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Hepatoprotective Impact of Seaweed (*Sargassum muticum*) Nanoparticles against Diethylnitrosamine Promoted Progression of Liver Tumor in Male Rats

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Abstract

There is now a greater concern in the marine organisms, especially algae, to find active ingredients to be used as therapeutic drugs in the medical applications. Delivery of drugs into the target organs with high specificity is now achieved when these compounds are coated on nanoparticles which improve the therapeutic properties of anti-tumor products. So, the current investigation aimed to assess the hepatoprotective capacity of silver nanoparticles (AgNPs) prepared by using ethanol extraction of *Sargassum muticum* (SmAgNPs) versus diethylnitrosamine (DEN) promoted liver tumor in male rats. Swiss albino male rats were allocated into several treated groups. Diethylnitrosamine (DEN, 20 mg per kg body weight) was used for 2 months in male rats to induce liver tumor and then treated with several concentrations (25, 50, and 75 mg per kg body weight) of algae extracts and SmAgNPsas well as with a reference drug (30 mg/kg of Silymarin) for 8 weeks. The results revealed that treatment of DEN-rats with SmAgNPsdecreased levels of the liver function enzymes, namely, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), decreased rates of the DNA damage, and increased activity levels of antioxidant enzymes such as catalase (CAT) and glutathione peroxidase (GPx). Additionally, DEN-rats treated with silver nanoparticles of *S. muticum* elevated the expression levels of tumor suppressor genes (such as ING3 and Akr1b10) and diminished the levels of oncogene (FoxP1) expression explaining that SmAgNPshas antitumor activity through alteration of the liver cancer-related genes. The results concluded that the high competence of *S. muticum* nanoparticles for protecting the DNA from damage and increase the antioxidant activities as well as enhance the up-regulation of tumor suppressor mRNAs is attributed to its rich active compounds of fucoxanthin (as marine carotenoids) and fucosterol (marine sterol).

Key words:*Sargassum muticum*, silver nanoparticles, liver cancer, antioxidants, DNA damage, Gene expression

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Introduction

Globally, liver malignant tumor, namely, hepatocellular carcinoma (HCC) is taking place in the top 10 most widespread cancers with rising incidence in some regions such as Gulf region, Europe and the USA (Jemal et al., 2011). As cited in the cancer incidence records, literature of the PubMed and the data of the national cancer, thirty five percent of the Arabian population comprises in the Gulf with population of about 40 million including Saudi Arabia, Kuwait, United Arab Emirate (UAE), Qatar, Oman and Bahrain (Alter, 2007; Daw and Dau, 2012).

The survival prognosis of the patients with HCC is recorded to be 36% of patients with degree 1 and 17% of patients of degree 3 cancer proliferation (Marrero et al., 2005). HCC patients are failing to receive successful therapeutic treatments (Rasul et al., 2010). Although there are many therapies available for the treatment of HCC patients, the decisions for treatment have become more complex. In general, HCC develops from secondary to chronic degree of liver disease and then cirrhosis (Munoz and Bosch, 1989). The data of liver disease in the Gulf area stated that the incidence of HCC varies in females between 1.8-3.1and in males between 3.4-8.1 casesper a population of 100,000 per year (Bosch et al., 2004; Ribes et al., 2004). In addition, the latest epidemiology data of HCC observes that the frequency of this liver tumor continues to rise quickly in most regions of the world including the Gulf area. In the West, the frequency of HCC disease is less widespread and mostly resulting from the infection with hepatic viruses such as hepatitis B virus (HBV) and alcoholic liver cirrhosis (Khoja, 2011; Rasul et al., 2013). However, in most of the countries of the Middle East including the Gulf area, HCC disease is considered as the most frequent cancer, regularly resulting from the infection with the hepatitis C virus (HCV) (Fasani et al., 1999; Lehman and Wilson, 2009).

For the pharmaceutical scientists, there is recently a greater interest in the marine organisms to find therapeutic drugs extracted from natural products in particular algae (Baumeister et al., 2019; Bousquet et al., 2019). From these organisms, the seaweeds are considered as the most attractive active natural compounds' sources for some reasons such as safety, massive biodiversity, and traditional Asian foods as well as for folk medicine.

In the orient regions, the dietary value of aquatic algae has been recognized more than in the western region with partial utilization as a dietary part (Gomez-Gutierrez et al., 2011; Taboada et al., 2013). The seaweeds have a lot of benefits for human consumption compared with plants. The seaweeds contain a high percentage of vitamins, low fat, and natural antioxidants (e.g. bioactive compounds including sulfate polysaccharides and terpenoids) (Hermund et al., 2016). Additionally, many seaweeds have a high percentage of free radical scavenging compounds such as *Sargassum* sp. (Raghavendran et al., 2005; Palanisamy et al., 2018). Moreover, the polysaccharides extracted from seaweeds neither in crude nor pure form revealed antitumor activity versus several types of cancers (Schumacher et al., 2011; Mohamed et al., 2012). However, numerous studies conducted on animal models showed that dietary algae exhibited protective roles against mammary, intestinal, and skin cancers (Noda et al., 1990; Funahashi et al., 2011; Namvar et al., 2012;Liu et al., 2012).The protective role of seaweeds against cancer in animal models might be involved in antioxidant capacity and suppression impacts on cell proliferation.

The action mechanism of algae against cancer is complex due to incredible structural diversity with multiple interactions (Deslandes et al., 2000; Namvar et al., 2012; Mentella et al., 2019). The antioxidant mechanism of algal bioactive compounds (Yuan and Walsh, 2006) enhances the immune system in the host cells by rising killer response (Ferna´ndezet al., 1989; Mayer and Gustafson, 1990), suppressing the cell expansion in the G1 phase and subsequently, differentiation (Mabeau and Fleurence, 1993; Gupta and Abu-Ghannam, 2011), prevention of the complicated pathway of angiogenesis (Souza et al., 2012) and inducing apoptosis (Kwon et al., 2007), which play as causative factors in the carcinogenesis suppression. So, different dietary antioxidants from algal species are remarkable effective agents against chronic diseases including cancer. Therefore, modulation in dietary behavior for sinking the frequency of these chronic diseases, particularly by increasing the consumption rate of the functional diets rich in marine antioxidants is markedly supported (Mentella et al., 2017).

Nowadays, nanomaterials have been applied in the therapeutic regimes of the medical application and pharmacotherapy as a novel promising tool (Karakoti et al., 2006; Bhattacharyya et al., 2010). They are used in the clinical applications and pharmacotherapy due to having a wide surface area, very low toxic effects, having delivery bioactive compounds possibility and could be used in very low doses. Therefore, the present study aimed to find novel drugs against liver cancer using nanoparticles of polyphenol-rich seaweed (*Sargassum muticum*) against the liver cancer rat models.

Materials and Methods

Collection and extraction of S. muticum

Green seaweeds were collected from the Arabian Gulf of Aqaba, Haql (29º20'25.4"N 34º56'53.5"E to 29º14'47.6"N 34º56'02.5"E) KSA. The algae samples were carefully washed in tap water and air-dried for 3-4 days. Afterward, the dried sample was powdered using a blender. Then, the *S. muticum* extract was prepared using 25 grams of dried powder samples with 100 ml of ethanol, as reported by Aziz et al (Aziz et al., 2018).

Green synthesis and characterization of S. muticum-AgNPs

Freshly prepared one millimolar silver nitrate ($AgNo_3$) solution was thoroughly mixed with 100 ml of aqueous extract of *S. muticum*. The pale yellow color in the aqueous medium confirms the formation of Ag nanomaterials. Further, the green fabricated silver nanomaterials were characterized through UV-visible spectrophotometry (UVD 3200), X-rayDiffractometer(Equinox 3000), scanning electron microscopy (Hitachi S-4160), Nno-z 590 Malvern-Zetasizer and FTIR spectrum (Bruker Tensor 27).

Animals

Adult Swiss albino male rats (n=63, 120-150 g), were purchased from the animal supplier and were fed regular laboratory diet and water *ad libitum*. Following 7 days of adaptation time, 9 animal groups were designed (7 rats each) in which the animals were housed separately in plastic cages. The animals were housed in a contamination-free room which was controlled for temperature and light periods.

Experimental Design

Swiss albino male rats (n=63) were allocated in 9 experimental groups (7 rats each). Additionally, a toxicity investigation was conducted to determine the lethal dose values (LD_{50}) of the nanoparticles doses which were fixed using five rats from each group. So, the treatment schedule was performed as follows; Group1: animals served as negative control received NaCl solution (0.9 w/v%). To produce animals with liver cancer, male rats were intoxicated orally with 20 mg/kg b.wt. diethylnitrosamine (DEN) in NaCl solution once per week (Prasannaraj and Venkatachalam, 2017) for a duration of 8 weeks. Afterward, animal groups with liver cancer were treated with different concentrations of algae extracts and nanoparticles in addition to reference drug for one month as follows; Group 2: liver cancer-induced rats without any drug treatment served as the positive control. Groups 3 to 5: animals were treated with *S. muticum* extracts by oral administration at 25, 50, and 75 mg per kg body weight doses (Jeon et al., 2019), respectively. Male rats in Groups 6 to 8 were treated by oral administration with 25, 50, and 75 mg per kg of *S. muticum* silver nanoparticles (SmAgNPs), respectively. Group 9 animals were treated with 30 mg/kg b.wt. of Silymarin (Zhang et al., 2013) as a standard drug.

After treatment completion, overnight fasted animals were exposed to an overdose of ether anesthesia and sacrificed by cervical dislocation. Blood samples from treated animals were aspirated individually by puncture of the heart to determine the levels of liver markers. Afterward, the liver tissues were separated, washed with saline solution and kept at -20ºC for

assessment of the activity of the antioxidant enzymes and at -80ºC for expression analysis of liver cancer-related genes.

Determination of ALT and AST levels

Blood samples were obtained from all treated animals. For measurements of ALT and AST, collected blood samples were put in micro-centrifuge tubes (2 ml). After centrifugation (2000 *g*) for five min, serum samples were collected and kept at -20ºC until use. ALT and AST measurements were carried out following the commercial kit instructions (Huang et al., 2006).

Analysis of antioxidant enzymes

Assessment of GPx Activity

The activity of the GPx was assessed using the kit for Ransel assay (Randox Laboratories Ltd.) (Paglia and Valentine, 1967). The spectrophotometer at 340 nm was used for 3 min to monitor the transformation of NADPH to $NADP^+$. The transformation process of oxidized NADPH to $NADP^+$ as moles per milligram per min of protein sample was measured as GPx activity.

Assessment of Catalase Activity

The activity of catalase was conducted according to Aebi (Aebi, 1984) method, using a spectrophotometer with minor modifications depending on the measurements of the substrate of the H_2O_2 residual after the catalase reaction. The H_2O_2 concentrations used in the current analysis (fifty mm) gives an assessable signal, however, it does not raise any enzyme inhibition. The catalase enzyme activity was measured as H_2O_2 micromoles decomposed per milligram per min of protein sample.

Comet Assay

The comet protocol of Blasiak et al. (Blasiak et al., 2004) was carried out with minor modifications. low-melting-point agarose was mixed with liver cells of each treatment group and then pipetted on normal melting agarose pre-coated slides. Small pieces of liver cells were pipetted on slides containing normal and low melting agarose which were then kept horizontal for 30 min at 4°C in a dark environment. Afterward, pipetting with agarose of the low melting point was done on the above slides and then the slides were left to harden at 4°C for half h. Then, the slides covered with a layer of low melting agarose were put in lysis buffer at 4°C for 60 min. Afterward, the slides were submerged in alkaline unwinding fresh buffer in a dark room at 20°C for 60 min. Then, they were transferred into electrophoresis buffer to allow the DNA tail to migrate on the 0.8 V/cm, 300 mAmps for half an hour. Then, the slides were washed with a neutralizing solution and placed in 70% ethanol for a few seconds and air-dried. Then, ethidium bromide dye was used to stain the slides which examined using Zeiss epifluorescence microscope (barrier filter 590 nm, 510–560 nm) with ×400 magnification. For each animal 100 cells were examined, then analyzed with specific software for DNA damage (TriTek corp., Comet Score, Sumerduck, VA22742).

Expression analysis of diabetes-related genes

I. RNA isolation

TRIzol® extraction Chemical (Invitrogen) was utilized to isolate the total genomic RNA of liver tissues of all treated animals. After completion of the isolation procedures, the RNA pellet was stored in DEPC treated water. To digest the potential DNA residues, the pellet of isolated RNA was treated with RNAse-free DNAse kit (Invitrogen, Germany). RNA aliquots were stored at -20°C or utilized immediately for reverse transcription (Salem et al., 2017).

II. Reverse transcription reaction

First Strand cDNA Synthesis Kit (RevertAidTM, MBI Fermentas) was used to synthesize the cDNA copy from liver tissues via reverse transcription reaction (RT). An RT reaction program of 25°C for 10 min, one hour at 42 °C, then 5 min at 95°C was used to obtain the cDNA copy of the liver genome. Finally, tubes of the reaction containing cDNA copy were collected on ice to use for cDNA amplification (Khalil et al., 2018).

III. Quantitative Real Time-PCR

SYBR® Premix Ex TaqTM kit (TaKaRa, Biotech. Co. Ltd.) was used to perform the qRT-PCR analyses using the synthesized cDNA copies from liver tissues. For each reaction, a melting curve profile was conducted**.** The quantitative values of the target genes were normalized on the expression of the housekeeping gene (Table 1). The $2^{-\Delta\Delta CT}$ method was used to determine the quantitative values of the specific genes to the β-actin gene.

Statistical analysis

General Liner Models (GLM) of Statistical Analysis System (SAS) was used to analyze the data of enzyme activities, DNA damage, and gene expression assays. Afterward, Scheffé-test was used to determine the significant differences between tested groups. The values are expressed as mean±SEM. All significance statements were based on the probability of $P < 0.05$.

Table 1: Primer sequences used for qRT-PCR amplification.

Gene	Sequence of primer (5'-----'3)		References
AKR1B10	F- CCT GGG CAC CTG GAA GTC T	R- CAT CAA TGG CTG CCT TCA CA	Ahmed et al., 2014
ING3	F- AGT GGC AGG AAG AGC AAA AA	R- TCC CAC CAT CTC TCC ATA GG	
Foxp1	F- CAG GCA GAT CCC CTA TGC AA	R- GGA CAG AGG GCC TTC AGC TT	
β-actin	F- GTG GGC CGC TCT AGG CAC CAA	R- CTC TTT GAT GTC ACG CAC GAT TTC	Khalil et al., 2018

Results

Liver function parameters determination

The effect of *S. muticum* and SmAgNPs at different doses on the ALT and AST levels of male rats exposed to DEN inducing liver cancer is summarized in Table 2. The results revealed that the levels of ALT and AST increased significantly (P<0.001) in the group of male rats exposed to DEN compared to other treated groups. However, the treatment of DEN-exposed rats to low, medium and high doses of *S. muticum* extract decreased the levels of ALT and AST (P<0.05) compared with the DEN group. Additionally, the levels of ALT and AST were much more decreased (P<0.01) in the groups of DEN-rats treated with low, medium, and high doses of SmAgNPs than the DEN group. Moreover, the levels of ALT and AST in the group treated with a high dose of SmAgNPs reached values near to those from the control group. Also, the treatment of DEN-rats with silymarin decreased significantly (P<0.01) the levels of ALT and AST compared to the DEN group.

Table 2: Liver function levels in blood cells of DEN-exposed rats treated with different doses of *S. muticum* and SmAgNPs.

Groups	ALT (U/L)	AST (U/L)
Control	58.4± 3.2^{d}	121.4± 8.3^{f}
DEN	195.3±11.6^{a}	352.6± 24.2^{a}
DEN+ *S. muticum* L	164.1±12.2^{b}	298.7± 12.3^{b}
DEN+ *S. muticum* M	112.4±16.5^{c}	249.8± 17.5^{c}
DEN+ *S. muticum* H	104.9±17.3^{c}	206.9± 13.7^{d}
DEN+ SmAgNPs L	115.3±18.1^{c}	211.5± 14.1^{d}
DEN+ SmAgNPs M	85.6±14.7^{cd}	187.6± 19.2^{d}
DEN+ SmAgNPs H	69.7±15.2^{d}	158.7± 15.2^{e}
DEN+ Silymarin	94.2±10.4^{cd}	163.2± 13.3^{e}

^{a,b,c,d,e,f}: Different small superscript letters are differing significantly; *S. muticum* L, *S. muticum* M, and *S. muticum* L: Low, medium, and high dose of *S. muticum*, respectively; SmAgNPs L, SmAgNPs M and SmAgNPs H: Low, medium, and high dose of *S. muticum* nanoparticles, respectively.

Assessment of the DNA damage

Table 3 and Figure 1 represent the measurement of the DNA damage in liver tissues of DEN-exposed rats treated with *S. muticum* and SmAgNPs at different doses. The results showed that the rates of DNA damage in the liver tissues collected from male rats exposed to DEN increased significantly (P<0.001) compared with the rats of the control group. However, DNA damage in the liver tissues of DEN-exposed rats treated with low, medium and high doses of *S. muticum* extract decreased significantly (P<0.05 and P<0.01) compared with those in DEN group. Moreover, the rates of the DNA damage were greatly decreased (P<0.001) in the groups of DEN-rats treated with low, medium, and high doses of SmAgNPs than the DEN group. Furthermore, the mean values of DNA damage in the treated group with a high dose of SmAgNPs were fairly reached values close to those from the control group. Also, the treatment of DEN-rats with silymarin decreased significantly (P<0.01) the levels of DNA damage compared to the DEN group.

Table 3: Rate of DNA damage in liver tissues of DM-induced rats treated with different doses of *S. muticum* and SmAgNPs using comet assay.

Treatment	No. of cells		Class[¥] of comet				DNA damaged cells
	Analyzed	Total comets	0	1	2	3	(mean ± SEM)
Control	500	29	471	24	5	0	5.8±0.20^{e}
DEN	500	117	383	29	36	52	23.4±0.26^{a}
DEN+ *S. muticum* L	500	83	417	28	35	20	16.6±0.36^{b}
DEN+ *S. muticum* M	500	64	436	25	27	12	12.8±0.16^{c}
DEN+ *S. muticum* H	500	57	443	20	24	13	11.4±0.33^{c}
DEN+ SmAgNPs L	500	61	439	19	26	16	12.2±0.37^{c}
DEN+ SmAgNPs M	500	41	459	21	14	6	8.2±0.29^{d}
DEN+ SmAgNPs H	500	38	462	22	11	5	7.6±0.23^{de}

DEN+ Silymarin	500	44	456	24	13	7	8.8±0.21[d]

[¥]: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus.(*): No. of cells analyzed were 100 per animal. *S. muticum* L, *S. muticum* M and *S. muticum* L: Low, medium, and high dose of *S. muticum*, respectively; SmAgNPs L, SmAgNPs M and SmAgNPs H: Low, medium, and high dose of *S. muticum* nanoparticles, respectively. [a, b, c]: Within each column, means superscripts with different letters are significantly different (P < 0.05).

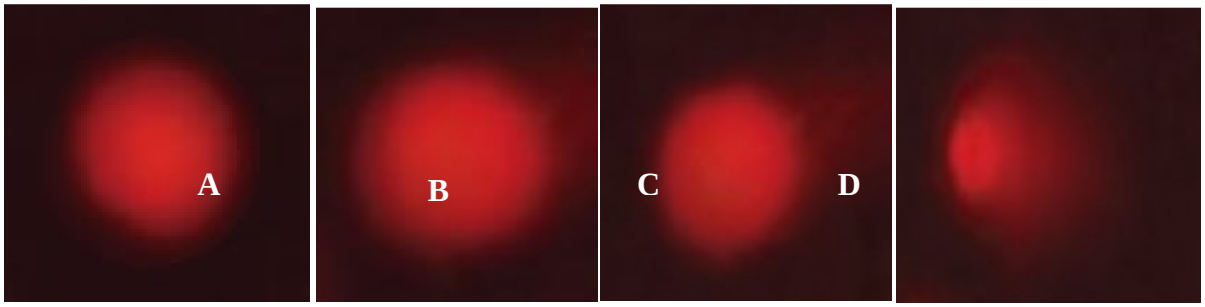

Figure 1: Classof DNA damage in liver cells of DEN-exposed rats treated with different doses of *S. muticum* and SmAgNPs. A: Class 0 represents normal cell; B: Class 1 of DNA damage; C: Class 2 of DNA damage; D: Class 3 of DNA damage.

Determination of antioxidant enzyme activities

The activities of glutathione peroxidase (GPx) and catalase (CAT) enzymes in the liver tissues of DEN-exposed rats treated with different doses of *S. muticum* and SmAgNPs are presented in Figure 2. The results found that the activity of GPx in the liver tissues collected from male rats exposed to DEN decreased significantly (P<0.05) compared with the rats of the control group. The GPx activity in the liver tissues of DEN-exposed rats treated with low and medium doses of *S. muticum* extract increased compared with those in the DEN group but without significant differences. However, the GPx activity in the liver tissues of DEN-exposed rats treated with a high dose of *S. muticum* extract increased significantly (P<0.05) compared with those in the DEN group. The GPx activity in the liver tissues of DEN-exposed rats treated with a low dose of SmAgNPs did not increase significantly compared with that in DEN. However, the activities of GPx enzyme in the group treated with medium and high doses of SmAgNPs increased significantly (P<0.01) compared with that in the DEN group. Treatment of DEN-rats with silymarin increased the activity of GPx enzyme compared with the DEN group but without significant differences.

Regarding the CAT enzyme, the results found that its activity in the liver tissues collected from male rats exposed to DEN decreased significantly (P<0.05) compared with the rats of the control group. Additionally, treatment of DEN-exposed rats with low, medium, and high doses of *S. muticum* extract slightly increased the activity of CAT without significant differences. The CAT activity in the liver tissues of DEN-exposed rats treated with low and medium doses of SmAgNPs increased compared with that in the DEN group but without significant differences. However, the activity of CAT enzyme in the group treated with a high dose of SmAgNPs increased significantly (P<0.05) compared with that in the DEN group. Treatment of DEN-rats with silymarin increased the activity of CAT enzyme compared with the DEN group but without significant differences.

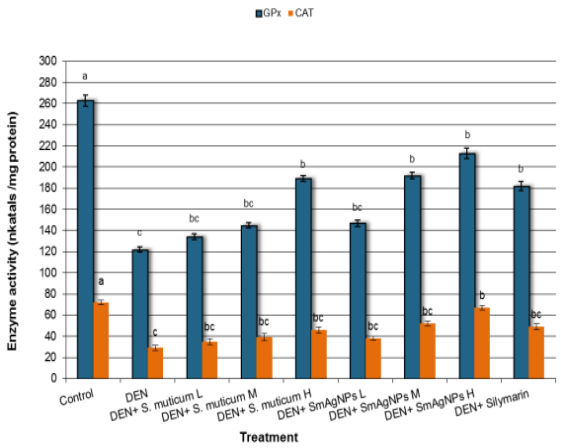

Figure 2: Glutathione peroxidase (GPx) and catalase (CAT) activities (nkatals/mg protein) in the liver tissues of DEN-exposed rats treated with different doses of *S. muticum* and SmAgNPs. *S. muticum* L, *S. muticum* M, and *S. muticum* L: Low, medium, and high dose of *S. muticum*, respectively; SmAgNPs L, SmAgNPs M and SmAgNPs H: Low, medium, and high dose of *S. muticum* nanoparticles, respectively. [a, b, c]: Within each column, means superscripts with different letters are significantly different (P < 0.05).

Expression of liver cancer-related genes

Expression levels of liver cancer-related genes including aldo-keto reductase 1B10 (Akr1b10), inhibitor of growth protein 3 (ING3) and forkhead box protein P1 (Foxp1) in liver tissues of DEN-exposed rats treated with *S. muticum* and SmAgNPs at different doses are stated in Figures 3-5. The expression levels of Akr1b10 gene in the liver tissues collected from male rats exposed to DEN decreased significantly (P<0.001) compared with the rats of the control group (Figure 3). Treatment of DEN-exposed rats treated with a low dose of *S. muticum* extract did not increase significantly the expression levels of Akr1b10 gene compared with those in the DEN group. However, the expression

levels of Akr1b10 gene in the liver tissues of DEN-exposed rats treated with medium and high doses of *S. muticum* extract increased significantly (P<0.05) compared with those in the DEN group. Moreover, the expression levels of Akr1b10 gene were markedly increased (P<0.05, P<0.01, P<0.001, respectively) in the groups of DEN-rats treated with low, medium and high doses of SmAgNPs than the DEN group. Additionally, the treatment of DEN-rats with silymarin increased significantly (P<0.001) the expression levels of Akr1b10 gene compared with the DEN group (Figure 3).

In the same line with Akr1b10 gene, expression levels of ING3 gene in the liver tissues collected from male rats exposed to DEN decreased significantly (P<0.001) compared with the rats of the control group (Figure 4). However, the expression levels of ING3 gene in the liver tissues of DEN-exposed rats treated with low, medium, and high doses of *S. muticum* extract and SmAgNPs increased significantly (P<0.05) compared with those in DEN group. Furthermore, the expression levels of ING3 gene in the group treated with a high dose of SmAgNPs fairly reached levels close to those from the control group. Additionally, the treatment of DEN-rats with silymarin increased significantly (P<0.001) the expression levels of ING3 gene compared with the DEN group (Figure 4).

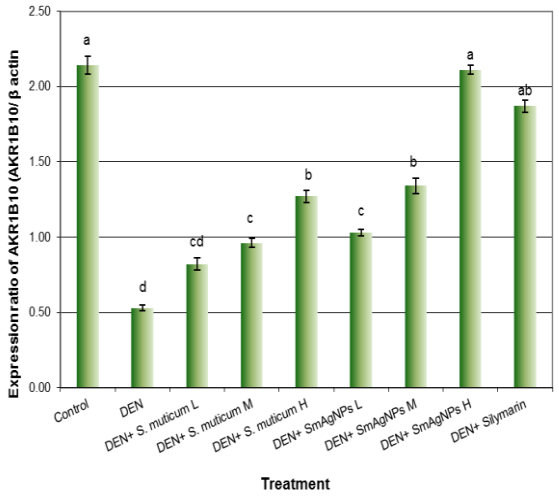

Figure 3: Quantitative values of *AKR1B10* genein the liver tissues of DEN-exposed rats treated with different doses of *S. muticum* and SmAgNPs. *S. muticum* L, *S. muticum* M, and *S. muticum* L: Low, medium, and high dose of *S. muticum*, respectively; SmAgNPs L, SmAgNPs M and SmAgNPs H: Low, medium, and high dose of *S. muticum* nanoparticles, respectively. [a, b, c]: Within each column, means superscripts with different letters are significantly different (P < 0.05).

Concerning the expression levels of Foxp1 gene in the liver tissues collected from male rats exposed to DEN increased significantly (P<0.001) compared with the rats of the control group (Figure 5). Treatment of DEN-exposed rats treated with a low dose of *S. muticum* extract did not decrease significantly the expression levels of Foxp1 gene compared with those in the DEN group. However, the expression levels of Foxp1 gene in the liver tissues of DEN-exposed rats treated with medium and high doses of *S. muticum* extract and all three doses of SmAgNPs decreased significantly compared with those in the DEN group. Additionally, the treatment of DEN-rats with silymarin decreased significantly (P<0.01) the expression levels of Foxp1 gene compared with the DEN group (Figure 5).

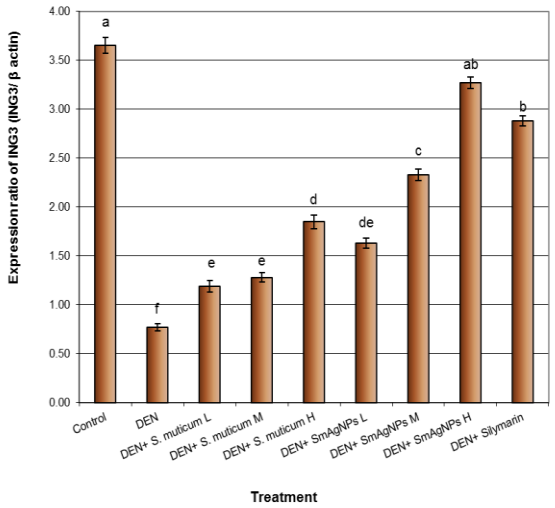

Figure 4: Quantitative values of *ING3*genein the liver tissues of DEN-exposed rats treated with different doses of *S. muticum* and SmAgNPs. *S. muticum* L, *S. muticum* M, and *S. muticum* L: Low, medium, and high dose of *S. muticum*, respectively; SmAgNPs L, SmAgNPs M, and SmAgNPs H: Low, medium, and high dose of *S. muticum* nanoparticles, respectively. [a, b, c]: Within each column, means superscripts with different letters are significantly different (P < 0.05).

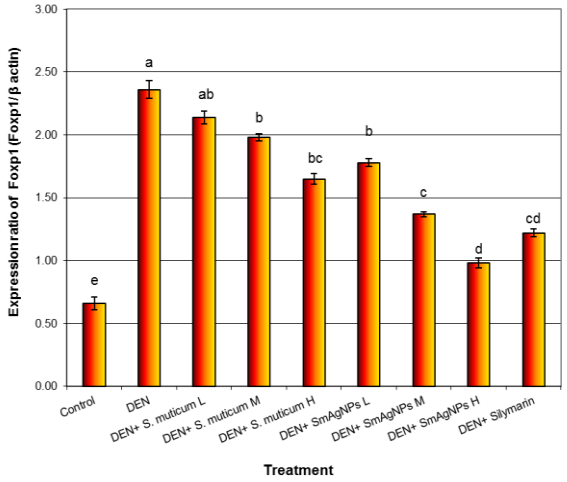

Figure 5: Quantitative values of *Foxp1*genein the liver tissues of DEN-exposed rats treated with different doses of *S. muticum* and SmAgNPs. *S. muticum* L, *S. muticum* M, and *S. muticum* L: Low, medium, and high dose of *S. muticum*, respectively; SmAgNPs L, SmAgNPs M, and SmAgNPs H: Low, medium, and high dose of *S. muticum* nanoparticles, respectively. [a, b, c]: Within each column,

means superscripts with different letters are significantly different ($P < 0.05$).

Discussion

The major primary malignant tumor of the liver (HCC), is one of the most life-threatening human cancers in the world. So, the principal goal of this study was to investigate the antitumor effect of SmAgNPs against liver cancer induced by DEN in an experimental animal model.

Hepatocellular carcinoma is considered as an aggressive malignant cancer with extremely poor diagnosis. Among all of the malignant tumors, HCC is the 7th most widespread cancer in men globally (Parkin et al., 1984). Among oncologists, there is currently agreement that the environmental factors are affecting the frequency of cancer. In this regards it has been reported surprising that between the environmental factors the nutrition regime is taking place to be responsible for cancer induction by 30 to 70% of the cancer reasons (Doll, 1990). Additionally, exposure to chemical pollution in our environment may also contribute to inducing multiple types of cancers. From these chemicals is the diethylnitrosamine (DEN) which is considered as one of the most vital environmental mutagens and carcinogens. DEN is mainly useful for the induction of liver cancer in laboratory animals due to its powerful carcinogenic activity and fairly simple metabolic pathway (Archer, 1989).

The present study found that levels of ALT and AST increased significantly in the group of male rats exposed to DEN compared to other treated groups. These findings are in the same line with Zayed Mohamed et al., 2019, who reported that DEN increased the liver function markers (ALT and AST) in DEN exposed rats. This elevation in the liver markers might be due to leakage from necrotic or damaged cells as a clear indication for HCC initiation in rats exposed to DEN (Kim et al., 2016).

On the contrary, the present study showed a decrease in the liver function markers in DEN-rats treated with SmAgNPs. These results commonly coincide with previous studies. Vazquez et al. 2012 showed that a *Sargassum sp.* aqueous extract decreased AST and ALT activities in rats treated with acetyl-para-aminophenol (APAP). Moreover, Hira et al. 2016 evaluated several extracts of *Sargassum* species such as *Sargassum binderi*, *Sargassum variegatum*, and *Sargassum terrarium* in an *in vivo* model. They found that ethanol extracts of *Sargassum sp.* exhibited high hepatoprotective activity. Raghavendran et al., 2004, found that the extract of *Sargassum polycystum* decreased the activities of AST and ALT in rats treated with acetyl-para-aminophenol induced liver damage compared to the positive control, and suggested that *Sargassum polycystum* has the hepatoprotective capability. The current study found that rates of DNA damage were increased in rats exposed to DEN which were in agreement with the results of Sadek et al. 2017, who observed that rates of DNA damage 3-fold increased in rats exposed to DEN compared to the damage levels of the DNA found in control group. One of the most important reasons caused DNA damage is the oxidation process which induces the cell to abnormal divide and prompts cancer (Hagen et al., 1994). Additionally, the carcinogenic capability of DEN in the liver tumor of rats might be attributed to its effect on changing DNA structure through inducing DNA adducts, micronuclei formation, and chromosome aberrations creation (Al-Rejaie et al., 2009).

On the other hand, the results of this study showed that the treatment of DEN-exposed rats with SmAgNPs decreased significantly the rates of DNA damage in the liver tissues compared with the DEN group. In the same line with these results, Ayyad et al. (Ayyad et al., 2011) indicated that *Sargassum* sp. prevented the DNA damage in the mammalian cells. They reported that *Sargassum* sp. has the antioxidant capacity and could be used as an antitumor compound. The efficiency of *Sargassum* sp. for protecting the DNA from damage is attributed to its active compounds, fucosterol and fucoxanthin, especially the last one.

The activity of the antioxidant enzymes (GPx and CAT) in the present study were decreased significantly in male rats exposed to DEN. The same results were also reported by Zayed Mohamed et al. 2019 and Sadek et al. 2017, who found that activity of GPx and CAT, as well as GSH and GSH-related antioxidant enzymes, were markedly declined in the group of rats exposed to DEN. These antioxidant enzymes play an important role in cancer suppression. These enzymes are acting on decrease in the generation of ROS species due to changing harmful H_2O_2 form to H_2O forms (Vasquez-Garzon et al., 2009). So, in the current study, DEN resulted in a significant decrease in the levels of GPx and CAT activities which may have resulted from the extreme lipid peroxidation (LPO) formed during the metabolism of DEN.

Thus, the antioxidants could act by inhibiting lipid peroxidation and ROS scavenging. It has been reported also that endogenous SOD, CAT, and GPx promote the cells to be protected against ROS (Basu, 2003). Furthermore, in comparison to other marine algae families, the Sargassaceae family has been found to reveal very high scavenging activity against free radicals (Basu, 2003). The present study found that antioxidant activities of GPx and CAT increased significantly in DEN-rats treated with nanoparticles of *Sargassum muticum* compared with the DEN group. These results were also consistent with the study of Balboa et al., 2019, which reported that *Sargassum muticum* extract has a high antioxidant capacity and increased the activity of antioxidant enzymes.

Several genes are playing an important role in the biological mechanism of HCC. For that reason, we selected three important genes including Akr1b10, ING3, and FoxP1. The gene encoding enzyme Akr1b10 is an important molecular biomarker candidate for HCC profiling. Identification of the expression patterns of this gene is very important especially in cirrhosis of liver or liver infected with virus hepatitis C. So, this gene could present a novel therapeutic target for multimodal therapy regimes. ING3 gene is an essential marker for HCC development and diagnosis and could be used in the therapeutic system. It is encoding a tumor suppressor protein which prevents cell growth and induction of apoptosis through interaction with TP53. FoxP1 gene has been

showed high expression levels in numerous kinds of cancers and has been coincided with the development of metastasis. Interestingly, it has been reported that FoxP1 gene acts as a tumor inhibitor candidate and also possible oncogene due to its differential levels of expression in several types of tumors (Ahmed et al., 2014). The present study found that male rats exposed to DEN showed a decrease in the expression levels of Akr1b10 and ING3 genes, while the expression levels of FoxP1 gene increased. However, the treatment of DEN-rats with silver nanoparticles of *Sargassum muticum* increased the expression of tumor suppressor genes (Akr1b10 and ING3) and decreased the expression of the oncogene gene (FoxP1) explaining that SmAgNPshas antitumor activity through alteration of the liver cancer-related genes.

Conclusion: *S. muticum* silver nanoparticles exhibited high competence for protecting the DNA structure from damage and increase the antioxidant activities as well as increase the over-expression of tumor suppressor genes which is attributed to its rich active compounds of fucoxanthin (as marine carotenoids) and fucosterol (marine sterol). Further research on the active compounds of *S. muticum* should be conducted to understand the biological mechanism versus liver cancer prevention.

References

Aebi, H.(1984). Catalase in vitro. *Methods Enzymol*, 105, 121–126.

Ahmed, H. H., Khalil, W. K., & Hamza, A. H. (2014). Molecular mechanisms of Nano-selenium in mitigating hepatocellular carcinoma induced by N-nitrosodiethylamine (NDEA) in rats. *Toxicology mechanisms and methods*, *24*(8), 593-602.

Al-Rejaie, S. S., Aleisa, A. M., Al-Yahya, A. A., Bakheet, S. A., Alsheikh, A., Fatani, A. G., ... & Sayed-Ahmed, M. M. (2009). Progression of diethylnitrosamine-induced hepatic carcinogenesis in carnitine-depleted rats. *World journal of gastroenterology: WJG*, *15*(11), 1373.

Alter, M. J. (2007). Epidemiology of hepatitis C virus infection. *World journal of gastroenterology: WJG*, *13*(17), 2436.

Archer, M. C. (1989). Mechanisms of action of N-nitroso compounds. *Cancer surveys*, *8*(2), 241-250.

Aziz AT, Alshehri MA, Panneerselvam C, Murugan K, Trivedi S, Mahyoub JA, Hassan MM, Maggi F, Sut S, Dall'Acqua S, Canale A, Benelli G. The desert wormwood (Artemisia herba-alba) - From Arabian folk medicine to a source of green and effective nanoinsecticides against mosquito vectors. J Photochem Photobiol B. 2018; 180:225-234.

Ayyad, S. E. N., Ezmirly, S. T., Basaif, S. A., Alarif, W. M., Badria, A. F., & Badria, F. A. (2011). Antioxidant, cytotoxic, antitumor, and protective DNA damage metabolites from the red sea brown alga Sargassum sp. *Pharmacognosy research*, *3*(3), 160.

Balboa, E. M., Millán, R., Domínguez, H., & Taboada, C. (2019). Sargassum muticum Hydrothermal Extract: Effects on Serum Parameters and Antioxidant Activity in Rats. *Applied Sciences*, *9*(12), 2570.

Basu, S. (2003). Carbon tetrachloride-induced lipid peroxidation: eicosanoid formation and their regulation by antioxidant nutrients. *Toxicology*, *189*(1-2), 113-127.

Baumeister, T. U., Staudinger, M., Wirgenings, M., & Pohnert, G. (2019). Halogenated anilines as novel natural products from a marine biofilm forming microalga. *Chemical Communications*, *55*(79), 11948-11951.

Bhattacharyya, S. S., Paul, S., & Khuda-Bukhsh, A. R. (2010). Encapsulated plant extract (Gelsemium sempervirens) poly (lactide-co-glycolide) nanoparticles enhance cellular uptake and increase bioactivity in vitro. *Experimental Biology and Medicine*, *235*(6), 678-688.

Blasiak, J., Arabski, M., Krupa, R., Wozniak, K., Zadrozny, M., Kasznicki, J., ... & Drzewoski, J. (2004). DNA damage and repair in type 2 diabetes mellitus. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *554*(1-2), 297-304.

Bosch, F. X., Ribes, J., Díaz, M., & Cléries, R. (2004). Primary liver cancer: worldwide incidence and trends. *Gastroenterology*, *127*(5), S5-S16.

Bousquet, M. S., Ratnayake, R., Pope, J. L., Chen, Q. Y., Zhu, F., Chen, S., ... & Luesch, H. (2019). Seaweed natural products modify the host inflammatory response via Nrf2 signaling and alter colon microbiota composition and gene expression. *Free Radical Biology and Medicine*. pii: S0891-5849(19)30996-7.

Daw, M. A., & Dau, A. A. (2012). Hepatitis C virus in Arab world: a state of concern. *The Scientific World Journal*, *2012*.

de Souza, L. A. R., Dore, C. M. P., Castro, A. J., de Azevedo, T. C., de Oliveira, M. T. B., Maria de Fátima, V. M., ... & Leite, E. L. (2012). Galactans from the red seaweed Amansia multifida and their effects on inflammation, angiogenesis, coagulation and cell viability. *Biomedicine & Preventive Nutrition*, *2*(3), 154-162.

Deslandes, E., Pondaven, P., Auperin, T., Guézennec, J., Stiger, V., & Payri, C. (2000). Preliminary study of the in vitro antiproliferative effect of a hydroethanolic extract from the subtropical seaweed Turbinaria ornata (Turner) J. Agardh on a human non-small-cell bronchopulmonary carcinoma line (NSCLC-N6). *Journal of applied Phycology*, *12*(3-5), 257-262.

Doll R. (1990). Symposium on diet and cancer. Proc Nutr Soc. 49:119–31.

Fasani, P., Sangiovanni, A., De Fazio, C., Borzio, M., Bruno, S., Ronchi, G., ... & Colombo, M. (1999). High prevalence of multinodular hepatocellular carcinoma in patients with cirrhosis attributable to multiple risk factors. *Hepatology*, *29*(6), 1704-1707.

Fernández, L. E., Valiente, O. G., Mainardi, V., Bello, J. L., Vélez, H., & Rosado, A. (1989). Isolation and characterization of an antitumor active agar-type polysaccharide of Gracilaria dominguensis. *Carbohydrate research*, *190*(1), 77-83.

Funahashi, H., Imai, T., Mase, T., Sekiya, M., Yokoi, K., Hayashi, H., ... & Hibi, Y. (2001). Seaweed prevents breast cancer?. *Japanese journal of cancer research*, *92*(5), 483-487.

Gomez-Gutierrez, C. M., Guerra-Rivas, G., Soria-Mercado, I. E., & Ayala-Sánchez, N. E. (2011). Marine edible algae as disease preventers. In *Advances in food and nutrition research* (Vol. 64, pp. 29-39). Academic Press.

Gupta, S., & Abu-Ghannam, N. (2011). Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. *Innovative Food Science & Emerging Technologies*, *12*(4), 600-609.

Hagen, T. M., Huang, S., Curnutte, J., Fowler, P., Martinez, V., Wehr, C. M., ... & Chisari, F. V. (1994). Extensive oxidative DNA damage in hepatocytes of transgenic mice with chronic active hepatitis destined to develop hepatocellular carcinoma. *Proceedings of the National Academy of Sciences*, *91*(26), 12808-12812.

Hermund, D. B., Karadağ, A., Andersen, U., Jónsdóttir, R., Kristinsson, H. G., Alasalvar, C., & Jacobsen, C. (2016). Oxidative stability of granola bars enriched with multilayered fish oil emulsion in the presence of novel brown seaweed based antioxidants. *Journal of agricultural and food chemistry*, *64*(44), 8359-8368.

Hira, K., Sultana, V., Ara, J., Ehteshamul-Haque, S., & Athar, M. (2016). Hepatoprotective potential of three sargassum species from Karachi coast against carbon tetrachloride and acetaminophen intoxication. *Journal of Coastal Life Medicine*, *4*(1), 10-13.

Huang, X. J., Choi, Y. K., Im, H. S., Yarimaga, O., Yoon, E., & Kim, H. S. (2006). Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. *Sensors*, *6*(7), 756-782.

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA: a cancer journal for clinicians*, *61*(2), 69-90.

Jeon, H., Yoon, W. J., Ham, Y. M., Yoon, S. A., & Kang, S. C. (2019). Anti-Arthritis Effect through the Anti-Inflammatory Effect of Sargassum muticum Extract in Collagen-Induced Arthritic (CIA) Mice. *Molecules*, *24*(2), 276.

Karakoti, A. S., Hench, L. L., & Seal, S. (2006). The potential toxicity of nanomaterials—the role of surfaces. *Jom*, *58*(7), 77-82.

Khalil, W. K. B., Booles, H. F., Hafiz, N. A., & El-Bassyouni, G. E. (2018). Ameliorative effects of Brachidontes variabilis calcium carbonate against bone loss in ovariectomized rats. *International Journal of Pharmacology*, *14*, 477-487.

Khoja T. (2011). Council of Health ministries of GCC, the Gulf center for cancer registration, 10 years incidence of cancers among nationals of GCC states Sep. 32-34.

Kim, N. H., Heo, J. D., Kim, T. B., Rho, J. R., Yang, M. H., & Jeong, E. J. (2016). Protective effects of ethyl acetate soluble fraction of Limonium tetragonum on diethylnitrosamine-induced liver fibrosis in rats. *Biological and Pharmaceutical Bulletin*, *39*(6), 1022-1028.

Kwon, H. J., Bae, S. Y., Kim, K. H., Han, C. H., Cho, S. H., Nam, S. W., ... & Kim, B. W. (2007). Induction of apoptosis in HeLa cells by ethanolic extract of Corallina pilulifera. *Food chemistry*, *104*(1), 196-201.

Lehman, E. M., & Wilson, M. L. (2009). Epidemiology of hepatitis viruses among hepatocellular carcinoma cases and healthy people in Egypt: A systematic review and meta-analysis. *International journal of cancer*, *124*(3), 690-697.

Liu, L., Heinrich, M., Myers, S., & Dworjanyn, S. A. (2012). Towards a better understanding of medicinal uses of the brown seaweed Sargassum in Traditional Chinese Medicine: A phytochemical and pharmacological review. *Journal of ethnopharmacology*, *142*(3), 591-619.

Mabeau, S., & Fleurence, J. (1993). Seaweed in food products: biochemical and nutritional aspects. *Trends in Food Science & Technology*, *4*(4), 103-107.

Marrero, J. A., Fontana, R. J., Barrat, A., Askari, F., Conjeevaram, H. S., Su, G. L., & Lok, A. S. (2005). Prognosis of hepatocellular carcinoma: comparison of 7 staging systems in an American cohort. *Hepatology*, *41*(4), 707-715.

Mayer, A. M., & Gustafson, K. R. (2006). Marine pharmacology in 2003–2004: anti-tumour and cytotoxic compounds. *European Journal of Cancer*, *42*(14), 2241-2270.

Mentella, M. C., Scaldaferri, F., Ricci, C., Gasbarrini, A., & Miggiano, G. A. D. (2019). Cancer and Mediterranean Diet: A Review, 11(9), 2059.

Mohamed, N. Z., Aly, H. F., moneim El-Mezayen, H. A., & El-Salamony, H. E. (2019). Effect of co-administration of Bee honey and some chemotherapeutic drugs on dissemination of hepatocellular carcinoma in rats. *Toxicology reports*, *6*, 875-888.

Mohamed, S., Hashim, S. N., & Rahman, H. A. (2012). Seaweeds: a sustainable functional food for complementary and alternative therapy. *Trends in Food Science & Technology*, *23*(2), 83-96.

Muñoz, N., & Bosch, X. (1987). Epidemiology of hepatocellular carcinoma. In *Neoplasms of the Liver* (pp. 3-19). Springer, Tokyo.

Namvar, F., Mohamed, S., Fard, S. G., Behravan, J., Mustapha, N. M., Alitheen, N. B. M., & Othman, F. (2012). Polyphenol-rich seaweed (Eucheuma cottonii) extract suppresses breast tumour via hormone modulation and apoptosis induction. *Food chemistry*, *130*(2), 376-382.

Noda, H., Amano, H., Arashima, K., & Nisizawa, K. (1990). Antitumor activity of marine algae. *Hydrobiologia*, *204*(1), 577-584.

Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal of laboratory and clinical medicine*, *70*(1), 158-169.

Palanisamy, S., Vinosha, M., Manikandakrishnan, M., Anjali, R., Rajasekar, P., Marudhupandi, T., ... & Prabhu, N. M. (2018). Investigation of antioxidant and anticancer potential of fucoidan from Sargassum polycystum. *International journal of biological macromolecules*, *116*, 151-161.

Parkin, D. M., Stjernswärd, J., & Muir, C. S. (1984). Estimates of the worldwide frequency of twelve major cancers. *Bulletin of the World Health Organization*, *62*(2), 163.

Prasannaraj, G., & Venkatachalam, P. (2017). Hepatoprotective effect of engineered silver nanoparticles coated bioactive compounds against diethylnitrosamine induced hepatocarcinogenesis in experimental mice. *Journal of Photochemistry and Photobiology B: Biology*, *167*, 309-320.

Raghavendran, H. R. B., Sathivel, A., & Devaki, T. (2004). Hepatoprotective nature of seaweed alcoholic extract on acetaminophen induced hepatic oxidative stress. *Journal of health science*, *50*(1), 42-46. doi: 10.1248/jhs.50.42.

Raghavendran, H. R. B., Sathivel, A., & Devaki, T. (2005). Protective effect of Sargassum polycystum (brown alga) against acetaminophen-induced lipid peroxidation in rats. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *19*(2), 113-115.

Rasul, K. I., Al-Azawi, S. H., & Chandra, P. (2013). Hepatocellular carcinoma in qatar. *Gulf J Oncolog*, *1*(14), 70-5.

Rasul, K. I., Saleh, M. M., & Bener, A. (2010). Epidemiology of cancer in Qatar. *Asian Pacific Organization for Cancer Prevention (APOCP) Cancer Report*, *391*.

Ribes, J., Clèries, R., Borràs, J., Galceran, J., & Bosch, F. X. (2004). Time trends in incidence and mortality for chronic liver disease and liver cancer in the interval 1980–1997 in Catalonia, Spain. *European journal of gastroenterology & hepatology*, *16*(9), 865-872.

Sadek, K. M., Abouzed, T. K., Abouelkhair, R., & Nasr, S. (2017). The chemo-prophylactic efficacy of an ethanol Moringa oleifera leaf extract against hepatocellular carcinoma in rats. *Pharmaceutical biology*, *55*(1), 1458-1466.

Salem, N. A., Wahba, M. A., Eisa, W. H., El-Shamarka, M., & Khalil, W. (2018). Silver oxide nanoparticles alleviate indomethacin-induced gastric injury: a novel antiulcer agent. *Inflammopharmacology*, *26*(4), 1025-1035.

Schumacher, M., Kelkel, M., Dicato, M., & Diederich, M. (2011). Gold from the sea: marine compounds as inhibitors of the hallmarks of cancer. *Biotechnology advances*, *29*(5), 531-547.

Taboada, C., Millan, R., & Miguez, I. (2013). Evaluation of marine algae Undaria pinnatifida and Porphyra purpurea as a food supplement: Composition, nutritional value and effect of intake on intestinal, hepatic and renal enzyme activities in rats. *Journal of the Science of Food and Agriculture*, *93*(8), 1863-1868.

Vásquez-Garzón, V. R., Arellanes-Robledo, J., Garcia-Roman, R., Aparicio-Rautista, D. I., & Villa-Treviño, S. (2009). Inhibition of reactive oxygen species and pre-neoplastic lesions by quercetin through an antioxidant defense mechanism. *Free radical research*, *43*(2), 128-137.

Yuan, Y. V., & Walsh, N. A. (2006). Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. *Food and chemical toxicology*, *44*(7), 1144-1150.

Zhang, W., Hong, R., & Tian, T. (2013). Silymarin's protective effects and possible mechanisms on alcoholic fatty liver for rats. *Biomolecules & therapeutics*, *21*(4), 264–269.