RESEARCH ARTICLE

Red Seaweed (*Hypnea Bryodies* and *Melanothamnus Somalensis*) Extracts Counteracting Azoxymethane-Induced Hepatotoxicity in Rats

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Abstract

Background: Azoxymethane (AOM) is a well-known colon cancer-inducing agent in experimental animals via mechanisms that include oxidative stress in rat colon and liver tissue. Few studies have investigated AOM-induced oxidative stress in rat liver tissue. Red seaweeds of the genera *Hypnea Bryodies* and *Melanothamnus Somalensis* are rich in polyphenolic compounds that may suppress cancer through antioxidant properties, yet limited research has been carried out to investigate their anti-carcinogenic and antioxidant influence against AOM-induced oxidative stress in rat liver. **Objective:** This study aims to determine protective effects of red seaweed (*Hypnea Bryodies* and *Melanothamnus Somalensis*) extracts against AOM-induced hepatotoxicity and oxidative stress. **Materials and Methods:** Sprague–Dawley rats received intraperitoneal injections of AOM, 15 mg/kg body weight, once a week for two consecutive weeks and then orally administered red seaweed (100 mg/kg body-weight) extracts for sixteen weeks. At the end of the experiment all animals were overnight fasted then sacrificed and blood and liver tissues were collected. **Results:** AOM treatment significantly decreased serum liver markers and induced hepatic oxidative stress as evidenced by increased liver tissue homogenate levels of nitric oxide and malondialdehyde, decreased total antioxidant capacity and glutathione, and inhibition of antioxidant enzymes (catalase, glutathione peroxidase, glutathione S-transferase, glutathione reductase and superoxide dismutase). Both red seaweed extracts abolished the AOM-associated oxidative stress and protected against liver injury as evidenced by increased serum levels of liver function markers. In addition, histological findings confirmed protective effects of the two red seaweed extracts against AOM-induced liver injury. **Conclusion:** Our findings indicate that red seaweed (*Hypnea Bryodies* and *Melanothamnus Somalensis*) extracts counteracted oxidative stress-induced hepatotoxicity in a rat model of colon cancer.

Keywords: Azoxymetahne- hepatotoxicity- red seaweeds- oxidative stress

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Introduction

Liver is the main metabolic organ for detoxification of drugs and xenobiotic, such mechanism is crucial to combat oxidative stress inducing agents that are circulating in blood circulation (Mello et al., 2016). Pathological and physiological insults lead to liver injury and damage that is accompanied by loss of liver capacity to tolerate reactive oxygen inducing agents and loss of detoxifying mediated pathways (Arauz et al., 2016). Azoxymethane (AOM) is commonly used as colon cancer inducing agent in various experimental animals, and followed by oxidative damage of liver organ (Al-Numair et al., 2011). Our previous studies have shown that AOM enhanced lipid peroxidation in colon and liver tissues of rats, and also significantly increased nitric oxide production (Waly et al., 2012; Guizani et al., 2013; Waly et al., 2015).

In biological systems nitric oxide is produced from L-arginine amino acid by the enzyme nitric oxide synthase and involved in many oxidative stress-mediated processes (Al-Nahdi et al., 2016). Reactive oxygen species induce oxidative damage in liver tissue by inhibiting antioxidant enzymes (catalase, glutathione peroxidase, glutathione S-transferase, and superoxide dismutase) and depleting intracellular glutathione (Valsalva et al., 2016). Red seaweed of the genera *Hypnea Bryodies* and *Melanothamnus Somalensis* are rich in bioactive compounds that might exhibit radical scavenging activity and reserve the consumption of glutathione and other cellular antioxidants (Al-Alawi et al., 2011; Al-Nahdi et al., 2015). Recent studies have reported the protective effect of seaweed against AOM-induced colon carcinogenesis through attenuation of oxidative stress (Waly et al., 2014), and supplementation of seaweeds extracts suppresses AOM-induced aberrant DNA methylation in colon and liver of experimental animals (Bu et al., 2014).

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Seaweeds constitute one of the major components of diet in several Asian countries and the consumption of seaweed as part of diet has been shown to be one of the reasons for low incidence of cancer (Shigihara et al., 2014). Addition of seaweed in food products would improve the nutritional quality and total antioxidant capacity, yet limited research was carried out to investigate its potential health effects. With this background, the current study aimed to determine the in vivo potential health effect of two red seaweeds (*Hypnea Bryodies* and *Melanothamnus Somalensis*) against AOM-induced oxidative stress and histological changes in liver tissue of rats as an experimental model for the study.

Materials and Methods

Red seaweed extraction

Both red seaweeds (*Hypnea Bryodies* and *Melanothamnus Somalensis*) were collected from the the southern coast of Oman, Mirbat (16° 59' 28.7'' N, 54° 41' 27.7'' E), during the period from March to June 2015. The samples were transferred to the laboratory in cold boxes, washed with distilled water, cleaned from extraneous matter (other seaweeds, invertebrates, molluscs and crustaceans) and were identified at the Department of Food Science and Nutrition, College of Agricultural and Marine Sciences, Sultan Qaboos University, Oman. The samples were air-dried and ground to a fine powder then extracted by 80% aqueous ethanol. Following filtration, the filtrate was concentrated under reduced pressure in a rotary evaporator and then lyophilized. The dry yields obtained were 1.1 g/100 g of *Hypnea Bryodies* and 3 g/100 g of Melanothamnus Somalensis. Extracts were stored at -20°C till used.

Experimental design and treatment protocol

Forty male Sprague–Dawley rats (average weight 200 ± 20g) were used in the present experiment. Animals were obtained from the small animal unit at Sultan Qaboos University. The local ethics and research committee approved the design of the experiments and the protocol conforms to the guidelines of the National Institutes of Health (NIH). The animals were housed in plastic well-aerated cages at normal atmospheric temperature (25 ± 5°C) and normal 12 h light/dark cycle, and all rats were caged individually and given feed and water ad-libitum. After two weeks of acclimation, rats were divided into four groups (10 rats per group); the first group was used as control. Group 2 received two intraperitoneal injections of AOM (Sigma Chemical Co., St. Louis, MI) dissolved in physiological saline once a week (15 mg/kg body weight) for 2 weeks. While groups 3 and 4 were injected with AOM and orally treated (1.5 ml/day) with extracts of *Hypnea Bryodies* or *Melanothamnus Somalensis*, respectively, at a dose of 100 mg/kg body-weight of rats, the dose was selected based on our previous study (Waly et al., 2014). Rats were orally administered their respective dose every day for sixteenths weeks, and at the end of treatment period, rats of each group were overnight fasted, euthanized under ether and sacrificed.

Liver function tests

Blood was taken from the heart and collected into plain redtop blood tubes and the serum was separated by centrifugation (6000 rpm for 20 min) and transferred into Eppendorf tubes, stored at -80 °C. Serum albumin, total bilirubin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assayed using automated analyzer, Cobas c 111 analyzer (Roche Diagnostics, USA).

Liver tissue oxidative stress markers

The liver tissue of each rat (~50 mg) was removed and immediately homogenized in 5 mL of 100 mM potassium phosphate buffer (pH 7.2) by a glass-Teflon homogenizer with an ice-cold jacket and centrifuged at 6,000 g at 4°C for 60 minutes. The resulting supernatant was used for protein content measurement (Lowry et al., 1951) and for oxidative stress markers. In this study, the following parameters were measured in the liver tissue homogenates according to the manufactures' instructions: Lipids peroxides, malondialdehyde (MDA) with commercial kit (Thiobarbituric Acid Reactive Substances (TBARS) Assay Kit from Cayman Chemical), nitric oxide concentration was determined with a spectrophotometric assay kit from Cayman Chemical. Meanwhile glutathione (GSH) with glutathione assay kit catalog #K251, catalase (CAT) with assay kit catalog #K773, glutathione peroxidase (GPx) with assay kit catalog #K762, glutathione S-transferase (GST) with assay kit catalog #K263, and superoxide dismutase (SOD) with assay kit catalog number catalog # K335 were purchased from BioVision, Inc., California 95035, USA.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 (version 5.03; GraphPad Software Inc. San Diego, CA, USA). The results are expressed as means ± standard deviation (SD) of 10 independent observations from each subgroup. The statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey's test and t-test for means comparisons and a P-value of less than 0.05 was considered significant.

Results

Treatment of rats with AOM induced marked impairment of liver function evidenced by a significant elevation of serum AST and ALT as compared to control group, $P<0.05$, Table 1. Meanwhile, the oral supplementation of AOM treated group with either *Hypnea Bryodies* or *Melanothamnus Somalensis* significantly ameliorated the altered serum aminotransferases activity, $P<0.001$. The same trend was observed for serum albumin level and our data revealed that AOM treatment caused a decrease on serum albumin level and the treatment with the two red seaweeds abrogated the observed AOM-associated albumin diminished level. This effect was significantly different when compared to control group, $P<0.05$, Table 1.

The AOM treatment caused a significant liver tissue depletion on glutathione level, increase in the lipid

Table 1. Serum Liver Function Markers in Control and Experimental Treated Groups

Group Parameter	Control	AOM	AOM & *Hypnea Bryodies*	AOM & Melanothamnus Somalensis
ALT (U/L)	25.7 ± 3.1	60.5 ± 3.7*	26.8 ± 2.7**	27.1 ± 2.4**
AST (U/L)	15.2 ± 0.9	30.2 ± 1.4#	16.2 ± 1.1##	16.6 ± 1.2##
Albumin (g/dl)	3.2 ± 0.9	0.92 ± 0.04^	2.7 ± 0.08^^	2.8 ± 0.06^^

Azoxymethane, (AOM); alanine aminotransferase, (ALT); aspartate aminotransferase, (AST); Results are expressed as mean ± SD; *, # Significantly higher than control group; **, ## Significantly lower than AOM group; ^, Significantly lower than control group; ^^, Significantly higher than AOM group; P <0.05, Based on one way ANOVA analysis

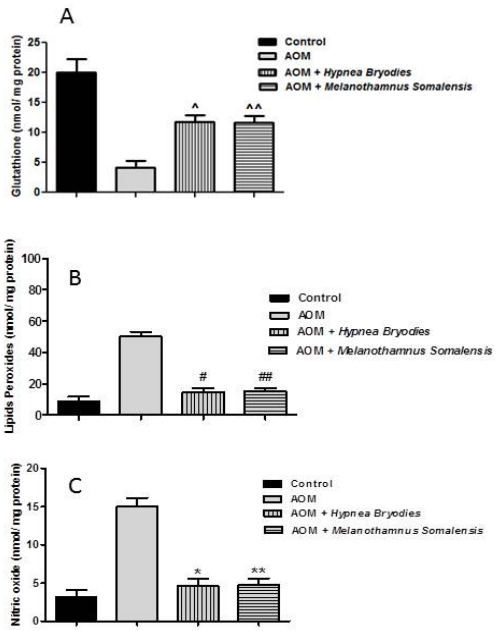

Figure 1. Measurements of Oxidative Stress Indices in Liver Homogenates. (A) Glutathione, (B) Lipid peroxides, and (C) Nitric oxide. Data are expressed as Mean ± SD. ^, ^^ Significantly higher as compared to AOM group, P<0.05. #,##,*,**Significantly lower than AOM group, P<0.05.

peroxides, and release of nitric oxide level (Figure 1A, 1B and 1C respectively), P<0.05. The AOM groups concomitantly treated with red seaweed, *Hypnea Bryodies* , or *Melanothamnus Somalensis* extracts combated the observed AOM-oxidative stress indices, P<0.05, Figure 1. The same trend was observed for hepatic antioxidant enzymes, where AOM caused a significant decline in the activities of CAT, GPx, GST and SOD as illustrated in Table 2. Meanwhile concomitant treatment of the AOM group with either *Hypnea Bryodies* or *Melanothamnus Somalensis* significantly alleviated the AOM-associated inhibition of these hepatic antioxidant enzymes activities, P<0.05, Table 2.

Discussion

Several studies revealed that AOM is a potent carcinogen for the liver and at low doses induce hepatotoxicity (Bémeur et al., 2010; Megaraj et al., 2014). The present study elucidated the role of red seaweeds in alleviating AOM-induced hepatotoxicity, we report that rats treated with AOM developed liver injury that is characterized histologically by extensive fatty degeneration, necrosis, dilation and congestion of blood vessels; these findings are consistent with previous studies demonstrated that AOM induces chronic injury in murine liver (Lahouar et al., 2014). Further, our findings confirm previous observations that AOM-induced hepatic damage as evidenced by elevation of the circulating levels of aminotransferases and total bilirubin (Gürocak et al., 2013), it is suggested that the observed high level of these enzymes is the consequence of AOM-induced loss liver cell membrane's integrity.

AOM as a carcinogen has been reported to initiate hepatic oxidative stress and modify the activities of antioxidant enzymes (Burlamaqui et al., 2013). The metabolism of AOM produces extremely reactive hydroxyl radical that induce oxidative stress (Lahouar et al., 2014). The resultant reactive oxygen species (ROS) induce lipid peroxidation, protein damage, DNA fragmentation, gene mutations and loss of membrane integrity (Anilakumar et al., 2010). In the present study, AOM-administered rats exhibited significantly elevated hepatic nitrates levels that reacted with superoxide anions to form the potent oxidant peroxinitrite. *Hypnea Bryodies* and *Melanothamnus Somalensis* have significantly augmented the observed AOM- decreased hepatic content of MDA and NO, suggesting their antioxidant potential. AOM-administered rats exhibited reduced activities of the antioxidant enzymes, CAT, GPx, GST and SOD; these enzymes play a critical role in maintaining the body's

Table 2. Red Seaweeds (*Hypnea Bryodies* and *Melanothamnus Somalensis*) Extracts Protect Against Azoxymethane (AOM)-Induced Antioxidant Enzymes Inhibition in Rat Liver Tissue Homogenates

Group Parameter	Control	AOM	AOM & *Hypnea Bryodies*	AOM & Melanothamnus Somalensis
CAT	124.3 ± 5.1	46.7 ± 5.3 *	124.6 ± 4.6 **	125.4 ± 6.8**
GR	9.3± 0.9	5.2 ± 0.7^	8.9 ± 0.8**	9.2 ± 0.6
GPx	22.6 ± 2.2	10.8 ± 1.9#	21.4 ± 2.4b	21.3 ± 1.2
GST	19.6± 1.3	11.2 ± 0.8&	18.9 ± 1.1b	20.2± 0.9
SOD	67.5 ± 4.3	31.5 ± 3.2+	66.7 ± 3.3b	66.2 ± 1.8

Antioxidant Enzymes CAT, Catalase; GR, Glutathione Reductase; GPx, Glutathione Peroxidase; GST, Glutathione Transferase; SOD, Superoxide Dismutase; Unit of enzymes activities is µmol/min/mg protein; Results are the mean ± SD; *, ^, #, &, +, Significantly lower than corresponding control group; **, ^^, ##, &&, ++ ,Significantly higher than AOM; P< 0.05, Based on one way ANOVA analysis.

defense mechanism against the destructive effects of ROS and free radicals. The assayed *Hypnea Bryodies* and *Melanothamnus Somalensis* extracts abolished the observed GSH depletion and improved activities of the assayed hepatic antioxidant enzymes in consistence with our previous studies (Waly et al., 2014).

In conclusion, our findings indicate that red seaweeds (*Hypnea Bryodies* and *Melanothamnus Somalensis*) extracts counteracted oxidative stress-induced hepatotoxicity in a rat model of colon cancer. This observed effect might be attributed to their rich content of phenolic compounds.

Conflict of Interest

The authors have declared that no competing interests exist.

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