Scientific Research Publishing

World Journal of Nuclear Science and Technology, 2017, 7, 189-205
http://www.scirp.org/journal/wjnst
ISSN Online: 2161-6809
ISSN Print: 2161-6795

Response of COX2/PGE2 Inflammatory Pathway to Brown Seaweed Extract in Rats Exposed to Gamma Radiation

Khaled Sh. Azab[1*], Nefissa H. Meky[2], Eglal A. M. El-Deghidy[1], Ghada Azoz[1]

[1]Radiation Biology Department, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Cairo, Egypt
[2]Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt
Email: *khsha_65@hotmail.com

How to cite this paper: Azab, Kh.Sh., Meky, N.H., El-Deghidy, E.A.M. and Azoz, G. (2017) Response of COX2/PGE2 Inflammatory Pathway to Brown Seaweed Extract in Rats Exposed to Gamma Radiation. *World Journal of Nuclear Science and Technology*, **7**, 189-205.
https://doi.org/10.4236/wjnst.2017.73016

Received: May 7, 2017
Accepted: July 7, 2017
Published: July 10, 2017

Abstract

Background: Systemic inflammation due to radiation exposure has been identified in a biological system by certain metabolic and behavioral disorders. These anarchies mostly mediated under a regulation of cyclooxygenase 2 (COX2) induced production of an inflammatory mediator prostaglandin E2 (PGE2). Aim: This study was undertaken to investigate the anti-inflammatory impact of brown sea weed extract (BSWE) against induction of COX2/PGE2 inflammatory pathway in gamma-irradiated rats. Rats were orally administrated with BSWE (27 mg/kg body weight/day) for 7 consecutive days before exposure to 8 Gy fractionated gamma radiation (2 Gy × 4; every 3 days). Treatment with BSWE was extended along with and in-between irradiation doses for another 14 successive days. Our data demonstrated that the administration of BSWE to rats exposed to gamma radiation, following the regimen suggested, significantly neutralize the changes induced in the inflammatory molecules COX2, PGE2, tumor necrosis alpha (TNF-α), and nitric oxide (NO). In addition, it adjusted significantly the cellular redox tone via regulation of changes induced in malondialdehyde (MDA) reduced glutathione (GSH), superoxide dismutase (SOD) catalase (CAT) and xanthine oxidoreductase system (XOR). Credibly, from the results emerged in this study, it could be suggested that BSWE has substantial anti-inflammatory activities and gamma radiation protection capabilities. It is recommended to include BSWE in the treatment strategy of various inflammatory diseases especially cancer as a safe natural anti-inflammatory agent.

Keywords

COX2, PGE2, Brown Seaweed Extract, Systemic Inflammation, Gamma Irradiation

1. Introduction

Inflammation is part of the body's immune response. It happened when something harmful or irritating affects a part of the body and there is a biological response to try to remove it. The signs and symptoms of inflammation, specifically acute inflammation, revealed that the body is trying to heal itself. Inflammation does not mean infection, even when an infection causes inflammation. Infection is caused by a bacterium, virus or fungus, while inflammation is the body's response to it including the local reactions and the destruction changes; the destruction was affected by brown seaweed extract and the responses that lead to repair and healing [1]. Thus, inflammation is a fundamental pathologic process consisting of a dynamic complex of histological apparent, infiltration, cellular infiltration and mediator release occurred in affected blood vessels and adjacent tissues, also, in abnormal stimulation caused by physical, chemical, or biologic agent [2].

Ionizing Radiation (IR) induces beneficial, as well as possible harmful effects to human population, representing one of the most important physical causes of inflammatory responses [3]. Ionizing radiation is known to induce oxidative stress through generation of Reactive Oxygen Species (ROS) resulting in imbalance of the pro- oxidant, and antioxidant in the cells, which is suggested to culminate in cell death [4]. It activates both pro- and anti-proliferative signal pathways altering the homeostatic balance between survival and cell death regulated by several genes and factors involved in cell cycle progression, repair, inflammation and cell death induction [5]. Activation and production of TNF-α and other cytokines are contributed to the inflammatory cascades after radiation exposure and ROS generation [6].

The process of inflammation comprise activated inflammatory cells (neutrophils, eosinophils, monocytes, phagocytes, and macrophages)which secrete increased amount of inflammatory molecules such as nitric oxide (NO), and tumor necrosis factor (TNF-α) as well as Prostaglandin E2 (PGE2); another important inflammatory mediator produced from arachidonic acid metabolites by a reactions promoted by enhanced COX2 enzymatic system [7].

In particular, uncontrolled inflammatory responses certainly pave to serious inflammatory diseases. So, it is important to hypothesize diverse strategies to cope with cascades of incontinent inflammation. The use of the herbal medicine has become a useful approach due to the anti-inflammatory properties in conjunction with low toxicity risk. A variety of plants and seaweed have traditionally been used in oriental folk medicine to treat inflammatory diseases.

A diet rich in marine products is considered to result in a lower incidence of certain inflammatory diseases like cardiovascular disease, diabetes, cancer, and stroke. These protective effects are attributed to nutraceuticals contained in marine foods [8].

The present study is interested in Marine algae (seaweed) exacting the brown seaweed. Microalgae brown seaweed is photosynthetic like plants that lack many of the distinct organs found in terrestrial plants, and whole seaweed plants are

available as a biomass resource. Brown seaweed contains carotenoid present in the algae chloroplasts and fucoxanthin is the major carotenoid. The antioxidant properties present in BSWE might be attributed to its carotenoid (fucoxanthin) potent free radicals scavenging capacity [9].

Following to the previous, in the present study we interested in evaluating of BSWE impact on the COX2/PGE2 inflammatory pathway in irradiated albino rats as well as the role of oxidative imbalance.

2. Material and Methods

2.1. BSWE

Fucoidan, products of Pacific standard distributor were purchased from maxwell health, USA. A bottle contains 60 capsules; each capsule contains 300 mg brown seaweed aqueous extract. A dose for human health maintaining (one capsule; 300 mg/day) was extrapolated to rat dose (*After to Paget and Baurns* 1964). The appropriate concentration was dissolved in distilled water and administrated by gavages to animals (≅27 mg/kg/day).

2.2. Animals

Male Wistar albino rats (weighing 100 - 120 g) were obtained from the animal farm of the Egyptian Holding Company for Biological Products and Vaccines, Egypt. Upon arrival, the animals were allowed to acclimatize for 1 week before starting the experiment. Food and water was available throughout the experiment to *ad libitum*.

2.3. Animal Welfare

Animal experimentation was consistent with the guidelines of Ethics by the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) in accordance with the recommendations for the proper care and use of laboratory animals approved by animal care committee of the National Center for Radiation Research and Technology (NCR RT), Cairo, Egypt.

2.4. Irradiation

Whole body gamma-irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt, using (137 cesium) Gamma cell-40 biological irradiator. Animals were housed in a special designed cage and the cage was inserted in the specified place of the gamma cell. The ventilation system of gamma cell was running before the cage was inserted into cell. The dose rate of the gamma source was 0.46 Gy/min. Animals were not anesthetized before the first dose of gamma radiation.

2.5. Experimental Design

The experiment duration divided into two stages, (pre-irradiation stage) 7 days before irradiation and the other 14 days (radiation exposures stage) started post exposure to the 1st dose of gamma irradiation until the end of the experiment.

Rats were divided into 4 groups (n = 6). Group I (control group, C) neither included rats neither treated nor irradiated. Rats of this group are received orally an equivalent volume of distilled water (vehicle of BSWE) during the period of BSWE administration. Rats in group II (Irradiated group, R) were exposed to 4 fractions of gamma radiation (2 Gy dose every 3 days) and rats of this group received orally an equivalent volume of distilled water during the period of BSWE administration. In group III (BSWE group), rats were received 27 mg/kg/day aqueous extract of brown seaweed via oral tube along the experimental period. Group IV (BSWE + R) included rats that were administered 27 mg/kg/day BSWE aqueous extract orally for 7days before gamma irradiation, and the administration of the extract was extended during radiation exposure period (14 days) Rats were immolated on the1st and 7th days post last radiation fraction and subjected to liver and blood biochemical analysis.

2.6. Biochemical Assays

After an overnight fast, rats were anesthetized with diethyl ether and then immolated via cervical dislocation. The liver was directly separated and washed in ice-cold saline then the liver samples were homogenized in double distilled water (10% W/V) using homogenizer then the cell debris was removed by centrifugation at 3000 rpm for 10 min. The homogenates supernatant were subjected to the following biochemical analysis.

COX-2 enzyme activities were detected spectrophotometrically in cell lysates and tissue extracts. Cyclooxygenases catalyze the synthesis of prostaglandins from arachidonic acid. The COX component converts arachidonic acid to Prostaglandin G2 (PGG2) whereas the peroxidase component reduces the PGG2 to the corresponding alcohol, PGH2. In this kit the TMPD serves as a reducing agent, TMPD gets oxidized, so electrons flow from the TMPD to PGG2 and the appearance of the oxidized TMPD is monitored at 590 nm. The rate of increase in absorbance was taken for calculating the activity of the enzyme [10].

PGE2 concentration was assessed by using multiple steps standard assay Prostaglandin E2 (PGE2) in vitro competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit which designed for the accurate quantitative measurement of Prostaglandin E2 in serum, saliva, urine and tissue culture media and other biological fluids. A mouse IgG antibody has been pre coated onto 96-well plates. Standards or test samples are added to the wells, along with an alkaline phosphatase (AP) conjugated-Prostaglandin E2 antibody. After incubation, the excess reagents are washed away and para-Triphenylphosphate (pNpp) substrate is added and is catalyzed by AP to produce a yellow color. The intensity of the yellow coloration is inversely proportional to the amount of Prostaglandin E2 captured in the plate [11].

The serum TNF-α (Rat) (pg/ml) was measured by a solid phase sandwich Enzyme Linked- immuno-Sorbent Assay (ELISA) kit (BMS 622, Vienna, Austria). A monoclonal antibody specific for Rat TNF-α has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Rat TNF-

α content, control specimens, and unknowns, are pipetted into these wells.

Nitric oxide (NO) was measured (nmole/g wet tissue) as a stable end product, nitrite, according to the method of Miranda *et al.* [12]. The assay is based on the reduction of nitrate by vanadium trichloride combined with detection by the acidic Griess reaction. The diazotization of sulfanilic acid with nitrite at acidic pH and subsequent coupling with N-(10 naphthyl)-ethylenediamine produced an intensely colored product that is measured at 540 nm.

Lipid peroxidation product, malondialdehyde (MDA), was measured (nmole/g wet tissue) by thiobarbituric acid assay, which is based on MDA reaction with thiobarbituric acid forming thiobarbituric acid reactive substances (TBARS), a pink colored complex exhibiting a maximum absorption at 532 nm [13].

Xanthine oxidoreductase system (XOR), including XD and XO activities (U/mg protein), were assayed by measurement of uric acid formation in the presence or absence of NADP at 37˚C, as described by Waud and Rajagopalan [14]. XO and XD activities were expressed as nmol uric acid formed per mg protein.

Superoxide dismutase (SOD) activity was assayed via the method of Kakkar *et al.* [15], based on the inhibition of superoxide ions generated by phenazine methosulfate that converts nitroblue tetrazolium (NBT) to NBT-diformazan, which absorbs light at 560 nm. SOD activity was defined as the amount of enzyme required to give 50% inhibition of NBT reduction and expressed as units/min/mg protein.

Catalase (CAT) activity was assayed using the method of Sinha ([16], based on the utilization of H_2O_2 by the enzyme. One unit of the enzyme is expressed as mmoles of H2O2 utilized per minute per mg protein.

The GSH content was determined photometrically at 412 nm using 5, 5-dithiobis-2-nitrobenzoic acid [17].

2.7. Statistical Analysis

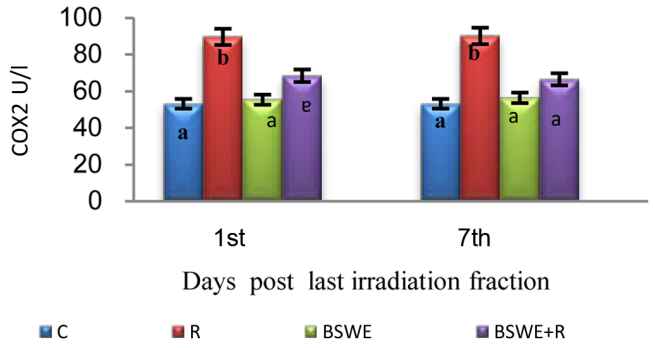
Statistical analysis was performed using one-way analysis of variance (ANOVA) and post hoc Duncan test using the Statistical Package for the Social Sciences (SPSS) Version 15.0 for windows. The values are mean ± S.E. (n = 6). Differences between groups were considered statistically significant at $P < 0.05$.

3. Results

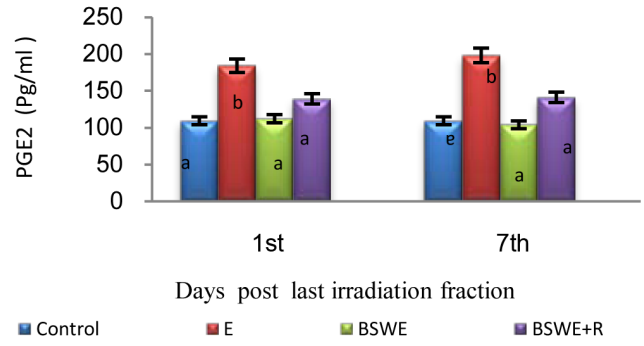
To achieve the goals of the present study, we have executed the following cohesive biochemical assessments: 1) Inflammatory response signs (NO, TNF-*α*, COX2, and PGE2), 2) Pro oxidant indicator and ROS generator system (MDA and XOR system) 3) Atioxidants parameters (GSH, SOD, and CAT).

3.1. Inflammatory Signs

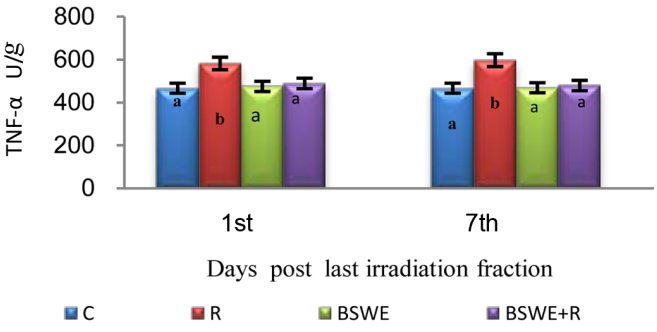
Data for inflammatory parameters were illustrated by **Figures 1(a)-(d)**. The administration of SW to normal rats does not induce any significant changes in COX2, and PGE2, TNF-*α*, and NO concentration comparing with control rats



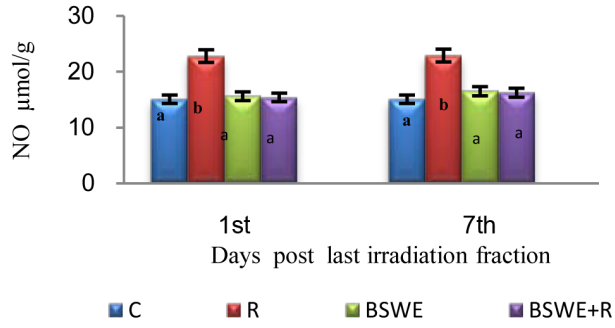
(a)



(b)



(c)



(d)

Figure 1. Impact of BSWE and/or gamma irradiation on the inflammatory profiles; a) activities of COX2 U/l homogenate b) concentration of PGE2 pg/ml homogenate c) a concentration of TNF-α U/g fresh tissue and d) NO concentrations µmol/g fresh tissue.

along the two-time intervals Figures 1(a)-(d). In regard to COX2, PGE2 and TNF-α, the exposure of rats to gamma radiation significantly increase the activity of COX2 and the concentrations of PGE2 and TNF-α as compared to their equivalents in controls. Also, exposure of rats to gamma radiation significantly increased NO concentrations in liver tissue and this increase was genuinely marked on the second interval. In addition, and according to our collected data the administration of 27 mg/kg body weight/day BSWE for 7 consecutive days before starting exposure to gamma radiation and for 14 successive days along the period of exposure to fractionated gamma radiation (2 Gy × 4 every other 3 days) persuade significant amelioration ($P < 0.05$) in all parameters comprise our chosen inflammatory profile Figures 1(a)-(d).

3.2. Pro Oxidant Indicator and ROS Generator System (MDA and XOR System)

Activities of XO and XDH, as well as the concentration of lipid peroxidation end product (MDA), were illustrated by Figure 2. We observed that there are no notable changes in XO, XDH activities and MDA concentration in a group of rats received several successive doses of BSWE according to the present experimental protocol (Figures 2(a)-(c)). The exposure of rats to fractionated gamma radiation (2Gy × 4) every other 3 days induced significant increases ($P < 0.05$) in the activities of XO along the 2 experimental intervals (Figure 2(a)). The activity of XDH exhibited significant decline ($P < 0.05$) as compared to normal control along the 2 experimental times (Figure 2(b)) in addition to significant increases ($P < 0.05$) in the concentration of MDA (Figure 2(c)). Administration of BSWE to rats exposed to fractionated gamma radiation brings on outstanding improvement in the status of XOR system as well as the liver pro- oxidant indicator (MDA concentration) Figures 2(a)-(c).

3.3. Antioxidant Parameters

The antioxidant manifestation (CAT, SOD and GSH) were illustrated by Figures 3(a)-(c). Figures 1(a)-(c) demonstrated that the administration of BSWE to normal rats according to the regimen followed in the present study does not bring any significant changes in these antioxidant considerations.

However, the exposure of rats to fractionated dose of gamma radiation (2Gy x4; 2Gy every other 3 days) significantly ($P < 0.05$) decreased the liver CAT activities on the 1st and 2nd intervals comparing to normal control rats (Figure 3(a)). Data of SOD activities pointed out to significant increases in the 1st interval followed by a significant decrease on the 2nd experimental hiatus (Figure 3(b)). The concentration of GSH was decreased significantly ($P < 0.05$) at the 2 times intervals of the experiment (Figure 3(c)). In group of rats received SW treatments and exposed to fractionated gamma radiation, the activities of antioxidant enzymes SOD and CAT as well as the GSH concentration are significantly ameliorated when compared with its equivalent values in irradiated rats (Figures 3(a)-(c)). Furthermore, we have been observed that, the activities of

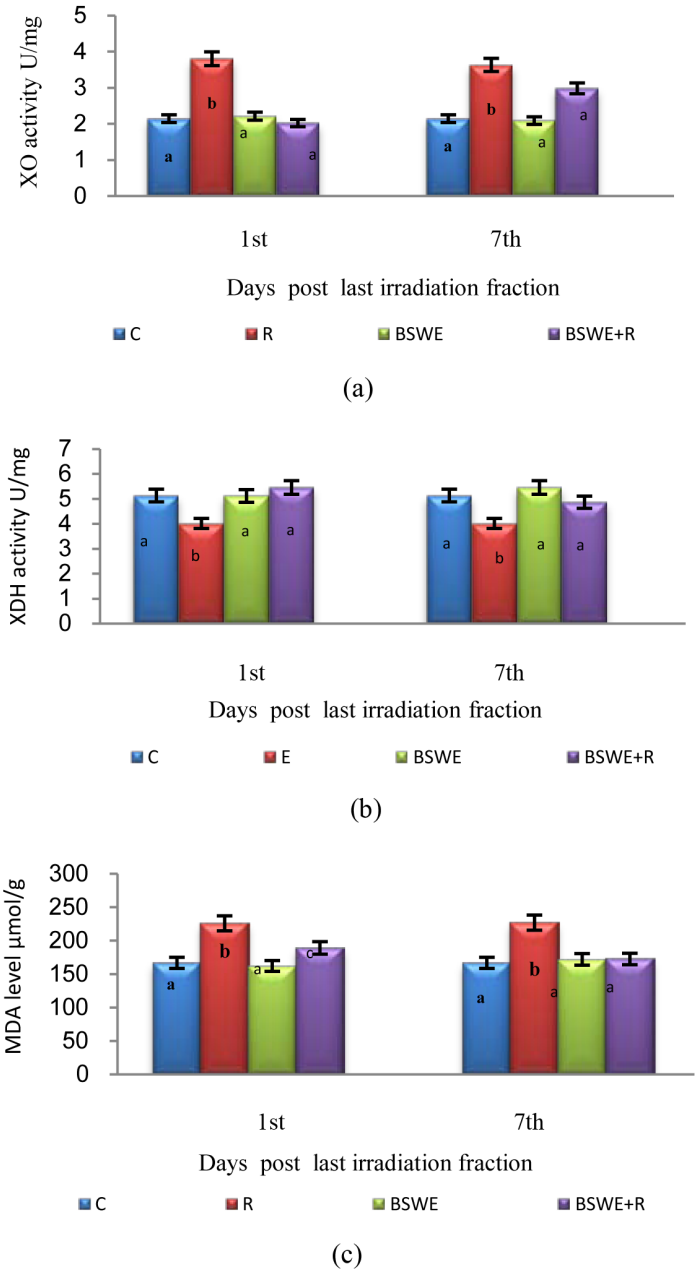


Figure 2. Impact of BSWE on the liver XOR system (U/mg Fresh Tissue) and MDA concentration (µmol/g fresh tissue) in irradiated rats.

SOD and CAT in addition to GSH concentration are matched to their values in normal control.

4. Discussion

In order to evade the development of serious inflammatory complications, we have to shed light on the mechanisms of inflammation targeting the suggestion of adequate solutions which includes the using of different anti-inflammatory agents preferring the natural nonsteroidal anti-inflammatories. Alterations to COX2 expression and the abundance of its enzymatic products PGE2 (COX2/

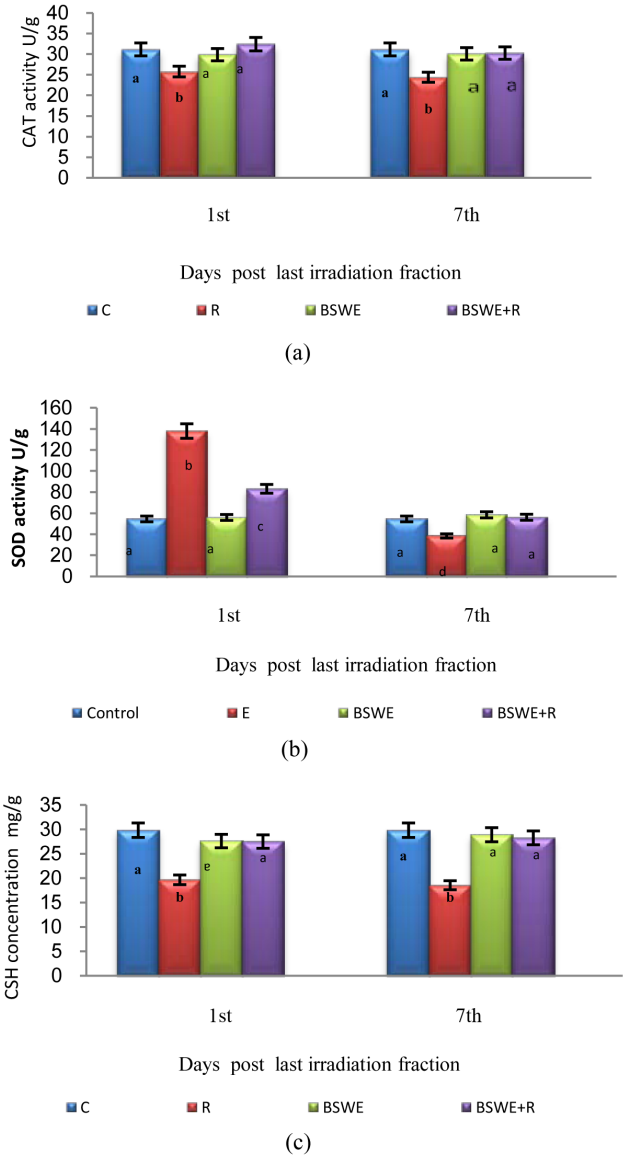


Figure 3. Impact of BSWE on a liver antioxidant system, activities of CAT (U/g Fresh Tissue), and SOD (U/mg Fresh Tissue) and concentration of GSH concentration (mg/g fresh tissue) in irradiated rats.

PGE2 pathway) have key roles in developments of many serious inflammatory diseases [18]. In the present study, significant increases in COX2 activities and PGE2 concentrations were observed in rats exposed to fractionated gamma radiation 8 Gy; 2 Gy × 4 every other 3 days) (**Figure 1(a)** & **Figure 1(b)**). The COX2 enzymatic activity upregulation could be attributed to the activation of several TNF-α dependent intracellular signaling pathway which plays a key role in the control of COX-2 induction. Nam *et al.* [19] stated that different protein kinases and transcriptional factors that are greatly regulated by TNF-α are contributed to COX2 upregulation. The expression of COX2 mRNA and protein is often enhanced in various human cell types by inflammatory cytokines such as interleukin-1beta (IL-1beta) and TNF-α [20]. Further, TNF-α induced PGE2 release that

caused pain and support inflammatory process is mediated by activation of COX2 transcription. Arachidonic acid is transformed into PGE2, important lipid mediators of numerous physiological and pathophysiological processes via cyclooxygenase (COX) enzymes and terminal prostaglandin E synthases (PGES) [21]. Likewise, PG syntheses can be stimulated in response to distinct pathophysiological situations, including inflammation, pain, and cancer. Undoubtedly, prostaglandin E2 (PGE2) is a principal mediator of inflammation in diseases. The nonsteroidal anti-inflammatory medications (NSAIDs) and selective cyclooxygenase-2 (COX-2) inhibitors reduce PGE2 production to diminish the inflammation seen in these diseases [22]. Subsequently, inhibition of COX-2 expression and blocking of pro-inflammatory cytokines (TNF-alpha, production are likely included in the anti-inflammatory process. In addition, as the interaction of various enzymes in the PGE2 synthetic pathway is complex and tightly regulated, the potential of targeting PGES is a more precise strategy for inhibiting PGE2 production [19].

In the same issue, Ionizing radiation (IR) activates both pro-and antiproliferative signal pathways producing an imbalance in cell Fate decision. Our data reveals a kind of these imbalances manifested by significant increases in TNF-α protein expression as well as in the concentration of proinflammatory mediator NO in rats after gamma radiation exposure (**Figure 1(c)** & **Figure 1(d)**). Tissue fixed macrophages, such as liver Kupffer cells, are believed to be major sources of TNF-α. However, other cell types, including endothelial cells, epithelial cells, monocytes, T-cells, smooth muscle cells, adipocytes, and fibroblasts, secrete significant amounts of TNF-α when exposed to the appropriate stimuli [23]. Schaue *et al.* [24] reported that exposure to ionizing radiation has a significant effect on the immune system modulation through the activation of cytokine cascades. The reported variation in TNF-α concentration along the 2 experimental times could be interpreted in the light of cytokine production is time-dependent, peaking usually at 4 - 24 hrs after irradiation with a subsequent decrease to baseline levels within 24 hrs to a few days [25]. Furthermore, enhancement of TNF-α release in response to exposure to gamma radiation as recorded in the present study could participate in the induction of NO release. Nitric oxide (NO) is a signaling molecule undergoes over production in abnormal situations and plays a key role in the pathogenesis of inflammation [26]. The increased NO generation could be attributed to radiation stimulation of inducible nitric oxide synthase (i-NOS) [27]. Multiple reports demonstrate that TNF-α regulated NOS (Nitric oxide synthases) expression and/or increased activity directly affect NO production [28]. TNF-α markedly augmented the expression of iNOS which is, an enzyme not normally produced in most cells. It is provoked and presented under certain conditions [29]. The inducible (calcium-independent) isoform (iNOS) produces much larger amounts of NO and is only expressed during inflammation [30]. It was demonstrated that iNOS, activated by pro-inflammatory cytokines, can radiosensitize tumor cells through endogenous production of NO [31]. The generation of NO and reactive nitrogen species

(NO/RNS) by iNOS is a critical feature of the inflammatory environment [32]. Worth mention, in the study of Gorbunov *et al.* [33] the induction of iNOS expression and iNOS-dependent release of nitric oxide in bone marrow stromal cells was observed within 24 h after irradiation and were similar in magnitude to that observed in cultures incubated with IL-1β and TNF-α.

One of the most important factors affecting the systemic inflammation in response to radiation exposure is cellular redox status. Our previous researchers pointed to alteration in redox status post radiation exposure perceptible by an accumulation of lipid peroxidation products MDA (measured as TBARS) and antioxidant decline [34] [35]. The plenty of ROS production and/or decreased antioxidant activity results in this deleterious status is comprehensible. Side-effects of radiation are linked to the imbalance between ROS and the antioxidant defense system. Also, Rao *et al.* [36] stated that ROS have been implicated in the induction and complications of many diseases.

In this study, exposure of rats to fractionated whole body gamma radiation produce significant increases of TBARS (**Figure 2(a)**), alteration in XOR system and significant decrease in antioxidants system (GSH, CAT, and SOD) (**Figures 3(a)-(c)**). Thiobarbituric reactive substances (TBARS), one of the important indices of oxidative stress appeared due to overproduction of ROS that elicit vigorous lipid peroxidation process ended with an accumulation of thiobarbituric reactive substances; malondialdehyde (MDA) [37]. Important sources of ROS as both signaling molecules and mediators of inflammation in the living cell are include mitochondria, NAD (P)H oxidase, cyclooxygenase (COX), lipo-oxidase (LOX), xanthine oxidase (XO), cytochrome P450 (CYP450) as well as a number of other physiologically relevant systems [38]. Our results reveal significant changes in XOR system (constitute of 2 inter convertible enzymes) represented by significant increases of XO associated with significant decrease of XDH in irradiated rats (**Figures 2(a)-(c)**). This could interpret the oxidative stress elicited in the cells after radiation exposure where the XO, an enzyme react with molecular oxygen and produce ROS, is plentiful over XDH an enzyme form dominant in normal conditions [33]. In addition, ROS such as superoxide radicals can rapidly combine with NO present in abundant as that reported in our data (**Figure 1(d)**) to form reactive nitrogen species (RNS). RNS, in turn, induces nitrosative stress, which adds to the pro-inflammatory burden of ROS. The decline occurred in antioxidant enzymatic activities (SOD and CAT) and also a nonenzyme antioxidant (GSH) participated in oxidative stress emerged after radiation exposure. The antioxidant system frustration might be due to lose a lot of GSH molecules in ROS neutralization process and/or denaturing of enzymatic antioxidant protein molecules attacked by free radicals. Wu *et al.* [39] reported that bursts of ROS and RNS may affect directly or indirectly proteins/genes that participate in oxidative metabolism. Largely, our data drive us to propose that oxidative stress is responsible for the induction of inflammatory cytokines where the alteration in cellular redox tune is accompanied with an increase in TNF-α after irradiation (**Figure 1(c)**). Shah and Channon [38] stated that oxidative stress can

activate a variety of transcription factors leading to the expression of over 500 different genes, including those for growth factors, inflammatory cytokines, chemokines, cell cycle regulatory molecules, and anti-inflammatory molecules. Among the commonly used inflammatory mediators to stimulate inflammation are cytokines (e.g., TNF-α) which induce only a subset of changes that are associated with full-blown inflammation [40].

In this study, we aimed to regulate the systemic inflammatory responses induced after radiation exposure by injection of adequate, adjustable doses of BSWE to irradiated animals. Targeted a successful anti-inflammatory process we have to include the inhibition of COX2 expression and blocking of pro-inflammatory cytokines in our practical protocol.

Analysis of our data could drive us to a perception that BSWE might revise balances between pro-inflammatory and anti inflammatory cytokines. In rats exposed to gamma irradiation and received several doses of BSWE, a significant amelioration of TNF-α and NO concentrations were observed (**Figure 1(a)** & **Figure 1(b)**). The modulatory action of BSWE on the concentrations of TNF-α could be exerted via the intervention of pathways participate in the induction of TNF-α, and NO production. Mhadhebi *et al.* [41] stated that, seaweeds are an excellent source of bioactive compounds polyphenols polysaccharides mero terpenoids and terpenoids which demonstrated a broad range of biological activities such as anti-inflammatory. Fung *et al.* [42] reported that fucoxanthin, a brown seaweed pigment (xanthophylls) found in most brown seaweeds has a molecule structurally similar to beta-carotene and vitamin A. Fucoxanthin is able to suppress the inflammatory response as assessed by NOS and COX-2 induction as well as cytokine and nitric oxide secretion [43]. Further, recent reports revealed seaweeds to be a rich source of antioxidant compounds [44]. The fucoxanthin inimitable structure carotenoids molecule could be responsible for the antioxidant activity of BSWE identified in our work by amelioration of SOD, CAT activities and GSH concentration as well as decreases in MDA (**Figure 2(a)**). It could be mention that adjustment of cellular redox tone might lead to ending pathways of TNF-stimulation and NO production related cascades. Moreover, Low molecular weight Fucoidan and fucoxanthin both appeared to reduce the level of pro-inflammatory mediators, including IL-1β and TNF-α via the inhibition of NF-κB activation. It seems that LMF, HS-Fucox, and LMF + HS − Fucox were trying to balance the immune disorder under LPS-induced inflammation [45]. Also, fucoidan downregulates the expression of pro-inflammatory genes involved in the synthesis of NO, PGE2, TNF-α and IL-1β by suppressing NF-κB activity [46].

Many carotenoids do not show direct free radical scavenging properties (β-carotene, β-cryptoxanthin, zeaxanthin, and lutein) making fucoxanthin somewhat unique in this regard, and the metabolite known as fucoxanthin appears to be more potent where fucoxanthin appears to scavenge free radicals with potency slightly less than vitamin C [47].

Overall, our data indicated that BSWE treatment significantly inhibited exces-

sive production of nitric oxide (NO) and prostaglandin E_2 (PGE_2) in gamma-irradiated rats. It also attenuated activation of COX2, and pro-inflammatory cytokines (**Figure 1(a)-(c)**). It seems that BSWE revising imbalances induced after irradiation between proinflammatory mediators and anti-inflammatory molecules appeared in our results through the down-regulation of TNF-α and NO in association with amelioration of antioxidants SOD, CAT, and GSH) could be contributed to attenuation of the inflammatory path COX2/PGE2. Pawel [48] declared that overproduction of PGE2 in response to pro-inflammatory molecules is associated with up-regulation of COX-2. In most physiologic conditions, the rate of PGE2 synthesis is controlled by local expression and activity of COX2.According to our data, inhibition of COX2 seemed to be a good candidate to regulate systemic inflammation after radiation exposure. It was affirmed that, mRNA of iNOS and COX-2 were affected by brown seaweed extract (fucoxanthin). Expression of TNF-α protein was also suppressed by fucoxanthin along with its mRNA [43].

Putting together all data obtained we can conclude that brown seaweed extract possesses a good potential against radiation-induced systemic inflammation via revising imbalance between pro-inflammatory mediators and anti-inflammatory factors manifested by inhibition of COX2/PGE2 inflammatory signal. It could recommend using brown seaweed extract as an adjuvant in therapeutics protocols that includes exposure to ionizing radiation.

Acknowledgements

Authors wish to express great appreciation to Radiation Biology Department, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority for introducing all equipments and chemicals used in this study.

Conflict of Interest

The authors declare they have no conflict of interest with the present study.

References

[1] Firestein, G.S. (2011) Mechanisms of Inflammation and Tissue Repair. In: Goldman, L. and Schafer, A.I., Eds. *Cecil Medicine*, 4th Ed., Chap. 47, Saunders Elsevier, Philadelphia, Pa.

[2] Vijayalakshmi, M., Kiruthika, R., Bharathi, K. and Ruckmani, K. (2015) Phytochemical Screening by LC-MS Analysis and *in Vitro* Anti-Inflammatory Activity of *Marselia quadrifolia* Plant Extract. *International Journal of PharmTech Research*, **8**, 148-157.

[3] Little, J.B. (2003) Genomic Instability and Bystander Effects: A Historical Perspective. *Oncogene*, **22**, 6978-6987. https://doi.org/10.1038/sj.onc.1206988

[4] Poljsak, B., Šuput, D. and Milisav, I. (2013) Achieving the Balance between ROS and Antioxidants: When to Use the Synthetic Antioxidants Borut. *Oxidative Medicine and Cellular Longevity*, **2013**, Article ID: 956792.

[5] Di Maggio, F.M., Minafra, L., Forte, G.I., Cammarata, F.P., Domenico, L., Messa,

C., Gilardi, M.C. and Bravatà, V. (2015) Portrait of Inflammatory Response to Ionizing Radiation Treatment. *Journal of Inflammation*, **12**, 14. https://doi.org/10.1186/s12950-015-0058-3

[6] Reuter, S., Gupta, S.C., Chaturvedi, M.M. and Aggarwal, B.B. (2010) Oxidative Stress, Inflammation, and Cancer: How Are They Linked? *Free Radical Biology and Medicine*, **49**, 1603-1616. https://doi.org/10.1016/j.freeradbiomed.2010.09.006

[7] Yoon, W.-J., Min, H.Y., Kim, S.-S., Yoo, B.-S., Moon, J.-Y., Baik, J.S., Lee, N.H. and Hyun, C.-G. (2009) Suppression of Pro-Inflammatory Cytokines, iNOS, and COX-2 Expression by Brown Algae *Sargassum micracanthum* in RAW 264.7 Macrophages. *EurAsian Journal of BioSciences*, **3**, 130-143.

[8] Lordan, S., Ross, R.P. and Marine, S.C. (2011) Bioactives as Functional Food Ingredients: Potential to Reduce the Incidence of Chronic Diseases Mar. *Drugs*, **9**, 1056-1100.

[9] Rodrigues, E., Lilian, R., Mariutti, B. and Mercadante, A.Z. (2012) Scavenging Capacity of Marine Carotenoids against Reactive Oxygen and Nitrogen Species in a Membrane-Mimicking System Mar. *Drugs*, **10**, 1784-1798. https://doi.org/10.3390/md10081784

[10] Kulmacz, R.J. and Lands, W.E.M. (1983) Requirements for Hydroperoxide by the Cyclooxygenase and Peroxidase Activities of Prostaglandin H Synthase. *Prostaglandins*, **25**, 531-540. https://doi.org/10.1016/0090-6980(83)90025-4

[11] Hwang, Y.J., Lee, E.J., Kim, H.R. and Hwang, K.A. (2013) NF-κB-Targeted Anti-Inflammatory Activity of Prunella Vulgaris var. Lilacina in Macrophages RAW 264.7. *International Journal of Molecular Sciences*, **14**, 21489-21503. https://doi.org/10.3390/ijms141121489

[12] Miranda, K.M., Espey, M.G. and Wink, D.A. (2001) A Rapid Simple Spectrophotometric Method for Simultaneous Detection of Nitrate and Nitrite. *Nitric Oxide*, **5**, 67-71. https://doi.org/10.1006/niox.2000.0319

[13] Yoshioka, T., Kawada, K., Shimada, T. and Mori, M., (1979) Lipid Peroxidation Inmaternal and Cord Blood and Protective Mechanism Against Activated-Oxygentoxicity in the Blood. *American Journal of Obstetrics & Gynecology*, **135**, 372-376. https://doi.org/10.1016/0002-9378(79)90708-7

[14] Waud, W.R. and Rajagopalan, K.V. (1976) Purification and Properties of the NAD Dependent (Type D) and O-Dependent (Type O) Forms of Rat Liver Xanthine Dehydrogenase. *Archives of Biochemistry and Biophysics*, **172**, 354-364. https://doi.org/10.1016/0003-9861(76)90087-4

[15] Kakkar, P., Das, B. and Viswanathan, P.N. (1984) A Modified Spectrophotometric Assay of Superoxide Dismutase. *Indian Journal of Biochemistry & Biophysics*, **21**, 130-132.

[16] Sinha, K.A. (1972) Colorimetric Assay of Catalase. *Analytical Biochemistry*, **47**, 389-394. https://doi.org/10.1016/0003-2697(72)90132-7

[17] Ellman, G.L. (1959) Tissue Sulphydryl Groups. *Archives of Biochemistry and Biophysics*, **82**, 70-77. https://doi.org/10.1016/0003-9861(59)90090-6

[18] Greenhough, A., Smartt, H.J., Moore, A.E., Roberts, H.R., Williams, A.C., Paraskeva, C. and Kaidi, A. (2009) The COX-2/PGE 2 Pathway: Key Roles in the Hallmarks of Cancer and Adaptation to the Tumour Microenvironment. *Carcinogenesis*, **30**, 377-386. https://doi.org/10.1093/carcin/bgp014

[19] Nam, K.W., Je, K.H., Lee, J.H., Han, H.J., Lee, H.J., Kang, S.K. and Mar, W. (2003) Inhibition of COX-2 Activity and Proinflammatory Cytokines (TNF-Alpha and IL-1beta) Production by Water-Soluble Sub-Fractionated Parts from Bee (Apismel-

lifera) Venom. *Archives of Pharmacal Research*, **26**, 383-388. https://doi.org/10.1007/BF02976695

[20] Dubois, R.N., Abramson, S.B., Crofford, L., Gupta, R.A., Simon, L.S., van de Putte, L.B. and Lipsky, P.E. (1998) Cyclooxygenase in Biology and Disease. *The FASEB Journal*, **12**, 1063-1073.

[21] Kuwano, T., Nakao, S., Yamamoto, H., Tsuneyoshi, M., Yamamoto, T., Kuwano, M. and Ono, M. (2004) Cyclooxygenase 2 Is a Key Enzyme for Inflammatory Cytokine-Induced Angiogenesis. *The FASEB Journal*, **18**, 300-310. https://doi.org/10.1096/fj.03-0473com

[22] Nørregaard, R., Kwon, T.H. and Frøkiær, J. (2015) Physiology and Pathophysiology of Cyclooxygenase-2 and Prostaglandin E2 in the Kidney Kidney Research and Clinical Practice. *Kidney Research and Clinical Practice*, **34**, 194-200. https://doi.org/10.1016/j.krcp.2015.10.004

[23] Chen, X., Andresen, B.T., Hill, M., Zhang, J., Booth, F. and Zhang, C. (2008) Role of Reactive Oxygen Species in Tumor Necrosis Factor-Alpha Induced Endothelial Dysfunction. *Current Hypertension Reviews*, **4**, 245-255. https://doi.org/10.2174/157340208786241336

[24] Schaue, D., Kachikwu, E.L. and McBride, W.H. (2012) Cytokines in Radiobiological Responses: A Review. *Journal of Radiation Research*, **178**, 505-523. https://doi.org/10.1667/RR3031.1

[25] Formenti, S.C. and Demaria, S. (2009) Systemic Effects of Local Radiotherapy. *The Lancet Oncology*, **10**, 718-726. https://doi.org/10.1016/S1470-2045(09)70082-8

[26] Yakovlev, V.A. (2013) Nitric Oxide-Dependent down Regulation of BRCA1 Expression Promotes Genetic Instability, *Cancer Research*, **73**, 706-715. https://doi.org/10.1158/0008-5472.CAN-12-3270

[27] Ghosh, S., Maurya, D.K. and Krishna, M. (2008) Role of iNOS in Bystander Signaling between Macrophages and Lymphoma Cells, *International Journal of Radiation Oncology*, **72**, 1567-1574. https://doi.org/10.1016/j.ijrobp.2008.08.006

[28] Moncada, S., Palmer, R.M. and Higgs, E.A. (1991) Nitric Oxide: Physiology, Pathophysiology, and Pharmacology. *Pharmacological Reviews*, **43**, 109-142.

[29] Bereta, J., Bereta, M. and Allison, A.C. (1997) Inhibitory Effect of Dicatecholroperol on VCAM-1 and iNOS Expression in Cytokine-Stimulated Endothelium. *Life Science*, **60**, 325-334. https://doi.org/10.1016/S0024-3205(96)00633-9

[30] Lowenstein, C.J. and Padalko, E. (2004) iNOS (NOS2) at a Glance. *Journal of Cell Science*, **117**, 2865-2867. https://doi.org/10.1242/jcs.01166

[31] De Ridder, M., Verellen, D., Verovski, V. and Storme, G. (2008) Hypoxic Tumor Cell Radio Sensitization through Nitric Oxide. *Nitric Oxide*, **9**, 164-169. https://doi.org/10.1016/j.niox.2008.04.015

[32] Vane, J.R., Mitchell, J.A., Appleton, I., Tomlinson, A. and Bishop-Bailey, D. (1994) Inducible Isoforms of Cyclooxygenase and Nitric-Oxide Synthase in Inflammation. *Proceedings of the National Academy of Science*, **91**, 2046-2050. https://doi.org/10.1073/pnas.91.6.2046

[33] Gorbunov, N.V., Pogue-Geile, K.L., Epperly, M.W., Bigbee, W.L. and Draviam, R. (2000) Activation of the Nitric Oxide Synthase 2 Pathway in the Response of Bone Marrow Stromal Cells to High Doses of Ionizing Radiation. *Journal of Radiation Research*, **154**, 73-86. https://doi.org/10.1667/0033-7587(2000)154[0073:AOTNOS]2.0.CO;2

[34] Azab, K.S., Mostafa, A.A., Ali, E.M.M. and Abdel-Aziz, M.A.S. (2011) Cinnamon Extract Ameliorates Ionizing Radiation-In Duced Cellular Injury in Rats. *Ecotox-*

icology and Environmental Safety, **74**, 2324 -2329. https://doi.org/10.1016/j.ecoenv.2011.06.016

[35] Azab, K.S., Salama, A.F. and Rizk, A.M.M. (2014) Cranberry Extract Modulate Inflammation in Irradiated Rats. *Sci-Afric Journal of Scientific Issues, Research and Essays*, **2**, 392-398.

[36] Rao, A.L., Bharani, M. and Pallavi, V. (2006) Role of Antioxidants and Free Radicals in Health and Disease. *Advances in Pharmacology and Toxicology*, **7**, 29-38.

[37] Lovell, M.A., Ehmann, W.D., Buffer, B.M. and Markesberry, W.R. (1995) Elevated Thiobarbituric Acid Reactive Substances and Antioxidant Enzyme Activity in the Brain in Alzemers Disease. *Neurology*, **45**, 1594-1601. https://doi.org/10.1212/WNL.45.8.1594

[38] Shah, A.M. and Channon, K.M. (2004) Free Radicals and Redox Signalling in Cardiovascular Disease. *Heart*, **90**, 486-487. https://doi.org/10.1136/hrt.2003.029389

[39] Wu, C., Parrott, A.M., Fu, C., Liu, T., Marino, S.M., Gladyshev, V.N., Jain, M.R., Baykal, A.T., Li, Q., Oka, S., Sadoshima, J., Beuve, A., Simmons, W.J. and Li, H. (2011) Thioredoxin 1-Mediated Post-Translational Modifications: Reduction, Transnitrosylation, Denitrosylation, and Related Proteomics Methodologies. *Antioxidants & Redox Signaling*, **15**, 2565-2604. https://doi.org/10.1089/ars.2010.3831

[40] Manish, M., Mohammad, R.S., Khiem, T., Sekhar, P.R. and Asrar, B.M. (2014) Reactive Oxygen Species in Inflammation and Tissue Injury. *Antioxid Redox Signal*, **20**, 1126-1167. https://doi.org/10.1089/ars.2012.5149

[41] Mhadhebi, L., Mhadhebi, A., Robert, J. and Bouraoui, A. (2014) Antioxidant, Anti-Inflammatory and Ant Proliferative Effects of Aqueous Extracts of Three Mediterranean Brown Seaweeds of the Genus Cystoseira. *Iranian Journal of Pharmaceutical Research*, **13**, 207-220.

[42] Fung, A., Hamid, N. and Lu, J. (2013) Fucoxanthin Content and Antioxidant Properties of Undariapinnatifida. *Food Chemistry*, **136**, 1055-1062. https://doi.org/10.1016/j.foodchem.2012.09.024

[43] Heo, S.J., Yoon, W.J., Kim, K.N., Ahn, G.N., Kang, S.M., Kang, D.H., Abu affan, Oh, C., Jung, W.-K. and Jeon, Y.J. (2010) Evaluation of Anti-Inflammatory Effect of Fucoxanthin Isolated from Brown Algae in Lipopolysaccharide-Stimulated RAW 264.7 Macrophages. *Food and Chemical Toxicology*, **48**, 2045-2051. https://doi.org/10.1016/j.fct.2010.05.003

[44] Duan, X.J., Zhang, W.W., Li, X.M. and Wang, B.G. (2006) Evaluation of Antioxidant Property of Extract and Fractions Obtained from a Red Alga. Polysiphonia Urceolata. *Food Chemistry*, **95**, 37-43. https://doi.org/10.1016/j.foodchem.2004.12.015

[45] Kim, K.N., Heo, S.J., Yoon, W.J., Kang, S.M., Ahn, G. and Yi, T.H. (2010) Fucoxanthin Inhibits the Inflammatory Response by Suppressing the Activation of NF-Kappab and MAPKs in Lipopolysaccharide Induced RAW 264.7 Macrophages. *European Journal of Pharmacology*, **649**, 369-375. https://doi.org/10.1016/j.ejphar.2010.09.032

[46] Yong, S.J., Jisang, P., Seung, M.H., Eun, S.K., Kyung, M.C. and Jeong, D.C. (2016) Fucoidan Prevents LPS-Induced Production of Nitric Oxide and Prostaglandin E2 by Inhibiting MAPK-Mediated Signal Pathways in RAW 264.7 Macrophage Cells. *Journal of Immunology*, **196**, 25-31.

[47] Sachindra, N.M., Sato, E., Maeda, H., Hosokawa, M., Niwano, Y., Kohno, M. and Miyashita, K. (2007) Radical Scavenging and Singlet Oxygen Quenching Activity of

Marine Carotenoid Fucoxanthin and Its Metabolites. *Journal of Agricultural and Food Chemistry*, **55**, 8516-8522. https://doi.org/10.1021/jf071848a

[48] Pawel, K. (2012) Regulation of Immune Responses by Prostaglandin E2. *Journal of Immunology*, **188**. 21-28. https://doi.org/10.4049/jimmunol.1101029