanied metabolic syndromes by evaluating its antioxidant and anti-inflammatory activities.

Materials and methods

Chemicals

Fucus vesiculosus L. (Bladderwrack) high quality powder form was provided from USDA organic (USA).

Animals and diets

The current protocol was conducted in accordance with the internationally accepted guidelines for laboratory animal use and care as found in the

European community guidelines/EEC directive of 1986; 86/609/EEC.

Forty adult male C57BL/6J mice, 6-8 weeks old, weighing 25-30g, were obtained from the research unit of pharmacology and chemistry, Misr University Science and Technology, Cairo, Egypt. Animals were housed maximum four in polyurethane cages in the breeding unit of the Medical Research Center (Faculty of Medicine, Ain Shams University, and Cairo, Egypt). Room temperature, relative humidity, and light/dark conditions were set to be maintained between 21± 2°C; 30–70%, and 12-hour light/12-hour dark respectively. Animals were divided according to the type of diet they fed. The standard diet consists of 20 kcal% protein; 10 Kcal% fat and 70 Kcal% carbohydrate. HFD consists of 20 Kcal% protein; 45 Kcal% fat and 35 Kcal% carbohydrate.

Experimental design

All groups had free access to the standard diet for one-week acclimatization. Then, animals were randomly divided into four groups of ten mice each. The control group (group1), animals continue to feed with the standard diet until the end of experiment. HFD group (group 2), mice fed with HFD for 6 consecutive weeks. *Fv* group (group 3), mice were supplemented with 575 mg/kg b.w./day along with the standard diet. HFD + *Fv* group (group 4), animals were ingested with *Fv* (575 mg/kg b.w./day) orally by gavage along with HFD till the end of the 6th week (ventura *et al.,* 2018). At the end of the experimental period, animals were fasted overnight, euthanized by sodium thiopental (30 mg/kg in saline) and subjected to gross necropsy by cervical decapitation (Brookes *et al.,* 2000). Blood was collected from the abdominal aorta, allowed to clot, centrifuged and the serum was stored at -20°C until use. At necropsy, livers were collected immediately, weighted and divided into three parts. One part was immersed in 10% formalin solution for histological investigations, another part was kept in saline for ELISA and DNA-based ELISA assays, and the rest was used for RNA extraction and enzyme activity assay.

Body and liver weights determination

Body weights of mice in all groups were recorded on the first day of the experiment, taken periodically once a week throughout the study and recorded on the last day before sacrifice. Likewise, the liver's weights from all groups were taken after necropsy.

Biochemical parameters assessment

Serum alanine transaminase (ALT) and aspartate transaminase (AST) activities were assayed according to the colorimetric methods described by Reitman and Frankel, 1957. Serum triglycerides (TGs) were assessed according to Fossati and Prencipe, 1982. Total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-c) were determined in serum according to the described methods of Allain *et al.,* 1974 and Finley *et al.,* 1978 respectively.

Evaluation of oxidative stress markers

The lipid peroxidation level was denoted by the amount of thiobarbituric acid reactive substances (TBARS) upon the reaction of thiobarbituric acid with malondialdehyde (MDA). MDA and total antioxidant activity (TAA) were determined according to the method of Yoshioka *et al.,* 1979 and Koracevic *et al.,* 2001 respectively.

Histological investigations

Fixed liver tissue samples were embedded in paraffin cubes, sliced into serial 4 µm-thick sections. Then, the obtained tissue sections were stained with hematoxylin and eosin (H&E) stains, examined and photographed under a light microscope (Bancroft and Stevens, 1990).

Real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

Total RNA was isolated from liver tissue samples using the BIOLINE TRIsure™ kit (Cat.no. BIO-38032), according to the manufacturer's instructions. For cDNA synthesis, one microgram of the total RNA was used using the BIOLINE SensiFast™ cDNA synthesis kit (Cat. no. BIO-65053) following the manufacturer's instructions. The relative expression levels of mRNA encoding the studied genes specifying

the fatty acid metabolism (FAS and SREBP-1c); the inflammatory mechanism (TNF-α and IL-6); the oxidative stress (HO-1), along with the housekeeping gene, β-actin were assessed using the SensiFAST™ SYBR® No-ROX kit (2X) (Cat. no. BIO-98005), according to the manufacturer's protocol. The results were computerized using the Stratagene (Mx 3000PTM) machine. The expression level of each target gene was normalized to the β-actin and presented as fold change relative to the control group. The forward and reverse primers for all selected genes used in this study are illustrated in Table 1.

ELISA assays

The hepatic content of the pro-inflammatory cytokines TNF-α and IL-6 were determined using commercial kits. Mouse TNF-α and IL-6 ELISA Kits were purchased from CUSABIO (Baltimore, USA).

DNA-Binding-based transcription factors ELISA assays

NF-kB and Nrf2 transcriptional activities were determined using the DNA-binding dependent enzyme immunoassay TransAM (Active Motif) kits purchased from Carlsbad, CA, USA; according to the manufacturer's instructions. Briefly, liver tissues of all groups were homogenized in ice-cold lysis buffer, incubated for 10 min, and centrifuged at 14,000Xg for 5 min at 4°C. The resulting nuclear pellet was suspended in extraction buffer, kept on ice for 15 min, and centrifuged at 14,000Xg for 5 min at 4°C. Nuclear extracts (5µg) were incubated in a 96-well plate containing the corresponding immobilized consensus binding sites.

The antioxidant response element (ARE) consensus site oligonucleotide sequence: (5′-GTCACAGTGACTCAGCAGAATCTG-3′) is the specific binding site for the Nrf2. The NF-κB specific binding consensus site oligonucleotide sequence is 5'-AGTTGAGGGGACTTTCCCAGGC-3'. Wells were washed three times and bound to the primary antibody and then secondary antibody conjugated with horseradish peroxide. The signals of both NF-κ B and Nrf2 were detected spectrophotometrically at 450 nm.

Heme oxygenase-1 enzymatic activity in liver

The activity of HO-1 enzyme was determined in the liver tissue as described by Kutty and Maines, 1982. Shortly, ~0.1 g of the liver sample from each group was homogenized in 0.5 ml pre-chilled 0.25 M sucrose solution containing 50 mM potassium phosphate buffer (pH 7.4). Then, the reaction mixture containing 0.5 mg/ml of liver cytosol as a source of biliverdin reductase, 0.2 mM of the substrate hemin, 500 µg/ml of cell lysate, 0.2 mM $MgCl_2$, 2 mM glucose-6-phosphate,1 U/ml glucose-6-phosphate dehydrogenase, 1 mM NADPH and 50 mM potassium phosphate buffer (pH 7.4) was incubated at 37°C for 1 h. HO-1 enzyme activity was then expressed as nmol bilirubin/h/mg protein.

Statistical analyses

The software program, Statistical Package for Social Science (SPSS), version 23.0 for Windows (SPSS® Chicago, USA) was used for data analysis. Data were expressed as mean ± standard deviation (SD). Statistical analysis for differences in means of variables between groups was performed using Student *t*-test; a probability of $P<0.05$ was considered significant while $P>0.05$ insignificant.

Results

Effect of Fucus vesiculosus L. on HFD-induced gain in body and liver weights

While all groups of mice were matched by their mean initial body weights, results showed a continuous and significant increase ($P<0.05$) in the weight of HFD group starting from the second week until the end of the experiment compared to the control group (Fig. 1A). Conversely, *Fv* ingestion exhibited a regulatory effect on the uncontrollable HFD-induced weight gain, as evidenced by the significant reduction recorded in the body weight of the HFD + *Fv* group compared to the HFD group. Additionally, *Fv* supplementation had significantly reduced the weight of liver gained due to the HFD ingestion, while the liver weight of the *Fv* group recorded no changes compared to the control one (Fig. 1B).

Table 1. Primers sequence for the studied genes.

Gene	Forward primer	Reverse primer
SREBP-1c	5'-GGGGCCTGACAGGTGAAATC-3'	5'- TGTCAGCAGCAGTGAGTCTG-3'
FAS	5'-TTGCTGGCACTACAGAATGC-3'	5'-AACAGCCTCAGAGCGACAAT-3'
TNF-alpha	5'TACTGAACTTCGGGGTGATTGGTCC3'	5'-CAGCCTTGTCCCTTGAAGAGAACC-3'
IL-6	5'-GAGGATACCACTCCCAACAGACC -3'	5'-AAGTGCATCATCGTTGTTCATACA-3'
HO-1	5'- AGCCCCACCAAGTTCAAACA -3'	5'- CATCACCTGCAGCTCCTCAA -3'
ß-actin	5′-AGCCATGTACGTAGCCATCC-3′	5′-CTCTCAGCTGTGGTGGTGAA-3′

SREBP-1c: sterol regulatory element binding protein-1c; FAS: fatty acid synthase; TNF-alpha: tumor necrosis-alpha; IL-6: interleukin-6; HO-1: heme oxygenase-1.

Effect of Fucus vesiculosus L. *on HFD-induced biochemical alterations*

Table 2 illustrated the impacts of *Fv* on the HFD-induced biochemical alterations. Results revealed no changes of the serum ALT and AST levels in the *Fv* group of mice, compared to control group. However, HFD group showed a marked and significant increase (p<0.001) in the ALT and AST activities compared to the control group. But these elevations were highly suppressed upon *Fv* supplementation.

As shown in Table 2, the group of mice supplemented with *Fv* along with HFD exhibited significant reduction (p<0.001) in the TC and TG levels with a concomitant increase (p<0.001) in the HDL-c level compared to the HFD group of mice.

Table 2. Effects of *Fv* on the HFD-induced biochemical alterations.

Parameters	Control group	HFD group	*Fv* group	HFD + *Fv* group
ALT (IU/L)	25.41 ± 2.5	52.51 ± 3.2*	23.5 ± 1.8$^{\#}$	30.60 ± 3.0$^{*\#}$
AST (IU/L)	15.13 ± 3.5	59.30 ± 4.5*	13.4 ± 4.0$^{\#}$	24.71 ± 2.4$^{*\#}$
TG (mmol/L)	126.9 ± 7.9	230.25 ± 8.4*	120 ± 5.5$^{\#}$	140.33 ± 8.5$^{*\#}$
TC (mmol/L)	8.13 ± 2.5	52.52 ± 5.3*	7.8 ± 3.5$^{\#}$	11.83 ± 4.5$^{*\#}$
HDL-c (mmol/L)	4.83 ± 3.1	2.53 ± 2.5*	4.0 ± 2.6$^{\#}$	3.42 ± 2.8$^{*\#}$
MDA (nmol/ml)	13.5 ± 1.5	25.23 ± 2.3*	14.2 ± 2.0$^{\#}$	17.57 ± 1.2$^{*\#}$
TAA (nmol/ml)	2.25 ± 1.4	0.87 ± 1.5*	2.0 ± 3.5$^{\#}$	1.77 ± 1.8$^{*\#}$

Data are represented as mean ± SD of the mean of the 10 mice in each group. ALT: alanine aminotransferase; AST: Aspartate aminotransferase; TG: triglycerides; TC: total cholesterol; HDL-c: high density lipoprotein cholesterol; MDA: malondialdehyde; TAA: total antioxidant activity. *p< 0.001, compared to control group. #p< 0.001, compared to HFD group.

Effect of Fucus vesiculosus L. *on HFD-induced oxidative stress*

The impact of *Fv* on the HFD-induced oxidative stress was evaluated via MDA and TAA levels. Results revealed a significant increase (P< 0.001) in the MDA level with a concomitant decrease in the TAA among HFD group of mice compared to the control animals (table 2). Obviously, *Fv* ingestion in parallel with HFD significantly suppressed the elevation of MDA and raised the TAA almost to their normal levels as compared to the HFD group. These findings denoted the capacity of *Fv* in promoting the cellular antioxidant defenses and signified its potent antioxidant activity against fat accumulation-induced oxidative stress.

Fucus vesiculosus L. *improves the HFD-induced histopathological alterations*

Histological examination of liver tissue samples from all studied groups was conducted in order to

investigate the impact of *Fv* on the HFD-induced histopathological fatty changes. In the control group, the liver showed an average portal tract (PT) with normal portal veins (PV), bile ducts and average central vein (CV). Normal-sized hepatocytes were arranged in single-cell cords with normal intervening blood sinusoids (Fig. 2, i). Similarly, no histopathological changes in the liver of the *Fv* group were detected (Fig. 2, ii). Conversely, the liver of HFD group revealed many histological changes; including dilation in the portal and central veins, wide spreading lipid vacuoles, degeneration in many hepatocytes with marked micro-vesicular steatosis in both peri-portal and peri-venular zones (Fig.2, iii-vi).

On the other hand, *Fv* supplementation modulates all these alterations as observed by the fewer and small cytoplasmic lipid vacuoles in the peri-venular area with an average portal and central veins compared to the HFD group (Fig. 2, vii & viii). These findings indicate the impact of *Fv* in improving the HFD-induced histopathological alterations.

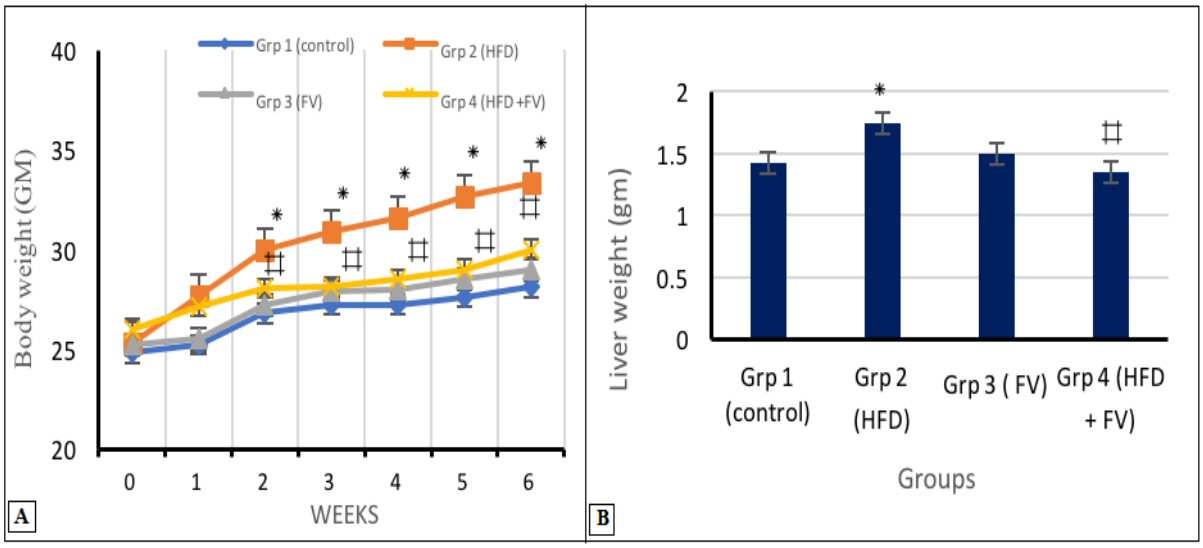

Fig. 1. Effects of *Fv* on HFD-induced alterations on body weight (A) and liver weight (B). Data are represented by mean ± SD from each group (n = 10). **p*<0.05 compared to the control group. ⌗*P*<0.05 compared to HFD group.

Effect of Fucus vesiculosus L. *on the HFD-induced dysregulation in lipogenic-related genes expression*

The mRNA expression levels of SREBP-1c and FAS were analyzed in all studied groups. The ß-actin gene was used as an internal standard for normalization of the target genes expression levels. As shown in Fig.3, the mRNA expression levels of SREBP-1c and FAS genes were significantly increased by 2.5 and 2-folds respectively in the HFD group compared to the control group. However, daily supplementation of *Fv* along with HFD significantly downregulated these expression levels to below those expressed in mice fed on the standard diet.

Effect of Fucus vesiculosus L. on HFD-induced oxidative stress signaling pathway

Oxidative stress signaling pathway was investigated via the Nrf2 transcriptional activity by the DNA-binding Nrf2 ELISA (Active Motif). The Nrf2 transcriptional activity was evaluated by its nuclear translocation and rate of binding with ARE site. This specific binding site for Nrf2 was within the promoter sequence of the gene encoding HO-1. Results showed that the rate of Nrf2: ARE binding in the HFD group was approximately lowered to its half, compared to the control group (Fig. 4C). Notably, this Nrf2:ARE binding rate in the HFD+*Fv* group shown to be 70% higher than the HFD group. This great binding rate triggers the expression of HO-1, the antioxidant phase II defense gene. The mRNA expression level of HO-1 enzyme was two folds downregulated in the HFD group of mice with a significant decrease in its activity in their liver tissues, compared to the control group (Fig. 4A). On the other hand, *Fv* supplementation

significantly shifted this situation by upregulating the HO-1 mRNA expression levels with notable induction in its activity in the HFD + *Fv* group, compared to the HFD group (Fig.4B).

Effect of Fucus vesiculosus L. on HFD-induced inflammatory signaling pathway

The pro-inflammatory cytokines, TNF-α and IL-6 were evaluated at both mRNA and protein levels. A dramatic increase in the hepatic contents of TNF-α and IL-6 was revealed in the HFD group at both levels. Indeed, this increment was reversed and significantly downregulated upon *Fv* supplementation in the HFD + *Fv* (Fig.5B). The expression of these cytokines is greatly dependent upon the activation of the transcription factor NF-κB and the rate of binding to its specific oligonucleotide. Results revealed the inducible effect of HFD ingestion to the transcriptional factor NF-κB as evidenced by two time's high binding rate over the control group. Conversely, *Fv* supplementation suppresses the NF-κB transcriptional activity and hence downregulates the expression of its targeted genes, TNF-α, and IL-6 (Fig.5A -C).

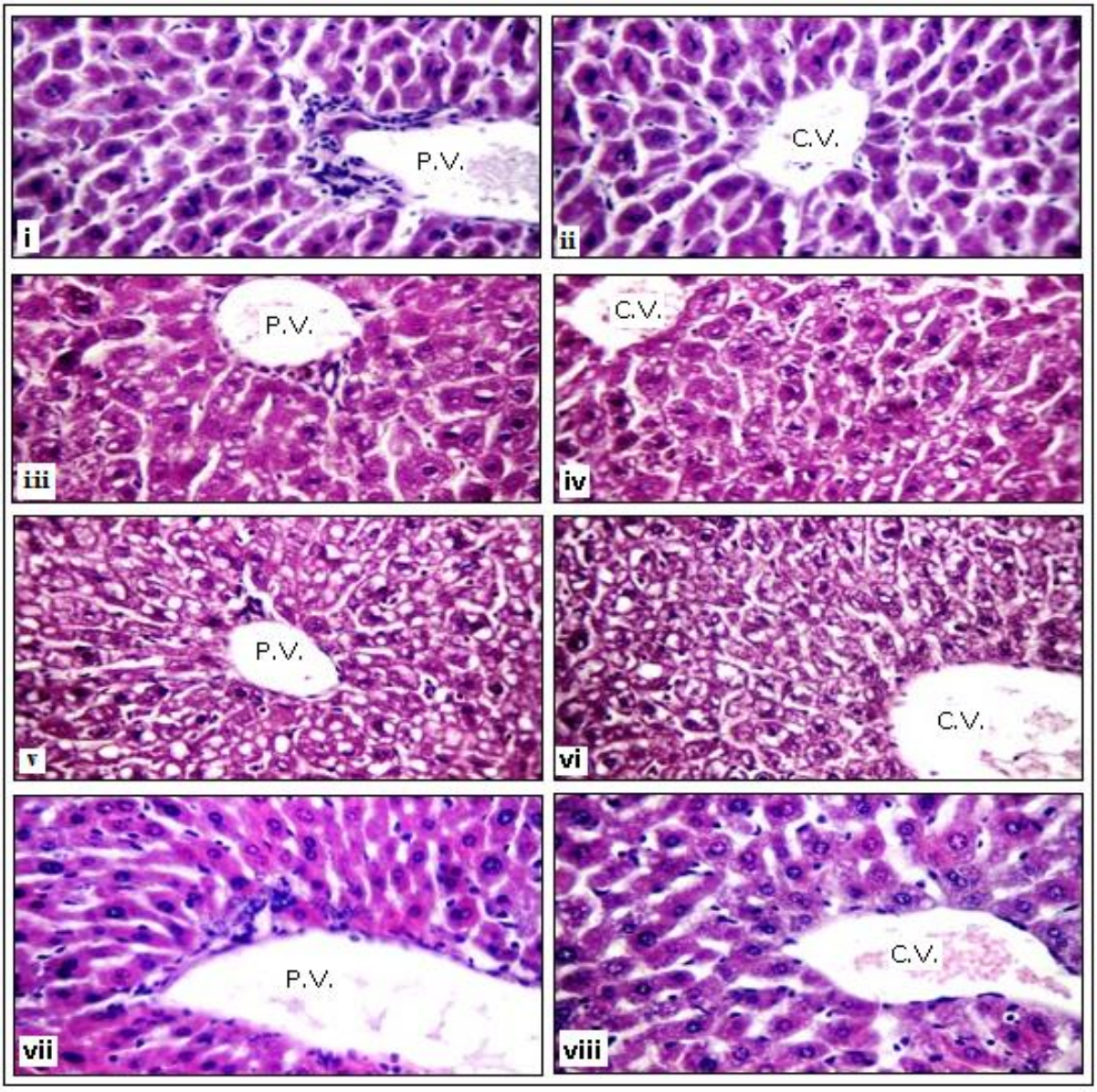

Fig. 2. Effects of *Fv* on HFD-induced histopathological changes in liver of mice. (i&ii): liver section from control and *Fv* groups respectively; (iii–vi): liver sections from HFD group, (vii & viii): liver sections from HFD + *Fv* group. cv: central vein; pv: portal vein.

Discussion

Fucus vesiculosus L. controls weight gain Weight gain is an apparent sign of fat accumulation. The significant increase in the body weight of the HFD group of mice reflects the uncontrollable rate of fatty acid synthesis. It is noteworthy to note that *Fv* controls the HFD-induced weight gain due to the effects of two of its active constituents; fucoxanthin and alginates. Accordingly, fucoxanthin, which is the major carotenoid found in the *Fv* extract helps in reducing body weight by upregulating the gene expression of the mitochondrial uncoupling protein 1 in white adipose tissue (Maeda *et al.,* 2005).

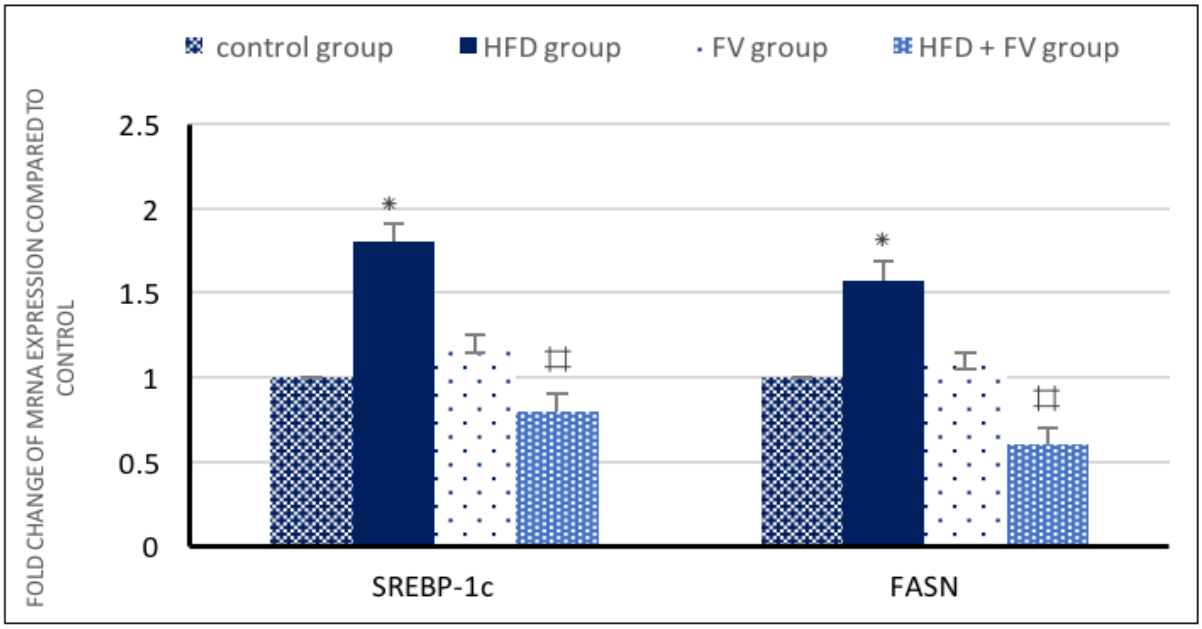

Fig. 3. Effects of *Fv* on the mRNA expression levels of SREBP-1c and FAS genes. Data are presented as the mean ± SD (n=10 mice from each group). **P*<0.001, compared to the control group. ⌗*P*< 0.001, compared to the HFD group.

This upregulation, in turn, leads to an increase in energy expenditure through uncoupling step during cellular metabolism. Fucoxanthin also plays a crucial role in fatty acids oxidation (Miyashita, 2009). Additionally, *Fv* was rich with alginic acid (reaching up to 59% of its dry weight), the most abundant polysaccharides which were normally present in the *Fv* cell wall's structure and contributing to its flexibility. Dietary alginates have been recognized with their valuable biological benefits in the gastrointestinal tract. They modulate the signals that control hunger and food intake and hence regulate appetite besides their regulating effect on the intestinal flow while stimulating the growth of favorable microbiota (Jensen *et al.,* 2012). This implied the effect of *Fv* on the prevention and/or management of obesity and metabolic syndrome due to the high percentage of alginates in its extract.

Fucus vesiculosus L. modulates HFD-induced biochemical alterations

Liver injury is one of the main consequences of ectopic fat accumulation. ALT and AST enzymes are among the sensitive biochemical markers of liver injury and the main glue of obesity and NAFLD development (Marchesini *et al.,* 2008). These enzymes are normally found inside liver tissue when tissues are injured, they are released into the bloodstream, which increases their serum levels.

Notably, *Fv* ingestion alleviated this liver injury by lowering serum enzymes level, reducing the number of fatty vacuolesin tissues and improving liver steatosis as well as the histological structure of hepatocytes. The porphyrin, free phenolic acids, and polysaccharides are among the hepatoprotective compounds found in the *Fv* extract.

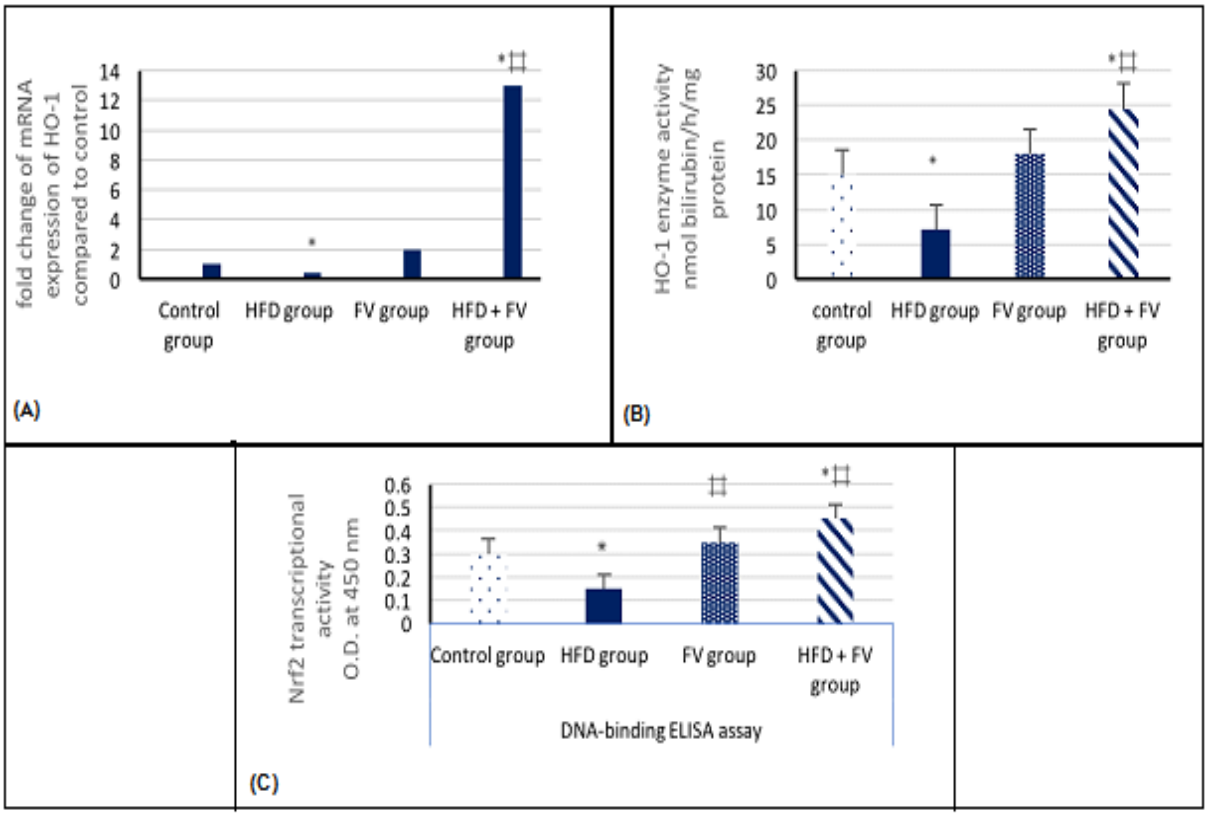

Fig. 4. Effects of *Fv* on the HFD-induced oxidative stress; mRNA expression level of HO-1 (A), hepatic HO-1 enzyme activity in mice (B) and hepatic Nrf2 transcriptional activity (C). Values are expressed as means ± SD (n=10). **P*<0.001, compared to the control group. ⌗*P*< 0.001 compared to the HFD group.

Abnormal lipid profile is the first hallmark specifying obesity and NAFLD. The current study showed that consuming HFD for 6 consecutive weeks trigger dyslipidemia. Notably, *Fv* supplementation reversed the HFD-induced lipid profile disturbances which would be ascribed to the presence of phlorotannins, omega-3 fatty acids, polyunsaturated fatty acids (PUFAs) and polysaccharides in its extract. Phlorotannins ingestion have the ability to suppress the cholesterol reabsorption in the enterohepatic circulation (Brown *et al.,* 2014). Omega-3 fatty acid reduces the serum TC, TGs and LDL-c levels *via* altering the cholesterol absorption and metabolism. Meanwhile, the relevance of PUFAsin hepatic lipid accumulation liesin its ability to decrease the very low-density lipoprotein (VLDL) levels (Catarino *et al.,* 2018). Moreover, the high Polysaccharides level of *Fv* renders this brown alga a good source of dietary fiber that contributes to the regulation of the blood cholesterol. This regulation, in turn, prevents the development of obesity, hypercholesterolemia and related metabolic syndrome (Brown *et al.,* 2014).

Lipogenic genes expression

SREBP-1c and FAS genes are two of the fatty acid synthesis-involved genes contributing to the development of obesity. Their abnormal expressions were greatly associated with body weight gain, TG-enriched fatty liver and therefore NAFLD progression. SREBP-1c, a well-recognized lipogenic transcription factor was involved in the biosynthesis of cholesterol, fatty acid and triglyceride in mammals and played a crucial role in regulating the transcription of its target lipogenic genes among which FAS gene (Horton *et al.,* 2002). The present results demonstrated that *Fv* ingestion in parallel with HFD downregulated the FAS gene expression *via* the SREBP-1c dependent mechanism. These findings are not in accordance with (park *et al.,* 2016), who reported that fucoidan extracted from *Fv* didn't affect the mRNA expression level of SREBP-1c. This verifies that the regulatory impact of *Fv* on

lipogenesis would be attributed to the synergistic effects of all its components.

Besides the direct hepatotoxic effect of free fatty acid, its overload induces reactive oxygen species (ROS) production. ROS overproduction-inducing lipid peroxidation resulted in mitochondrial dysfunction and contributed to the hepatocellular damage. Conversely, *Fv* ingestion along with HFD alleviate the endogenous lipid peroxidation *via* reducing the MDA and boosting the TAA levels. The antioxidant activity of *Fv* would be ascribed to the free radicals scavenging ability of its active constituents such as iodine, vitamins, and fucoidans. The inorganic iodine neutralized hydrogen peroxide by converting it first to hypoiodous acid and then to water (Bogolitsyn *et al.,* 2014). Furthermore, Vitamin C participates in the ascorbate-glutathione cycle to reduce H_2O_2 to water (Martínez and Araya, 2010) and contributes to the recycling of tocopherol (vitamin E). *Fv* was the richest seaweed *sp* with Vitamin E (600 mg/kg dry weight), which was involved in the prevention of lipid oxidation and protection of the membranes from reacting with ROS (Niki, 2014). Meanwhile, fucoidans act either by scavenging ROS or stimulating the activity of cellular endogenous antioxidant defense enzymes (Brown *et al.,* 2014).

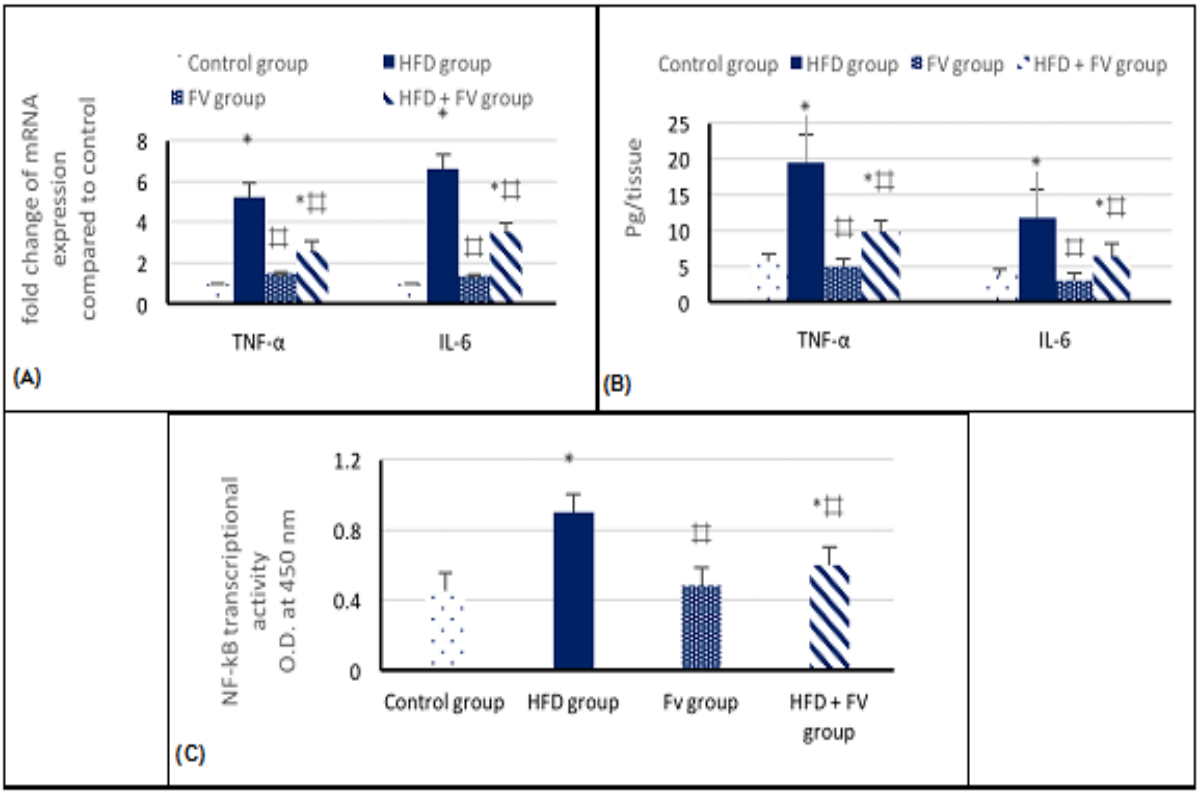

Fig. 5. Effects of *Fv* on the HFD-induced inflammation; mRNA expression levels of TNF-α and IL-6 (A), hepatic contents of TNF-α and IL-6 in mice (B) and hepatic NF-κB transcriptional activity (C). Values are expressed as mean ± SD (n=10). **P*<0.001, compared to the control group. ⌗*P*< 0.001, compared to the HFD group.

Fucus vesiculosus L. *modulates HFD-induced oxidative stress*

In the present study, HFD consumption was shown to downregulate the mRNA expression level of HO-1 with a concomitant decrease in its activity in liver tissue. HO-1 which was largely involved in protecting the liver against oxidative stress was induced by the vital transcription factor Nrf2 upon its translocation to nucleus and binding to ARE (Shin *et al.,* 2009). *Fv* supplementation restores these downregulations based on its active constituent, fucoxanthin. The protective effect of fucoxanthin against oxidative stress-related lipid peroxidation and DNA damage was accomplished by up-regulating the Nrf2/ARE

binding rate and its down-stream phase II anti-oxidant enzymes (Peng *et al.,* 2011). These upregulations, in turn, inhibit the expression of different pro-inflammatory mediators such as TNF-α, IL-1β, IL-6 and, NF-κB. It is noteworthy to note that FAS is among genes which are largely repressed by Nrf2 transcriptional activation (Shin *et al.,* 2009), which supported our findings of interdependence between FAS downregulation and the transcriptional factor Nrf2 activation.

Fucus vesiculosus L. alleviates HFD-induced inflammation

Free fatty acids accumulation trigger inflammation *via* activating NF-κB pathway. In normal conditions, NF-κB binds with its specific inhibitor (IkB) in the cell cytosol. In response to fat-accumulation-inducing oxidative stress, NF-κB was activated, translocated to the nucleus and stimulated the transcription of an array of inflammatory-involved genes, among which TNF-α and IL-6 (Ellullu *et al.,* 2017). Obviously, *Fv* ingestion in parallel with HFD downregulated the mRNA expression levels of TNF-α and IL-6 and hence abolishing their inducing effect for NF-κB transcriptional activation along with translocation to the nucleus. Fucoidans and phlorotannins are among the bioactive constituents of *Fv* responsible for its anti-inflammatory activity (Catarino *et al.,* 2018). Ingestion of fucoidans from *Fucus* sp. exhibited promising inhibitory effects against important pro-inflammatory signaling pathways, through suppression of nuclear factor-κB (NF-κB), protein kinase B (Akt), c-Jun *N*-terminal kinase (JNK), and p38 mitogen-activated protein kinase (p38-MAPK) (Park *et al.,* 2011). Additionally, Phlorotannins are a group of marine exclusive phenolic compounds abundant in brown seaweeds. They have been described for their good anti-inflammatory effects by counteracting various pro-inflammatory mediators including several cytokines such as IL-1β, IL-6, IL-17, and TNF-α, as well as chemokines such as monocyte chemoattractant protein-1(MCP-1), iNOS and COX-2 enzymes. These results illuminate the potent protective impact of *Fv* against liver injury on HFD-induced obesity in mice *via* its anti-inflammatory activity.

Conclusion

In conclusion, *Fucus vesiculosus L.* (Bladderwrack) has a great impact on HFD-induced liver injury. It controls body weight gain, regulates liver enzymes and lipid profile, improves the liver steatosis. Moreover, it regulates lipogenic genes expression. *Fv* can modulate oxidative stress and pro-inflammatory events, the two interdependent pathophysiological processes induced by high-fat consumption and responsible for the onset of many diseases, including NAFLD. Indeed, *Fv,* with its praise-worthy bioactive constituents can be considered as a new promising gift from nature for NAFLD treatment and obesity management.

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