ISSN : 2347 - 2243

Indo - American Journal of Life Sciences and Biotechnology

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Assessing the potential toxicity of Assessing the potential toxicity of Assessing the potential toxicity

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Abstract

Using an animal model, this study aims to demonstrate that Sargassum plagiophyllum extract is safe for human consumption. Methods: An autoclave set at 121°C for 20 minutes was used to extract water from Sargassum plagiophyllum, which is known as SPE. For 21 days, four groups of adult male mice were gavaged with the SPE. A range of SPE doses—100, 500, 1000, and 2000 mg/kg—were administered to the treatment groups. Mice served as controls were given pure water. Individuals' dietary consumption, as well as their weight, were documented. Blood, biochemical, and histological indicators were used to evaluate the toxicity of SPE. Findings: Not even at 2000 mg/kg did 21 days of SPE ingestion affect body mass, feed intake, or water intake. Additionally, there was no change in hemodynamic parameters. The results of the biochemical examination of the blood and serum showed that all of the treatment groups, in comparison to the control group, had normal levels of creatinine, alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP). Organs such as the liver, kidneys, colon, and others were found to be in good health across all therapy groups, according to histological investigations. In conclusion, our mouse model findings broaden the potential medicinal application of Sargassum plagiophyllum extract by providing fundamental scientific proof that it is safe to consume, even at large dosages. Sargassum plagiophyllum, brown algae, animal testing, histopathology, and safety

INTRODUCTION

Brown algae (Phaeophyceae) are the most important seaweeds in temperate coastal ecosystems around the globe. In the class Phaeophyceae, the genus *Sargassum* is the largest brown algae present in large quantities in the coastal regions of Andaman Sea and Thai Gulf. Brown algae live in harsh environments which stimulate the formation of secondary metabolites, and in turn, these substances exert specific biological activities [1]. They constitute a rich source of bioactive molecules such as alginate, laminarin, and fucoidan, and have been used for a long time as food and folkloric medication in Asia.

The pharmacological activities of brown algae have gradually aroused scientific interest. There are reports on the anticancer activity of *Sargassum oligocystum* extract against human cancer cell lines [2]. Moreover, *Sargassum polycystum* extract exerted anti-melanogenic effect by inhibiting cellular tyrosinase activity in melanoma cells [3]. A study has shown that *Sargassum wightii* extract possesses anti-nociceptive and anti-inflammatory activities [4]. A more recent study reported the antioxidant activities of a *Sargassum plagiophyllum* extract [5]. The consumption of this extract showed potential to prevent constipation in mice by enhancing colon function and modulating the gut microbiota [6]. With respect to human health, it is perhaps not only the bioactive molecules in seaweed that have

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beneficial effects but also the dietary polysaccharides. It has been reported that the fermentation of plant-derived polysaccharides by gut microbiota produce short-chain fatty acids (SCFAs) [7]. The SCFAs have received attention in the development of health supplements due to their importance in hemostasis and recovery from the disease [7].

In view of the increasing interest in the potential of *Sargassum* spp. to supply therapeutic agents, basic evidence is required on the safety of long-term consumption of products derived from this genus. The present study was aimed at establishing a valid scientific evidence for the safety of *S. plagiophyllum* extract in a mouse model, especially at high doses. The toxicity of the extract was assessed using biochemical, hematological, and histopathological parameters.

EXPERIMENTAL

Animals

Adult male ICR/Mlac mice (*Mus musculus*) aged 4 - 5 weeks were supplied by the National Laboratory Animal Center, Mahidol University, and were maintain in animal house at Prince of Songkla University, Thailand. The animals were kept in a temperature-controlled room (25 ± 2 ºC) with an ambient humidity of 50 – 55 % in an environment with 12-h light/12- h dark cycle, and were allowed *ad libitum* access to water and feed. This study received approval from the Animal Ethical authority of the Faculty of Science, Prince of Songkla University, Songkhla 90110, Thailand (approval no. MOE 0521.11/1555, Ref. 68/2018). Animals were handled according to the National Institutes of Health Laboratory Animal Care and Use Guidelines [8].

Preparation of SPE powder

Mature brown algae sample was obtained from the Thai island of Lanta. The sample was dried at 60°C for 48 h and then ground to a fine powder [5]. To prepare the *S. plagiophyllum* extract (SPE), the powdered sample and distilled water were mixed at a ratio of 40 : 4 (g : L), followed by autoclaving for 20 min at 121 oC and passing through a filter cloth. The filtrate was clarified using approx 10-min centrifugation at 2220 *g* [6], followed by freeze-drying.

Design and treatment groups

After a week-long acclimatization, the experimental animals were assigned to five groups, four of which received SPE (100, 500, 1000, or 2000 mg/kg) via gavage once daily for 21 days. The control group received distilled water. Body weight, feed intake, and water intake were recorded daily. On day 22, blood samples were collected via cardiac puncture under thiopental sodium (*i.p.*) anesthesia (70 mg/kg), and the internal organs, i.e., colon, liver, kidney, heart, lung, spleen, and testes were excised and weighed. The gross morphology of each of these organs was examined, and the organs were preserved in 10 % formalin for 24 h at room temperature for histopathological studies.

Hematological and biochemical analyses

Whole blood samples were analyzed using the BC-2800Vet Auto Hematology Analyzer (Shenzhen, China). The analytical parameters determined were hemoglobin (HGB), red blood cell (RBC), hematocrit (HCT), red cell distribution width (RDW), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), platelet distribution width (PDW), mean platelet volume (MPV), white blood cells (WBCs), lymphocytes, monocytes, and granulocytes. In the determination of biochemical parameters, blood samples were centrifuged at 3000 rpm for 5 min and the plasma samples were collected. The levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and creatinine in blood plasma were determined using the BS-20 Chemistry Analyzer (Shenzhen, China).

Histopathological studies

The fixed internal organs were processed through a series of graded alcohol concentrations, followed by paraffin-embedding and sectioning at thickness of 5 µm. Following standard procedures, the tissue slices were subjected to routine staining with Masson's trichrome, H & E and PAS, followed by light microscopy.

Statistics

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Data are presented as mean ± SEM. Comparison amongst groups was performed using ANOVA and Tukey's multiple comparison. Values of $p < 0.05$ indicated statistical significance.

RESULTS

Effect of SPE treatment on body weight and feed and water intake

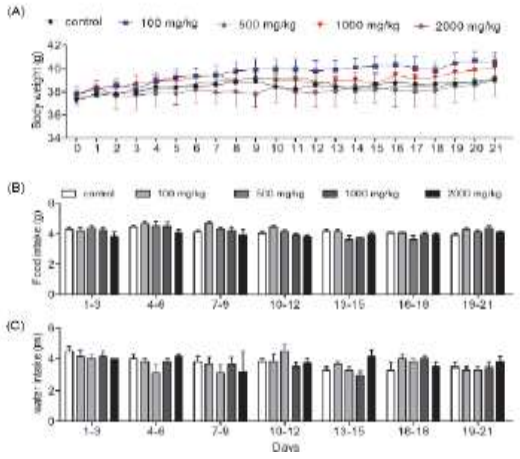

Figure 1: Body weights, and feed and water intakes of adult male mice treated with different doses of *Sargassum plagiophyllum* extract (SPE) for 21 consecutive days. The extract had no effect on the growth of the mice, as measured using body weight (A). The recorded feed intake (B), and water intake (C) were normal and consistent with the recorded body weight

Oral SPE administration at the 4 doses for 21 consecutive days did not affect body weight. The average body weights of all SPE-exposed mice and control mice were comparable (Figure 1 A). The body weights of mice in all groups gradually increased throughout the 21 days, which reflected normal growth. The feed and water intakes in all groups given SPE were consistent with the trends observed in body weight, and they were not significantly different from those of the control group (Figures 1 B and C). Moreover, no death or abnormal behavior of mice was noticed in any group given SPE. These results revealed that oral intake of SPE for 21 days did not affect growth in the mouse model, even at a dose of 2000 mg/kg.

Effect of SPE treatment on gross morphology of selected internal organs

The gross morphology, shape, texture, size, and color of the liver, kidneys, heart, testes, and spleen of mice treated with SPE at all doses were normal (Figure 2). In addition, the relative organ weights of all selected organs did not differ significantly between the control and the SPE-treated groups (Table 1). These results confirm that SPE intake for 21 days, irrespective of dose, had no adverse impact on the internal organs of the mice.

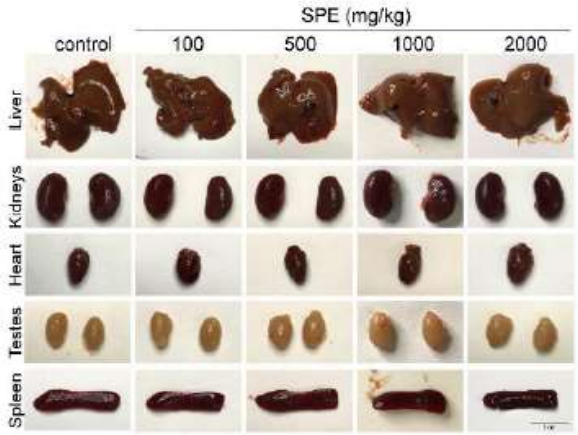

Figure 2: The internal organs of mice treated for 21 consecutive days with different doses of *Sargassum plagiophyllum* extract (SPE) were excised and photographed. The gross morphology of liver, kidneys, heart, testes, and spleen showed no adverse effects from the treatments. Scale bar = 1 cm

Influence of SPE treatment on hematological parameters

After administration of SPE for 21 days, the number of WBC was found to have increased in a dose-dependent manner (Table 2). The administration of SPE at a dose of 2000 mg/kg significantly increased WBC number, when compared to the control group. However, the levels of all other hematological parameters tested were not different from control values.

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Table 1: Impact of SPE treatment on relative organ weight

Organ	Control	SPE (mg/kg)			
		100	500	1000	2000
Liver	0.047±0.001	0.045 ±0.002	0.044±0.001	0.045±0.002	0.047±0.002
Kidney	0.015±0.000	0.014±0.000	0.014±0.001	0.015±0.001	0.015±0.000
Heart	0.005±0.000	0.004±0.000	0.004±0.000	0.005±0.000	0.005±0.000
Lung	0.005±0.000	0.005±0.000	0.005±0.000	0.005±0.000	0.005±0.000
Testes	0.008±0.001	0.007±0.000	0.007±0.000	0.008±0.000	0.007±0.000
Spleen	0.003±0.000	0.004±0.000	0.004±0.001	0.004±0.000	0.005±0.001

Values are presented as mean ± SEM

Table 2. Effect of SPE treatment on hematological parameters

Parameter	Control	SPE (mg/kg)			
		100	500	1000	2000
HGB (g/dL)	13.20±1.45	13.06±0.58	11.98±0.96	12.20±0.50	12.58±0.37
RBC (10^3/µL)	9.05±0.81	8.99±0.35	8.42±0.69	8.82±0.19	8.74±0.24
HCT (%)	47.34±4.73	46.96±1.96	43.20±3.47	44.95±2.10	46.14±1.51
RDW (%)	15.76±0.47	15.52±0.55	15.12±0.55	16.40±0.61	16.16±0.67
MCV (fL)	52.58±1.04	52.34±1.01	51.44±0.80	50.95±1.23	53.42±0.88
MCH (pg)	14.40±0.44	14.48±0.17	14.18±0.12	13.78±0.29	14.34±0.07
MCHC (g/dL)	27.68±0.68	27.76±0.35	27.70±0.40	27.13±0.36	27.26±0.44
PLT (10^3/µL)	1898±201	1944±280	1805±312	2598±464	1867±203
PDW (%)	16.24±0.22	15.86±0.05	15.98±0.11	16.18±0.19	15.98±0.15
MPV (fL)	5.58±0.14	5.54±0.13	5.44±0.22	6.18±0.21	5.56±0.29
WBC (10^3/µL)	2.44 ±0.54	2.84±0.32	2.98±0.22	3.40±0.41	4.44±0.55*
Lymphocytes (%)	75.44 ±1.57	70.42±4.14	65.36±2.68	74.98±0.75	68.68±3.70
Monocytes (%)	2.94±0.23	3.38±0.48	3.22±0.15	2.75±0.14	2.96±0.35
Granulocytes (%)	21.62±1.40	26.20±3.71	31.42±2.70	22.28±0.76	28.36±3.64

Data are presented as mean ± SEM; *$P < 0.05$

Impact of SPE on liver structure and function

Liver function was assessed using biochemical assays. Levels of ALT and AST in mice treated with all doses of SPE were lower than control values (Figures 3 A and C) but there were no significant differences. Levels of ALP (Figure 3 B) in all treatment groups were not significantly different from the corresponding control levels. Histopathological examination of the liver revealed normal liver histology in all treatment groups. The central vein (CV) characteristics were normal, with normal radiating hepatic cords, normal hepatic sinusoids, and normal portal triads (Figure 3 D). Moreover, Masson's trichrome staining revealed that no fibrosis was present in liver tissue from the control and all SPE-treated groups. There was only a small amount of collagen fiber distributed in liver tissue, most of which was accumulated in the portal triad to support the internal structures.

Effect of SPE treatment on kidney structure and function

The 21-day administration of SPE had no adverse impact on renal function. In all treatment groups, levels of the renal function parameters BUN and creatinine were comparable with control values (Figure 4 A and B). Histopathological examination revealed normal glomeruli, normal Bowman's capsules, and normal urinary spaces in kidney tissue of control and all treatment groups (Figure 4 C). Renal tubules showed simple cuboidal epithelium with brush borders in the proximal tubule (PT), and simple cuboidal epithelium in the distal tubule (DT) (Figure 4 C), which are the normal characteristics of mouse renal tubules. Moreover, Masson's trichrome

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staining showed that no fibrosis was present in kidney tissue from the control and all SPE-treated groups.

Influence of SPE treatment on colon

Since the animals received the SPE through the oral route, the extract had to pass through the gastrointestinal tract. Therefore, there was need to check its potential to cause intestinal inflammation. Since colonic length is the parameter normally used to determine colitis, the whole colon specimens were excised and measured. No effect on colon length was observed (Figure 5 A), indicating that the administration of SPE did not cause colitis. Moreover, colon histology revealed healthy architecture in SPE-treated mice. The epithelium remained simple columnar epithelium with normal cellular components (Figure 5 B). PAS staining revealed normal distributions of goblet cells. Although goblet cell populations were higher in SPE-treated groups than in control group, the differences were not significant (Figure 5 C).

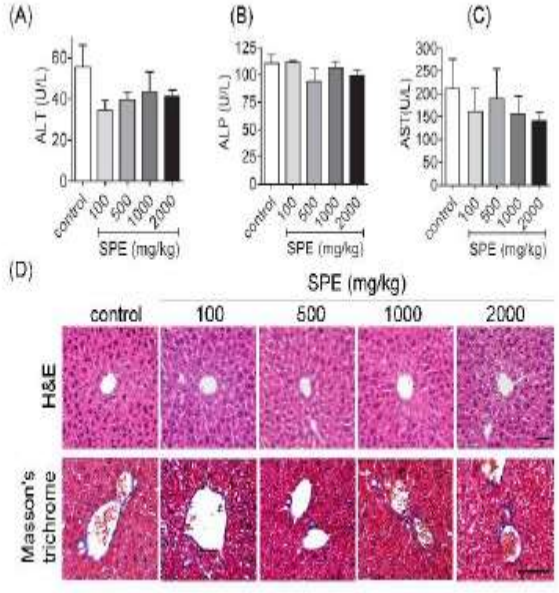

Figure 3: Photomicrographs of liver from adult male mice treated with different doses of *Sargassum plagiophyllum* extract (SPE) for 21 consecutive days. To evaluate the effect of the treatments on liver function, blood samples were collected using cardiac puncture, and were analyzed for levels of ALT (A), ALP (B), and AST (C). The liver slices were stained with Masson's trichrome and H & E (D). Values were expressed as mean ± SEM. Scale bar = 20 μm

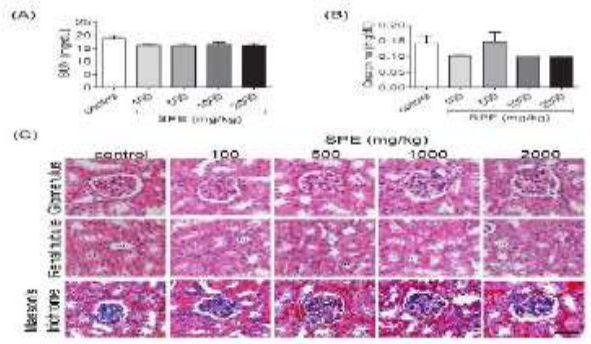

Figure 4: Adult male mice were treated with different doses of *Sargassum plagiophyllum* extract (SPE) for 21 consecutive days. To evaluate the effects of treatment on the kidney, blood specimens were analyzed for blood urea nitrogen (BUN) and creatinine levels (A and B). For histopathological examination, kidney slices were stained with Masson's trichrome and H & E (C). Values were expressed as mean ± SEM. Scale bar = 20 μm

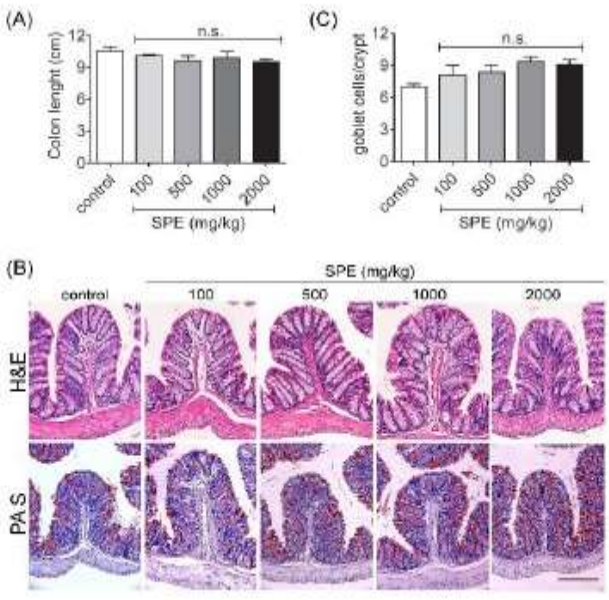

Figure 5: Adult male mice were treated for 21 days with different doses of *Sargassum plagiophyllum* extract (SPE). To investigate the effects of the treatments on the colon, whole colons were excised

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and their lengths were measured (A). The colon tissue was stained with H & E and periodic acid Schiff (PAS) (B), and the number of goblet cells per crypt was determined (C). Values were expressed as mean ± SEM. Scale bar = 100 µm

Impact of SPE treatment on histopathology of selected internal organs

No overt pathological lesions were observed in the internal organs examined. No abnormal signs of toxicity in heart, lung, testis, and spleen tissue were observed in any of the treatment groups (Figure 6). These histopathological examinations confirmed the normal cellular architectures of the tested organs.

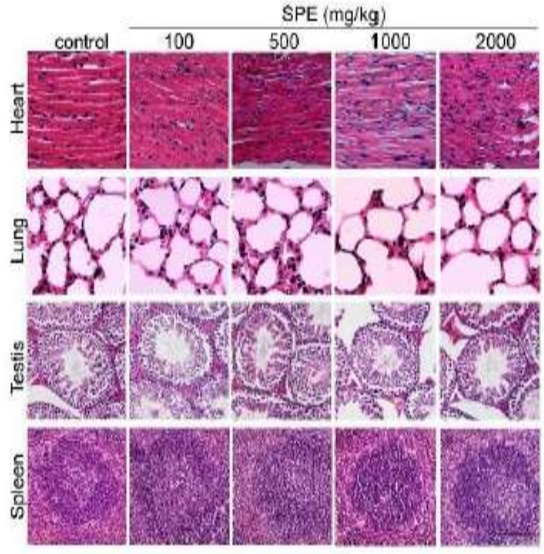

Figure 6: Photomicrographs of tissues from adult male mice treated for 21 days with different doses of *Sargassum plagiophyllum* extract (SPE). The impact of treatment on tissue of the heart, lung, testes, and spleen were determined using H & E staining. Scale bar = 50 µm

DISCUSSION

The present study has demonstrated that oral intake of SPE to a level of 2000 mg/kg for 21 days did not cause any detrimental effect, death, or abnormal behavior in mice. This finding is consistent with a previous report which showed that *Sargassum* extract administered at a high dose for 28 days was non-toxic to Wistar rats [9]. Seaweed extracts from a variety of species have been reported safe and non-toxic in different animal models [9,10].

Changes in body weight and the weights of internal organs which have previously been used as indices for toxicity assessment [9], were statistically insignificant. These results were in line with feed and water intakes which are indices of behavioral changes. Based on the present work, it may be postulated that *Sargassum* extract was non-toxic on the growth and wellness of the animal model. Until now, there have been no reported adverse effects of *Sargassum*. Indeed, the beneficial effects of *Sargassum* on health have been confirmed. The beneficial effects include immune response modulation [11], improved blood biochemistry profiles under stress conditions [12], facilitation of neuronal maturation and synaptogenesis [13], and protection against chemical-induced toxicity [14].

The administration of toxic substances has been reported to affect hematological parameters, but results have not been consistent [15,16]. Previous studies reported reductions in white blood cells, red blood cells, and platelets of rats exposed to insecticidal oils obtained from the leaves of *Cassia occidentalis* and *Euphorbia miliiwhite* [16]. Other research reported reduced packed cell volume, hemoglobin and platelets in rats administered *Erythrophleum Suaveolens* extract [15]. Moreover, reductions in packed cell volume, hemoglobin and red blood cells of rats treated with leaf extract of *Cassia occidentalis* have been reported [17]. In this study, there were no marked changes in the hematological parameters determined, except white blood cells, which increased in number. These results imply the safety of *Sargassum* extract consumption. The accentuation in population of white blood cells is most likely attributable to improved immune function. However, further studies are needed to elucidate the cause of this increase.

The kidney, liver, and colon are the organs that process ingested toxic substances. The effects of toxic substances on kidney tissue may lead to glomerular atrophy, tubular degeneration, and glomerular membrane damage, which in turn affect the glomerular filtration rate (GFR). A decrease in GFR leads to an increase in the levels of BUN and creatinine which are the markers of nephrotoxicity. In the liver, hepatotoxicity may be marked by the presence of fibrosis, cellular necrosis, and inflammation. The toxic effects on liver function are

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usually indicated by increases in the serum levels of liver enzymes such as AST, ALT, and ALP [18].

The safety of consuming *Sargassum* extract was confirmed in the present work by the histologic evidence of intact kidney and liver, and also by the normal serum levels of ALT, ALP, AST, BUN, and creatinine. These results are consistent with the findings of a research on the toxicity of *Sargassum wightii* Greville [9]. The administration of *Sargassum* extract produced no pathologies in the other organs, which is consistent with previous reports [5,9].

Since shortened colon length is a well-known marker of colitis [19,20], the normal length and histology of the colon seen in this study confirm that *Sargassum* extract did not cause colon inflammation. Furthermore, the extract did not cause constipation, but in contrast, it increased gut motility and number of goblet cells [5]. The absence of any adverse effect on the gastrointestinal tract could be due to the prebiotic properties of the *Sargassum* extract [21], and/or the metabolism of the extract in the gut. Following digestion and fermentation, the extract was broken down to SCFAs which are thought to modulate gut immunity, enhance colon functions, and modulate gut microbiota [6], thereby ultimately conferring a gut health benefit.

CONCLUSION

This research has demonstrated the safety of consumption of *Sargassum plagiophyllum* extract in a mouse model, based on the results of biochemical, hematological, and histopathological parameters. Further studies in humans will potentially expand the use of SPE as a medication for improving health.

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