Biomedicine & Pharmacotherapy 87 (2017) 296–301

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

The properties of red seaweed (*Kappaphycus alvarezii*) and its effect on mammary carcinogenesis

Vi-Sion Chang, Patrick N. Okechukwu, Swee-Sen Teo*

Department of Biotechnology, Faculty of Applied Sciences, UCSI University, No. 1, Jalan Menara Gading, UCSI Height, Cheras, 56000, Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:
Received 24 October 2016
Received in revised form 5 December 2016
Accepted 22 December 2016

Keywords:
Red seaweed
Kappaphycus alvarezii
Breast cancer
Toxicity

ABSTRACT

The edible red seaweed (*Kappaphycus alvarezii*) is one of the algae species which was found to be rich in nutrients and nutraceutical. Hence, *K. alvarezii* may have the ability to suppress cancer through its antiproliferative properties. The aim of this study was to investigate the potential compounds of *K. alvarezii*, cytotoxicity properties of *K. alvarezii* extract on breast cancer cell line (MCF-7), investigated toxicity effect of high dosage *K. alvarezii* extract in rats and determined the effect of *K. alvarezii* on 7, 12-dimethylbenz[a]anthracene (DMBA) mammary carcinogenesis in rats. The method of LCMS/MS and MTT assay were used. For animal study, sub-chronic toxicity method was used, the rats were supplemented with 2000 mg/kg body weight daily of *K. alvarezii* crude extracts by oral gavage. For the anticancer effect of *K. alvarezii* crude extracts, this study consisted of three groups of the experimental, untreated and normal group of rats. The experimental and untreated groups of rats were induced with mammary tumour with DMBA. The experimental group of rats was given with *K. alvarezii* crude extracts orally. The results were being used to compare with the untreated group of rats and normal group of rats. All the rats were fed with standard diet and water *ad libitum*. Mortality, behavior changes and tumour sizes were observed specifically. The differences between the three groups of rats were evaluated by using the ANOVA test. By using LCMS/MS method, six unknown compounds were analysed. *K. alvarezii* crude extract reduced the cell viability of MCF-7 from 84.91% to 0.81% and the IC_{50} value is 4.1 ± 0.69 mg/mL. For sub-chronic and heavy metal toxicity studies, no significant difference was found in haematological and biochemical values of the control group and experimental group. The growth rate of tumours in the untreated group of rats was found significantly higher than the experimental group of rats. Besides that, the white blood cells level in untreated group was found significantly higher than the experimental group and the normal group. In conclusion, *K. alvarezii* extract might able to slow down the growth rate of the tumour cells, therefore, identification of an active compound of inhibition growth rate of the tumour cells can be positively carried out in the future.

1. Introduction

Red seaweeds (Rhodophyta) can usually be found in a wide range of shoreline habitats and has about 6000 diversity species which is the highest among all three types of seaweeds. Previously, it is reported that there are about 2.8 million tons of red seaweeds being harvested annually. Species that are harvested for consumption purpose such as nori are principally from the genera *Porphyra*, whereas for carrageenan production, species involved includes *Eucheuma* and *Kappaphycus* [1]. One of the species abundantly found in East Malaysia is *Kappaphycus alvarezii*.

K. alvarezii is one of the main seaweed which cultivated in the world especially country such as Malaysia, Philippines, Indonesia and Tanzania [2]. *K. alvarezii* is one of an important red tropical seaweed which with highly demanded its cell wall polysaccharide, it is the most important source of kappa carrageenan [3]. Kappa carrageenan is a hydrocolloid that used as a food additive, acting as a gelling, emulsifying, thickening and stabilizing agent in both pharmaceutical and nutraceutical products [4].

Seaweed is found to be low in calorie content, high fiber and mineral content, significant amount of protein, vitamins, trace elements and a wide range of second metabolites not found in other organisms. Besides, *K. alvarezii* is proposed potentially can be used as dietary fiber, cholesterol reducer, a source of antioxidant, anti-viral and anti-cancer compounds, and hemagglutination activity [5,6].

* Corresponding author.
E-mail address: teoss@ucsiuniversity.edu.my (S.-S. Teo).

http://dx.doi.org/10.1016/j.biopha.2016.12.092
0753-3322/

K. alvarezii is rich in protein (16.2% w/w), fiber (29.4% w/w) and carbohydrates (27.4% w/w), with a high proportion of unsaturated fatty acids (44.5% of the total; 11.0% oleic acid, 13.5% cisheptadecenoic acid, 2.3% linoleic acid) and saturated fatty acids (37.0%, composed mainly of heptadecanoic acid) [7]. Besides, the carrageenan that found in *K. alvarezii* can act as a dietary fiber, clearing the digestive system, protecting the stomach surface membrane, and preventing the effects of potential carcinogens on the intestine. Besides, it also shows a good antiviral activity in against enveloped viruses, including human pathogens such as HIV (immunodeficiency virus), HSV (herpes simplex virus) and HCMV (cytomegalovirus). *K. alvarezii* is also found potentially in anti-tumour activity [8,9]. The aim of this study was to investigate the potential compounds of *K. alvarezii*, cytotoxicity properties of *K. alvarezii* extracts on breast cancer cell line (MCF-7), investigated toxicity effect of high dosage *K. alvarezii* extracts in rats and determined the effect of *K. alvarezii* on 7, 12-dimethylbenz[a] anthracene (DMBA) mammary carcinogenesis in rats.

2. Materials and methods

2.1. Sample preparation

K. alvarezii was obtained from Semporna, Sabah, Malaysia. The seaweed sample was washed and dried at 40 °C for 3 days. The dried seaweed was ground with liquid nitrogen [10]. A total of 10 g samples was ground and added into 100 mL 70% methanol and incubated for 2 h at room temperature with stirring at 200 rpm. The solvent extracts were filtered and the filtrate was concentrated by rotary evaporation at 40 °C. After the evaporation process, the resulting extract was kept in −20 °C for further usage.

2.2. Liquid chromatography-mass spectrometry

K. alvarezii crude extracts was fully scanned by using LCMS/MS method. The *K. alvarezii* crude extracts was diluted with 2 mL of methanol and filtered through nylon 0.22 μM. In a total of 20.0 μL of sample was injected for the analysis. The gradient runs program was set as 10% of buffer mixture (acetonitrile with 0.1% formic acid and 5 mM ammonium formate) to 90% of the buffer from 0.01 min to 8.0 min which hold for 3 min and back to 10% of the buffer in 0.1 min and re-equilibrated for 5 min. The column used was Aglient Zorbax C18, 15 mm × 4.6 mm × 5 μm. For this sample, negative ionization mode was used. AB Sciex 2100QTrap LCMS/MS with Perkin Elmer FX 15 uHPLC system with gradients program 10% B buffer to 90% B buffer from 0.01 min to 8.0 min, holds for 3 min and back to 10% B buffer in 0.1 min and re-equilibrated for 5 min.

2.3. Cell viability test with MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay

MCF-7 cells were counted by using cell quantitation with trypan blue exclusion assay and 1×10^4 cells were seeded into per well with total volume of 200.0 μL of MEM except for blank set. The 96 wells plate was then incubated overnight in a CO_2 incubator (37 °C, 5.0% CO_2 air humidified). Then, the spent cultured medium was removed from each well. Different concentration of *K. alvarezii* crude extracts (200.0 μL) was added into the well accordingly. The concentrations (1.0, 3.0, 5.0, 7.0, 9.0, 12.0 and 15.0 mg/mL) of the *K. alvarezii* crude extracts was prepared. The 96 wells plate was then incubated in a CO_2 incubator for 24 h. After 24 h, 50.0 μL of MTT reagent was added to each of the wells. The plate was then incubated for another 4 h. Next, DMSO was added to each well to dissolve the crystals. The absorbance was measured at 570 nm. The percentage of cell viability was calculated according to the following equation:

$$\text{Percentage of cell viability (\%)} = (\text{absorbance of sample}/\text{absorbance of solvent control}) \times 100 \quad (1)$$

2.4. Animal ethic declaration

All the animal experiment complied with the ARRIVE guideline and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986. Ethical clearance for research has been approved by UCSI University research committee (Proj-FAS-EC-13-031).

2.5. Sub-chronic toxicity

Adult male and female Sprague-Dawley rats (10 rats for each gender) were obtained. Each gender of the rat was divided into 2 groups i.e. test group (5) and control group (5). As OECD guidelines, the test group animal was supplemented with *K. alvarezii* crude extracts (2000 mg/kg body weight). The rats were supplemented with *K. alvarezii* crude extracts for 60 days. The rats were observed for physical changes in the body like hair fall, necrosis, infection, and overall activeness. The rats were fed with standard diet and water *ad libitium*. The blood samples that were kept in EDTA tubes were used for full blood count, whereas the blood samples that were kept in empty tubes were used for biochemical parameters, mineral and enzymes analysis (Table 1).

2.5.1. Assessment of heavy metals in organ samples

Liver from each of the animals was separated and rinsed with cold PBS solution. The samples were frozen in liquid nitrogen and stored in −80 °C freezers until heavy metal analysis was carried out by using AAS method. The analysis was carried out by using AOAC official method 999.10 to determine the heavy metals contents and other elements in the liver. The test parameters were Cadmium, Arsenic, Chromium, Iron, Manganese, Lead, Mercury, Nickel, Selenium and Zinc. All the test parameters were expressed in ppm.

2.6. K. alvarezii *effect on mammary tumour animal model*

Twenty (6 weeks old) female Sprague-Dawley rats were obtained. The rats were randomly assigned to negative control, positive control and test groups. Each of the groups consisted of 5 rats.

The positive control and test groups animals were initially induced to developed mammary tumour with 65 mg/kg of DMBA by oral gavage. A single dose of DMBA would produce mammary tumours [11]. DMBA was weighed and dissolved in olive oil. The *K. alvarezii* crude extracts was only given after tumour has developed.

Table 1
Biochemical parameters analysed by using serum.

Biochemical test	Biochemical parameters
Minerals	Sodium, potassium, chloride, phosphorus, calcium, and magnesium
Biochemical parameters	Glucose, cholesterol, total protein, urea nitrogen, albumin, globulin
Enzymes	Alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase

Table 2
LC–MS analytes, retention times (RT), and main product ions.

Peak No	Retention time (min)	$[M-H]^-$ (*m*/*z*)	Main Product ions (*m*/*z*)	Tentative Identification
1	4.60	371.20	353.20, 327.20, 269.10, 243.20, 225.20, 199.20, 197.10, 182.10, 100.10, 83.10, 81.10	Unknown
2	4.73	487.30	451.40, 225.20	Unknown
3	6.05	713.50	677.50, 649.60, 225.10	Unknown
4	8.17	399.20	335.20, 301.30, 287.00, 269.10, 257.30, 199.10, 119.90, 97.00, 82.00, 80.00, 77.40, 69.30, 64.00	Unknown
5	9.49	527.30	299.00, 225.00, 207.00, 165.00, 149.00, 81.00	Unknown
6	11.07	825.60	559.60, 537.05, 225.00, 165.00	Unknown

The test group animal was given *K. alvarezii* crude extracts with dosage 300 mg/kg for 11 weeks. The dosage of 300 mg/kg was suggested by researchers [12–15]. The *K. alvarezii* crude extracts was dissolved with Millipore water and was prepared fresh. All the rats were given normal diet and water for the entire of the study.

The rats were palpated every week after DMBA administration. A tumour is defined as a discrete palpable mass that is recorded for at least 2 consecutive weeks. Sites and approximate size of mammary tumours were recorded. If any tumour became large (3 cm) and ulcerated, or the animal became critically ill, the rat was sacrificed early. All the surviving rats were killed 11 weeks after tumours developed. The rats were sacrificed by cervical dislocation and the blood was collected and stored in EDTA tube. All the EDTA tubes were stored in −80 °C freezers for further analysis. Tumour volume and specific growth rate were calculated by using Eqs. (2) and (3) respectively.

$$\text{Tumour volume } (mm^3) = (a \times b^2)/2 \quad (2)$$

Where a = longest diameter of tumour (mm)
b = shortest diameter of tumour (mm)

$$\text{Specific growth rate } (mm^{3}/t) = (\ln(V_2/V_1)/(t_2 - t_1)) \quad (3)$$

Where V1 and V2 are two tumour volume estimates at two different occasions.

2.6.1. Biochemistry analysis

Biochemistry analysis for the rat's blood sample carried out and the parameters were red blood cells, white blood cells, neutrophils, lymphocytes, glucose, cholesterol, total protein, calcium and phosphorus.

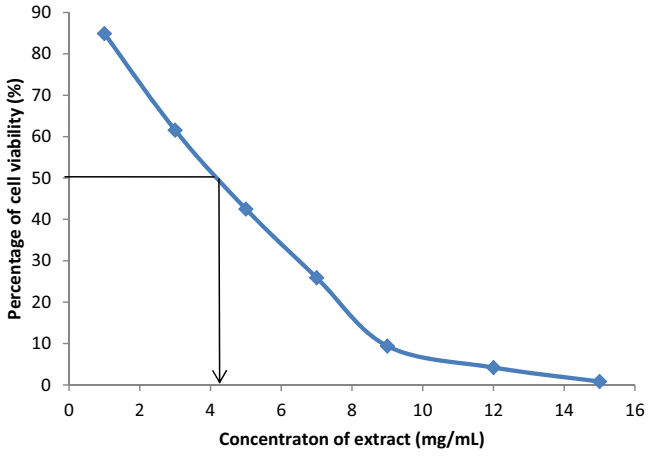

Fig. 1. Graph of percentage of cell viability versus concentration of *K. alvarezii* extract. The graph shows a descending graph where the cell viability decreases when the concentration increases.

3. Results

3.1. LCMS

Different compounds were found in *K. alvarezii*, the chromatogram from LCMS/MS shown different peak and there are six peaks were chosen as the study compound from the full chromatogram. There are only six peaks was chosen to study is because of from 9 min onwards most of the peak are matrices where the major interferences are from the sample matrices itself. Matrix refers to the components of a sample other than analyte. The detail of each peak is shows in Table 2.

3.2. Cytotoxicity properties of K. alvarezii extract

MTT assay was used to test for the cell viability of MCF-7 cell lines after treated with various concentration of *K. alvarezii* extract. *K. alvarezii* extract started to inhibit cell growth at 1.0 mg/mL where it shows a reduction of cells compared to the solvent control. The concentrations of *K. alvarezii* extract were 1.0 mg/mL, 3.0 mg/mL, 5.0 mg/mL, 7.0 mg/mL, 9.0 mg/mL, 12.0 mg/mL and 15.0 mg/mL. The percentage of cell viability of MCF-7 was reduced from 84.91% to 0.81% which showed a significant reduction of cell viability when the concentration of *K. alvarezii* extract is increased. Based on the observation, *K. alvarezii* extract is able to induce the apoptosis of the MCF-7. Fig. 1 shows the Graph of percentage cell viability versus concentration of *K. alvarezii* extract. The IC_{50} values for *K. alvarezii* extracts is 4.100 ± 0.690 mg/mL which means *K. alvarezii* extract able to reduce 50% of cell at the concentration of 4.100 ± 0.690 mg/mL.

3.3. Sub-chronic toxicity and heavy metal toxicity

The haematological values the 60th day of normal rats and rats that was fed with *K. alvarezii* extracts is presented in Table 3. There was no significant difference found in haematological parameters. The haematological parameters between the control and the treated group analysed were Red Blood Corpuscles (RBC), White Blood Corpuscles (WBC), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC).

Table 3
Hematological values of rats.

Parameters	Control group	Experimental group	Treatment effect
Hemoglobin (g/dL)	14.200 ± 0.780	13.600 ± 0.760	NS
RBC ($\times 10^{12}$/L)	7.700 ± 0.510	7.400 ± 0.450	NS
WBC ($\times 10^{9}$/L)	4.700 ± 2.300	5.900 ± 3.440	NS
PCV (%)	45.800 ± 2.170	44.000 ± 3.320	NS
MCV (fL)	59.400 ± 3.130	59.800 ± 1.790	NS
MCH (pg)	18.600 ± 0.550	18.600 ± 0.550	NS
MCHC (g/dL)	31.100 ± 1.420	30.900 ± 1.260	NS

$P < 0.05$, as compared to control group and Values are mean ± SD of n = 5 animals in each group. NS = Not significant.

Table 4
Biochemical values of rats.

Parameters	Control group	Experimental group	Treatment effect
Glucose (mmol/L)	10.800 ± 3.150	13.700 ± 5.020	NS
Cholesterol (mmol/L)	1.800 ± 0.210	1.800 ± 0.210	NS
Urea (mmol/L)	5.700 ± 0.380	6.700 ± 0.910	NS
Total protein (g/L)	72.900 ± 2.570	66.200 ± 5.090	NS
AST (IU/L)	199.000 ± 203.790	188.000 ± 140.110	NS
ALT (IU/L)	66.400 ± 19.500	74.600 ± 14.140	NS
Sodium (mmol/L)	142.000 ± 1.410	139.200 ± 1.300	NS
Potassium (mmol/L)	5.400 ± 1.870	6.500 ± 1.680	NS
Calcium (mmol/L)	2.700 ± 0.180	2.700 ± 0.110	NS
Phosphorus (mmol/L)	2.300 ± 0.590	2.200 ± 0.610	NS
Alkaline phosphatase (IU/L)	122.000 ± 40.850	170.400 ± 20.610	NS

$P < 0.05$, as compared to control group and Values are mean ± SD of n = 5 animals in each group. NS = Not significant.

Table 5
Heavy metals in organs of two groups of rats (ppm).

Organs	Control group				Experimental group			
	Hg	As	Cd	Pb	Hg	As	Cd	Pb
Liver	nd*	nd*	nd**	nd**	nd*	nd*	nd**	nd**

nd = not detected where n = 5 animals in each group. *detection limit less than 0.005. **detection limit less than 0.01.

Table 4 reveals the effect of *K. alvarezii* extracts on the biochemical parameters in rats. There are neither significant difference in the serum levels of glucose, cholesterol, total protein, albumin, AST, ALT, phosphorus, sodium, calcium, potassium, urea and alkaline phosphatase between control group and treated group.

The heavy metal content of liver was determined in both control and treated group of rats. The heavy metals contents determined were mercury, cadmium, lead and arsenic. In both of the groups, none of the heavy metals are detected in the liver of rats. The result of the test is shown in Table 5.

3.4. K. alvarezii *effect on mammary tumour animal model*

The specific growth rate of tumour for untreated group is $1.580 \pm 0.270\ mm^3/t$ and *K. alvarezii* extract treated group is $0.097 \pm 0.060\ mm^3/t$. The specific growth rate of tumour in untreated group is significantly higher than treated group. The mean growth rate of tumours in untreated group and treated group is shown in Table 6.

The haematological values of the three groups of rat are shown in Table 7. There was no significant difference found in Red Blood Corpuscles (RBC), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC). However, WBC in untreated group is significantly higher than treated group and negative control group.

Table 8 reveals the biochemical test for the three groups of rat, however there are no significant difference found in any of the parameters tested.

4. Discussion

For LCMS/MS, the peak at retention times 4.60, 4.73, 6.05, 8.17, 9.49 and 11.07 were identified as unknown compound which cannot be match with the database library. Besides, main product ions for the compounds were unable to match with the database therefore the six chosen compounds could be potential new compound. Although none of the compounds can be identified in *K. alvarezii* extract crude extracts but there are several compounds suspected to be found in red seaweed. The compounds that are proposed to be found in red seaweed are Chlorophyll *d*, Pycobiliproteins, Allophycocyanin, C-phycocyanin, R-phycocanin, B-phycoerythrin, β-carotene, α-carotene, sterols (C27 sterols, cholesterol, desmosterol, 22-dehydrocholestrol), unsaturated C20 fatty acid (20:4 ω6, 20:5 ω5), Vitamin B12 and Taurine [16]. Therefore, the unknown compounds could be the mentioned compounds and further analysis of *K. alvarezii* need to be carried out in order to determine the compounds. Since the compounds unable to match with the database in library, purification of sample is needed and further analysis can be carried out with NMR and FTIR together with complementary analysis from GCMS.

For *K. alvarezii* extract cytotoxicity effect on cell viability of MCF-7, the cell reduced 50% at the concentration of 4.100 ± 0.690 mg/mL. The lower the IC_{50} value the better the cytotoxicity effect of the compound. As compared to IC_{50} value for *Sargassum sp.* (250.0 μg/mL) and *Ulva lactuca* (37.0 μg/mL), *K. alvarezii* shown higher IC_{50} value [17,18]. Besides, Tamoxifen also shown a lower IC_{50} value (12.000 ± 0.520 μg/mL) compared to *K. alvarezii* where Tamoxifen is an anti-estrogen drug that is commonly used in breast cancer treatment which prevents the effect of estrogen on tissue [19]. The two species of seaweed and Tamoxifen show lower IC_{50} values than *K. alvarezii* extract, however *K. alvarezii* extract that was used in this study is a crude extract. Purify and identify the active compound *K. alvarezii* might give a better cytotoxicity effect than *K. alvarezii* crude extract. Therefore the potential for *K. alvarezii* extract on cytotoxicity effect on MCF-7 should not be under estimated.

There are eight elements (cadmium, arsenic, chromium, iron, manganese, lead, mercury, nickel and zinc) were tested for *K. alvarezii* extract. Three out of eight elements were found in *K. alvarezii* extract. The three elements were Arsenic (3.9 ppm), Iron (14.9 ppm) and Zinc (3.0 ppm) [20]. During the 60 days of treatment period, there were no differences observed among the rats in terms of nature of stool, colour of eyeballs, and the fur. Same as *A. spicifera*, *K. alvarezii* extract does not reveal any toxicological effect on dosage 2000 mg/kg body weight [21].

Table 6
Specific growth rates of tumour between untreated group and treated group.

	Untreated group	Treated group	p-value
Specific growth rate of tumour (mm^3/t)	1.580 ± 0.270	0.097 ± 0.060	0.017

$p < 0.05$, as compared specific growth rate of tumour between untreated group and treated group. Values are mean ± SD of n = 4 animals in each group.

Table 7
Hematological values of rats.

Parameters	Control group	Experimental group	Treatment group
Hemoglobin (g/dL)	13.800 ± 0.190^{a}	12.900 ± 2.360^{a}	12.600 ± 0.830^{a}
RBC ($\times 10^{12}$/L)	7.500 ± 0.130^{a}	6.800 ± 1.500^{a}	6.700 ± 0.500^{a}
WBC ($\times 10^{9}$/L)	2.050 ± 0.4900^{b}	11.800 ± 2.920^{a}	3.800 ± 0.4240^{b}
PCV (%)	44.600 ± 1.140^{a}	41.800 ± 7.630^{a}	41.200 ± 2.860^{a}
MCV (fL)	59.400 ± 2.300^{a}	61.500 ± 3.110^{a}	61.200 ± 3.190^{a}
MCH (pg)	18.400 ± 0.550^{a}	19.000 ± 0.820^{a}	18.800 ± 0.450^{a}
MCHC (g/dL)	31.000 ± 0.690^{a}	30.800 ± 0.590^{a}	30.800 ± 0.880^{a}

Values are mean ± SD of n = 4 animals in each group. Different superscript letter in each row indicate a statistical significantly different at $p < 0.05$.

Table 8
Biochemical values of rats.

Parameters	Control group	Experimental group	Treatment group
Glucose (mmol/L)	8.500 ± 2.040^{a}	9.800 ± 1.110^{a}	8.500 ± 1.530^{a}
Cholesterol (mmol/L)	1.800 ± 0.510^{a}	2.200 ± 0.150^{a}	2.300 ± 0.450^{a}
Total protein (g/L)	74.000 ± 6.140^{a}	71.900 ± 5.380^{a}	76.100 ± 1.280^{a}
Calcium (mmol/L)	2.600 ± 0.140^{a}	2.600 ± 0.070^{a}	2.600 ± 0.050^{a}
Phosphorus (mmol/L)	1.900 ± 0.280^{a}	1.800 ± 0.130^{a}	1.700 ± 0.150^{a}

Values are mean ± SD of n = 4 animals in each group. Different superscript letter in each row indicate a statistical significantly different at $p < 0.05$.

Besides, referring to the liver function and kidney functions as both of the groups, there were no significant difference observed in the creatinine, AST and ALT parameters. Although arsenic was found in the *K. alvarezii* sample, the amount is permissible tolerable as suggested by WHO. In addition no arsenic was found in the liver sample of the treated group.

For *K. alvarezii* effect on animal model of mammary tumour, 7, 12-dimethylbenz(a)anthracene (DMBA) was used to induce mammary tumour in the rats. DMBA is a polycyclic aromatic hydrocarbon (PAH) that is used to promote tumours in laboratory animal. Mammary tumours can be produced with administration of DMBA by oral gavage. It will lead to up-regulation of the cellular cytosolic receptor for DMBA which is aryl hydrocarbon receptor (AhR). The AhR translocates into the nucleus and associates with the cofactor AhR nuclear translocation protein (ARNT). The activated AhR/ARNT complex will bind to the specific DNA recognition sites upstream of AhR responsive genes and induces genes transcription. The early stage of tumorigenesis involved p450 enzymes which metabolize DMBA into a mutagenic epoxide intermediate that readily forms DNA adducts. The DNA adducts are associated with DNA mutations and the malignant transformation that is thought to be involved in PAH carcinogenesis [22–24].

The specific growth rate of tumour for untreated group is $1.580 \pm 0.270\ mm^3/t$ and treated group is $0.097 \pm 0.060\ mm^3/t$. The specific growth rate of tumour in untreated group is significantly higher than treated group. Similar result was observed by Fatemeh et al. [25] i.e. the tumour size of the untreated group is significantly higher than the tumour size of treated group. Besides, there are several reports stating that red and brown seaweeds have the anti-cancer effect [26,27], which indicate *K. alvarezii* to be a potential natural product of cancer treatment which has an advantage of causing less side effects to human.

Furthermore, according to Jane et al. [11], dietary of seaweed (*Laminaria* sp.) significantly delay the time of the first palpable tumour in the seaweed-fed rats. In their research, the test animal was fed with *Laminaria* enriched diet and the control group animal was fed with normal alphacel diet at the time of their arrival. The rats were given 5 mg DMBA at 55th days of age and for the next 26 weeks the rats were weighed and palpated weekly. Their results showed a significantly lower number of adenocarcinomas per adenocarcinoma-bearing rat for the seaweed-fed group. Based on their study, the rats fed with seaweed took a longer time to develop tumours and got fewer tumours than rats which were not fed with seaweed.

Jane et al. [11] suggested that the differences between the tumours between the groups might be altered by the seaweed compounds which directly affect mammary gland development. Besides, the seaweed might affect the development of the mammary glands or inhibit the initial metabolism of the DMBA which affect the promotion of tumour growth of the rats [28].

The WBC in untreated group is significantly higher than treated group and negative control group. High white blood cell counts could be a sign of an infection [29]. Seaweed is suggested to having antimicrobial and anti-inflammation properties, hence, this can explain why the reading in treatment group is lower than the untreated group [30]. High reading of WBC could be due to inflammation that occurs to mammary tumour development [31,32].

5. Conclusion

K. alvarezii is a potential therapeutic functional food for management of breast cancer. This study reveals that *K. alvarezii* extract might able to slow down tumour growth. *K. alvarezii* showed a good cytotoxic effect on MCF-7 cell line. The good cytotoxic effect of *K. alvarezii* shows that *K. alvarezii* is a potential natural product of cancer treatment. No toxicity effect was found in the rat which fed with *K. alvarezii* extract. *K. alvarezii* extract also showed good result of slowing down the growth rate of mammary tumour in rats. *K. alvarezii* extracting is also exerts a good anti inflammation effect.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

References

[1] S.M. Coelho, S. Heesch, N. Grimsley, H. Moreau, J.M. Cock, Genomics of marine algae, Intro. Mar. Genom. 1 (2010) 179–211.
[2] L. Hayashi, A.A. Santos, G.S.M. Faria, B.G. Nunes, M.S. Souza, A.L.D. Fonseca, P.L. M. Barreto, E.C. Oliveira, Z.L. Bouzon, *Kappaphycus alvarezii* (Rhodophyta, Areschougiaceae) cultivated in subtropical waters in Southern Brazil, J. App. Phys. 23 (2011) 337–343.
[3] H. Bixler, Recent developments in manufacturing and marketing carrageenan, Hydrobiologia 326/327 (1996) 35–57.
[4] T.D. Pickering, P. Skelton, J.R. Sulu, Intentional introductions of commercially harvested alien seaweeds, Botani. Mar. 50 (2007) 338–350.
[5] P. Ferraces-Casais, M.A. Lage-Yusty, A.R.B. Quirós, J. LópezHernández, Evaluation of bioactive compounds in fresh edible seaweeds, Food Anal. Met. 5 (2011) 828–834.
[6] A. Senthil, B.S. Mamatha, P. Vishwanath, K.K. Bhat, G.A. Ravishankar, Studies on development and storage stability of instant spice adjunct mix from seaweed (Eucheuma), J. Food Sci. Technol. 48 (2011) 712–717.
[7] M. Fayaz, K.K. Namitha, K.N. Chidambara Murthy, M. Mahadeva Swamy, R. Sarada, S. Khanam, P.V. Subbarao, G.A. Ravishankar, Chemical composition iron bioavailability, and antioxidant activity of Kappaphycus alvarezii (Doty), J. Agric. Food Chem. 53 (2005) 792–797.
[8] Y.S. Khotimchenko, Antitumor properties of nonstarch polysaccharides: fucoidans and chitosans, Russ. J. Mar. Biol. 36 (2010) 321–330.
[9] H. Yuan, J. Song, X. Li, N. Li, S. Liu, Enhanced immunostimulatory and antitumor activity of diffferent derivatives of k-carrageenan oligosaccharides from Kappaphycus striatum, J. Appl. Phycol. 23 (2011) 59–65.
[10] E. Taskin, Z. Caki, M. Ozturk, Assessment of *in vitro* antitumoral and antimicrobial activities of marine algae harvested from the eastern Mediterranean sea, Afr. J. Biotechnol. 9 (2010) 4272–4277.
[11] T. Jane, L. Margaret, Harbison, S.G. Rebecca, Dietary seaweed (*Laminaria*) and mammary carcinogenesis in rat, Am. Assoc. Cancer Res. 44 (1984) 2758–2761.
[12] P. Marina, S. Ranko, R.A. Matea, K.S. Jelena, J. Jure, V. Ksenija, Z. Biserka, R. Marko, Antitumor activity of novel *N*-sulfonylpyrimidine derivatives on the growth of anaplastic mammary carcinoma in vivo, J. Cancer Res. Clin. Oncol. 131 (2005) 829–836.
[13] M. Mahsa, E. Mahdi, Y.M. Goh, M. Patricia, M. Suhaila, *Sargassum polycystum* reduces hyperglycaemia, dyslipidaemia and oxidative stress via increasing

insulin sensitivity in a rat model of type 2 diabetes, J. Sci. Food Agric. 1 (2012) 1–7.
[14] M. Federica, R. Sabrina, C. Carlo, Biological functions and health promoting effects of brown seaweeds in swine nutrition, J. Dairy Vet. Anim. Res. 1 (2014) 1–3.
[15] H.G. Kim, D.W. Lim, S. Cho, D. Han, Y.T. Kim, The edible brown seaweed *Ecklonia cava* reduces hypersensitivity in postoperative and neuropathic pain models in rat, Molecules 19 (2014) 7669–7678.
[16] S.L. Christopher, J.W. Michael, The Biology of Seaweeds, University of California Press, Berkeley, 1981.
[17] J.S. Mary, P. Vinotha, A.M. Pradeep, Screening for in vitro cytotoxic activity of seaweed, Sargassum sp. against Hep-2 and MCF-7 cancer cell lines, Asian Pac. Org. Cancer Preven. 13 (2012) 6073–6076.
[18] D.J. Saraniya, B.B. Valentin, Anticancer activity of silver nanoparticles synthesized by seaweed Ulva lactuca Invitro, Sci. Rep. 1 (2012) 1–5.
[19] N.S. Ebrahimi, F.M. Yusoff, A.N.B. Mohamed, M. Rasoli, T.S. Keong, A.R. Omar, Cytotoxic effect of ethanol extract of microalga Chaetoceros calcitrans, and its mechanisms in inducing apoptosis in human breast cancer cell line, J. Biomed. Biotechnol. 2013 (2013) 1–8.
[20] V.S. Chang, S.S. Teo, Evaluation of heavy metal, antioxidant and anti-tyrosinase activities of red seaweed (*Eucheuma cottonii*), Int. Food Res. J. 23 (2016) 2370–2373.
[21] B.A. Thahira, M. Uma, Toxicity study of seaweed as sourced of nutritionally beneficial compounds, J. Food Sci. 45 (2011) 23–31.
[22] M. Dias, S. Cabrita, E. Sousa, B. Franca, J. Patricio, C. Oliveira, Benign and malignant mammary tumors induced by DMBA in female Wistar rat, Eur. J. Obst. Gyne. Oncol. 4 (1999) 285–288.
[23] C.S.D.B. Alfredo, N.M. Elisa, J.M. Lincon, H.T.P. Christina, I. Kiyoshi, G. Giovana, A.P. Jose, Induction of experimental mammary carcinogenesis in rats with 7,12-dimethlbenz(a)anthracene, Rev. Hosp. Clin. 59 (2004) 257–261.
[24] C. Nicholas, E.S. Sandra, G.D. Elizabeth, D.L.F. Chang, F. Magrant, H.Q. Ying, D. Isael, D.S. Gail, D.C. Robert, Z.X.J. Xiao, H.S. David, C.S. David, Oncogenic signaling pathways activated in DMBA-induced mouse mammary tumors, Toxic. Pathol. 33 (2005) 727–737.
[25] T.S. Fatemeh, K. Ali, G.F. Samaneh, A. Rasedee, H.O. Hemn, M. Suhaila, Comparison of tamoxifen with edible seaweed (*Eucheuma cottonii L.*) extract in suppressing breast cancer, Nutr. Cancer 65 (2013) 255–262.
[26] N. Farideh, M. Suhaila, G.F. Samaneh, B. Javad, M.M. Noordin, B.M.A. Noorjahan, O. Fauziah, Polyphenol-rich seaweed (*Eucheuma cottonii*) extract suppresses breast tumour via hormone modulation and apoptosis induction, Food Chem. 130 (2012) 376–382.
[27] Z.M. Soheil, K. Hamed, K. Ramin, R. Mahboubeh, F. Mohammad, Z. Keivan, A.K. Habsah, Anticancer and antitumor potential of fucoidan and fucoxanthin, two main metabolites isolated from brown algae, Sci. World J. 2014 (2014) 1–10.
[28] R. Russo, I.H. Russo, Influence of differentiation and cell kinetics on the susceptibility of the rat mammary gland to carcinogenesis, Cancer Res. 40 (1980) 267–2687.
[29] MedlinePlus, WBC count. http://www.nlm.nih.gov/medlineplus/ency/article/003643.htm, 2016 (accessed 16.10.10).
[30] J.S. Choi, H.J. Bae, S.J. Kim, I.S. Choi, In vitro antibacterial and anti-inflammatory properties of seaweed extracts against acne inducing bacteria, Propionibacterium acnes, J. Environ. Biol. 32 (2011) 313–318.
[31] I.F.V. Ana, M.D.S. Carlos, G.D. Neivys, M.S.A. Ana, S.O. Yulica, C.S. Hiran, Anti−inflammatory and analgesic activities of red seaweed Dichotomaria obtusata, J. Pharm. Sci. 47 (2011) 112–118.
[32] B. Walailuck, P. Yuwadee, K. Duangta, P. Jeeraporn, A. Doungporn, P. Chayakorn, S. Prasan, V. Panmuk, Antimicrobial and anti-inflammatory properties of various seaweeds from the Guf of Thailand, J. Agric. Biol. 13 (2011) 100–104.