

RESEARCH ARTICLE

Cancer Chemopreventive Effect of *Spirogyra Neglecta* (Hassall) Kützing on Diethylnitrosamine-Induced Hepatocarcinogenesis in Rats

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Abstract

Spirogyra neglecta, a freshwater green alga, is a local food in the northern and northeastern parts of Thailand. This investigation explored the anticarcinogenicity of *S neglecta* and its possible cancer chemopreventive mechanisms in rats divided into 14 groups. Groups 1 and 10 served as positive and negative control groups, respectively. Groups 1-9 were intraperitoneally injected with diethylnitrosamine (DEN) once a week for 3 weeks. Groups 10-14 received normal saline instead. One week after the last DEN injection, groups 2-5 were administered for 9 consecutive weeks various doses of *S neglecta* extract (SNE) and dried *S neglecta* (SND), mixed with basal diet. Groups 6-9 and 11-14 similarly were administered various doses of SNE and SND starting from the first week of the experiment. Administration of SNE and SND was not associated with formation of glutathione-S-transferase placental form (GST-P) positive foci in rat liver. SNE and SND during initiation phase significantly reduced the number of GST-P positive foci in rats injected with DEN. The number of GST-P also diminished in groups treated with SNE and SND after injection with DEN, except for the low dose extract group. SNE showed stronger anticarcinogenic potency than SND. Furthermore, SNE also decreased the number of Ki-67 positive cells. However, the numbers of TUNEL-positive cells in the liver of the SNE-treated groups were not statistically different from the controls. The GST activity in 50 mg/kg bw of SNE and 1% of SND groups was significantly increased as compared to the positive control. In conclusion, *Spirogyra neglecta* (Hassall) Kützing showed cancer chemopreventive properties at the early stages of diethylnitrosamine-induced hepatocarcinogenesis in rats. Possible inhibitory mechanisms include enhancement of the activities of some detoxifying enzymes and/or suppression of precancerous cells.

Keywords: Cancer chemoprevention - diethylnitrosamine - medium-term carcinogenicity test

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Introduction

Liver cancer is a major chronic health problem in Sub-Saharan Africa, East Asia, and Southeast Asia. It is the third most common cancer in males and the sixth most common cancer in females in Thailand (Attasara and Buasom, 2011). The risk factors of liver cancer include hepatitis B and C, viral infection, alcohol, and chemical carcinogens such as aflatoxin B1 (Ding and Wang, 2014). Avoiding exposure to either carcinogens or other risk factors can prevent cancer. Moreover, there are numerous reports of naturally occurring substances that inhibit cancer formation; sources of these substances include plants, animals, and microorganisms such as algae (Nobili et al., 2009; Lee et al., 2013; Muthuirulappan et al., 2013).

Several studies have reported the anticarcinogenic properties of various algae. *Lithothamnion calcareum*,

red seaweed, suppressed polyp formation and reduced inflammatory lesions in colon of high-fat treated mice (Aslam et al., 2010). Dimethylsulfoniopropionate isolated from green marine algae showed anti-cancer effects on Ehrlich ascites carcinoma-transplanted mice (Nakajima et al., 2009). However, there are a few studies that describe the anticarcinogenic properties of freshwater green macroalgae. *Spirogyra* spp, a filamentous green macroalga in freshwater, is found in many countries. It has been used as a food only in the northern and northeastern parts of Thailand. *Spirogyra neglecta* (Hassall) Kützing consists of proteins, lipids, carbohydrates, and dietary fibers. The water extract of *S neglecta* inhibits the formation of gastric ulcers and shows hypolipidemic, hypoglycemic, and renoprotective abilities in type 2 diabetic rats (Ontawong et al., 2013).

Our previous studies have shown that the water extract of *S neglecta* possesses antioxidant activity (Thumvijit et

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al., 2013b). This extract also exhibited antimutagenicity effects on *Salmonella typhimurium* strains TA98 and TA100 in the presence of the metabolic activator S9 system (Thumvijit et al., 2013a). The pharmacological properties of this alga might be effective in the prevention of cancer. Therefore, the aim of present study is to investigate the protective role of *Spirogyra neglecta* (Hassall) Kützing on diethylnitrosamine-induced hepatocarcinogenesis in rats. Here, we examine the effects of both the hot water extract of *S neglecta* (SNE) and also dried *S neglecta* (SND).

Materials and Methods

Chemicals

Reduced glutathione (GSH) and 1-chloro-2,4,-dinitrobenzene (CDNB) and diaminobenzidine (DAB) were purchased from Sigma-Aldrich Co. (MO, USA). Diethylnitrosamine (DEN) was obtained from Tokyo Chemical Industry Co., Ltd, (Tokyo Japan). The rabbit polyclonal anti-GST-P antibody was purchased from Medical and Biological Laboratories Co., Ltd (Nagoya, Japan). The rabbit polyclonal anti-Ki67 antibody was purchased from Merck Millipore (MA, USA), the ApopTag Peroxidase in situ kit was purchased from Chemicon International Inc. (CA, USA). EnVision Doublestain system was purchased from DAKO (Hamburg, Germany). The Vectastain Elite ABC kit (Universal) was from Vector Laboratories Inc. (CA, USA). Other chemicals used were purchased from AppliChem (Darmstadt, Germany).

Materials

Spirogyra spp. was collected from cultivation pool of Na Kuha village, Phrae Province, Thailand during March and May 2010. These algae were identified and authenticated according to the morphology of both vegetative cells and sex cells, and also habitat according to the method of John et al. (2002). Fresh algae were washed, dried, and milled into powder by a mechanical grinder. The dried algae were boiled in water for 2 hours, filtered through 4 layers of gauze, and then lyophilized by freeze-drying. The yield of the hot water extract of *Spirogyra neglecta* (Hassall) Kützing was about 25%. The chlorophyll content in the dried algae and the hot water extract were 15.45 and 9.91mg/g, respectively. The total phenolic contents in the dried algae and the hot water extract were 72.98±14.34 and 151.02±15.39 mg GAE/g, respectively. The total carbohydrates and sulfated polysaccharides of the hot water extract (SNE) were 33.94±1.46% and 1.08±0.01% respectively (Thumvijit et al., 2013b).

Experimental design

Male Wistar rats, weighing 100-150g, were used in the study. All animals were housed in stainless steel cages and maintained in 12hr light/dark cycles with constant temperature and humidity. The animals were acclimatized for one week before the experiments. This experiment was approved by The Animal Ethical Committee of Faculty of Medicine, Chiang Mai University.

The rats were randomly divided into 14 groups of 10 rats per group for Groups 1-9 and 6 rats per group for

Groups 10-14 as shown in Figure 1. Groups 1 and 10 were positive and negative control groups, respectively. Groups 1-9 were intraperitoneally injected with 100mg/kg body weight (bw) of diethylnitrosamine (DEN) in saline as a triple dose once a week for three weeks to induce preneoplastic lesions in the liver. After one week of DEN injections, Groups 2 and 3 were given 50 and 200mg/kg bw, respectively, of the water extract of *S neglecta* (SNE) via intragastric feeding, while Groups 4 and 5 were given 1% and 4% w/w, respectively, of dried *S neglecta* (SND) mixed with basal diet for 9 weeks. Groups 6 and 7 were fed with 50 and 200 mg/kg bw of SNE, respectively, while Groups 8 and 9 were fed with 1% and 4% w/w of SND, respectively, for the entire 13 weeks of the experiment. Groups 11 and 12 were fed with 50 and 200 mg/kg bw, respectively, of SNE for 13 weeks. Groups 13 and 14 were fed with 1% and 4% w/w SND, respectively, for 13 weeks. Water and food consumption and body weight were measured twice a week throughout the study. All rats were sacrificed by diethyl ether at the end of 13th week, and livers were removed and weighed. The livers were cut and fixed in 10% formalin for immunohistochemical examination. The remaining portion was kept at -80°C for further analysis. Blood was collected for measuring serum alanine transaminase and aspartate transaminase activity using commercial Olympus kits (Olympus Corp., Tokyo, Japan).

Determination of glutathione-S-transferase placental positive foci

Immunohistochemical staining for glutathione-S-transferase placental (GST-P) positive foci was conducted to evaluate preneoplastic lesions in the rat liver tissue. The detection of GST-P foci was performed using the avidin-biotin complex method reported by Puanachokchai et al. (2006). After deparaffinization and rehydration, liver sections were soaked in 3% H₂O₂ and skim milk to block endogenous peroxidase activity and non-specific protein binding, respectively. The slides were then incubated with polyclonal anti-rabbit GST-P antibody (1:1000) for 2 hours at room temperature and with goat anti-rabbit IgG biotinylated antibody conjugated with the avidin-biotin-

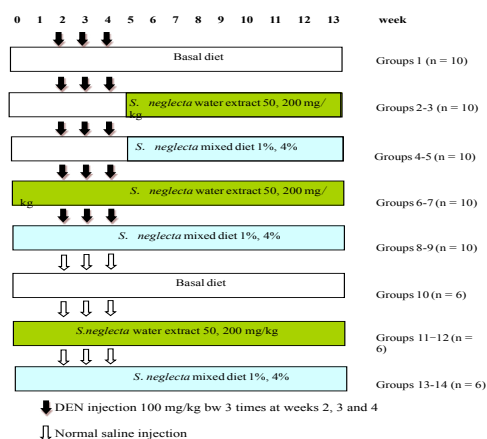


Figure 1. The Protocol for Investigation the Anticarcinogenic Effects of Spirogyra Neglecta (Hassall) Kützing on DEN-Induced Early Stages of Rat Hepatocarcinogenesis

peroxidase complex for 30min. Finally, the peroxidase binding sites were visualized with 3,3-diaminobenzidine hydrochloride. The tissue sections were counterstained with hematoxylin for microscopic examination. The total area of liver sections and numbers of GST-P positive foci, some of which contained more than 30 cells per foci and measured approximately $2\mu\text{m}^2$, were counted under a light microscope.

Determination of Ki-67

All samples were deparaffinized and retrieved in citrate buffer at pH 6 using an autoclave for 20min. After cooling to room temperature, the samples were rinsed in phosphate buffered saline (PBS) and incubated with rabbit polyclonal anti rat Ki-67 (1:250) overnight at 4°C. Sections were then incubated with biotinylated antibodies for 30min and followed by using an Elite avidin-biotin complex kit. Then, the samples were incubated with DAB chromogen, giving a brown product. Finally, the tissue sections were counterstained with hematoxylin for microscopic examination. Brown nuclei labeling cells were considered as positive cells for Ki-67. At least 2000 hepatocytes per liver were examined for Ki-67 positive cells. The Ki-67 proliferative index was expressed as the percentage of positive cells per 100 hepatocytes.

Determination of apoptosis

Apoptotic cells were determined by terminal deoxynucleotidyl transferase-mediated X-dUTP nick-end labeling (TUNEL) assay in Groups 1-5 and 10. The double immunostaining for TUNEL and GST-P was performed using an ApopTag Peroxidase in situ kit and EnVision Doublestain system. Liver sections were deparaffinized, rehydrated, and pretreated with proteinase K (20 $\mu\text{g}/\text{ml}$) for 15min and 3% H_2O_2 . After incubation in equilibration buffer for 5 min, working strength terminal deoxynucleotidyl transferase (TdT) enzyme was added and incubated at 37°C for 1 hour. The samples were treated with an anti-digoxigenin antibody conjugated to a peroxidase molecule that generated color when exposed to DAB. All slides were incubated with the Double-stain Block reagent followed by incubation with the anti-rabbit

GST-P antibody (1:1000). The alkaline phosphatase (AP) labeled polymer from the EnVision System kit was applied for 10min at room temperature. The secondary staining was visualized using red phosphatase chromogen solution. The numbers of TUNEL positive cells were counted both inside and outside regions of GST-P positive foci. The apoptotic index was assessed as apoptotic cells per 1000 hepatocytes.

Determination of glutathione-S-transferase activity

Rat liver was homogenized in ice-cold buffer containing 1.5% w/v KCl and 0.25mM phenylmethylsulfonyl fluoride, and centrifuged at 10,000g for 20min at 4°C to remove a part of pellet. The cytosolic fraction was obtained from further centrifugation of supernatant at 100,000g for 60 min at 4°C.

Glutathione-S-transferase (GST) activity was determined spectrophotometrically according to the method of Habig and Jakoby (1981). This assay was determined by monitoring rate of increase in conjugate formation between GSH and 1-chloro-2,4-dinitrobenzene (CDNB). One unit of enzyme is defined as the amount of glutathione-S-transferase catalyzes the formation of 1 μmol of CDNB-GSH conjugates formed.

Statistical analysis

The experimental results are expressed as mean of group \pm SD. The statistical significance of the difference between groups was analyzed by one-way analysis of variance (ANOVA) with least significant difference (LSD) for post hoc test.

Results

There were no significant differences in food and water intake observed among the experimental groups. Table 1 shows the effect of *S neglecta* on body and liver weights and liver functional test. The body weight change in groups treated with *S neglecta* was not significantly different from either DEN-treated or negative control groups. No significant difference in liver, kidney, and spleen relative weights was observed in groups treated

Table 1. Body and Liver Relative Weights and Blood Biochemistry of Rats

Group	Treatments	Administration period (weeks)	Final body weights (g)	% Body weight weights (g)	Relative liver weights (g/100g body weight)	AST (U/l)	ALT (U/l)
1	DEN	-	415 \pm 25	310.8 \pm 28.8	2.87 \pm 0.17	148 \pm 31	41 \pm 4
2	DEN+SNE 50mg/kg bw	9	404 \pm 25	298.3 \pm 32.6	2.80 \pm 0.25	112 \pm 21*	43 \pm 7
3	DEN+SNE 200mg/kg bw	9	406 \pm 27	294.5 \pm 24.0	2.80 \pm 0.34	103 \pm 21*	36 \pm 9
4	DEN+1% SND	9	412 \pm 19	305.9 \pm 16.8	3.02 \pm 0.25	128 \pm 27*	36 \pm 4
5	DEN+4% SND	9	418 \pm 36	308.2 \pm 36.1	3.11 \pm 0.46	134 \pm 23	44 \pm 9
6	DEN+SNE 50mg/kg bw	13	404 \pm 24	294.8 \pm 28.7	2.72 \pm 0.20	123 \pm 29*	39 \pm 7
7	DEN+SNE 200mg/kg bw	13	404 \pm 23	293.7 \pm 21.3	2.80 \pm 0.17	105 \pm 21*	37 \pm 7
8	DEN+1%SND	13	393 \pm 17	292.7 \pm 18.2	3.08 \pm 0.30	120 \pm 12*	43 \pm 7
9	DEN+4%SND	13	407 \pm 19	306.8 \pm 23.2	3.05 \pm 0.20	99 \pm 9*	40 \pm 9
10	NSS	-	428 \pm 10	324.4 \pm 18.4	3.00 \pm 0.44	76 \pm 7	32 \pm 8
11	SNE 50mg/kg bw	13	441 \pm 42	340.7 \pm 37.4	2.91 \pm 0.26	99 \pm 22	30 \pm 4
12	SNE 200mg/kg bw	13	461 \pm 43	360.8 \pm 37.6	2.92 \pm 0.48	83 \pm 12	31 \pm 6
13	1% SND	13	467 \pm 49	358.4 \pm 38.2	2.92 \pm 0.29	81 \pm 5	33 \pm 3
14	4% SND	13	435 \pm 17	324.9 \pm 21.7	3.06 \pm 0.35	83 \pm 8	33 \pm 6

*Significantly difference from the DEN group; $p < 0.05$; DEN: diethylnitrosamine; NSS: normal saline; SNE: water extract of *S neglecta*; SND: *S neglecta* mixed diet;

**values are means \pm SD

Table 2. Effects of *Spirogyra neglecta* (Hassall) Kützing on the GST-P-positive Foci Formation in the Liver of Rats

Group	Treatments	Administration period (weeks)	GST-P positive foci (foci/cm ²)	Distribution of GST-P+ size (foci/cm ²)		
				31-50 (cells/focus)	51-100 (cells/focus)	>100 (cells/focus)
1	DEN	-	18.20±11.26	7.76±4.39	6.10±4.39	4.34±3.22
2	DEN+SNE 50mg/kg bw	9	14.11±10.30	6.35±4.07	4.43±2.63	3.33±4.2
3	DEN+SNE 200mg/kg bw	9	6.58±3.49**	3.21±1.91	1.65±1.21	1.72±1.51
4	DEN+1% SND	9	9.89±7.78*	4.51±3.62	3.46±3.06	1.92±1.63
5	DEN+4% SND	9	9.25±6.32*	3.99±2.63	2.73±1.89	2.53±3.08
6	DEN+SNE 50mg/kg bw	13	5.76±4.17**	3.10±2.03	1.94±1.87	0.72±0.55
7	DEN+SNE 200mg/kg bw	13	7.88±5.04*	4.06±2.65	2.18±1.45	1.64±2.47
8	DEN+1% SND	13	9.44±6.04*	4.09±3.30	2.96±2.16	2.39±1.85
9	DEN+4% SND	13	7.95±4.73*	3.58±2.60	2.86±1.85	1.51±1.13
10	NSS	-	0±0	0±0	0±0	0±0
11	SNE 50 mg/kg bw-	13	0±0	0±0	0±0	0±0
12	SNE 200 mg/kg bw	13	0±0	0±0	0±0	0±0
13	1% SND	13	0±0	0±0	0±0	0±0
14	4% SND	13	0±0	0±0	0±0	0±0

*Significantly difference from the DEN group p<0.05, **p<0.005, DEN; diethylnitrosamine, NSS; normal saline, SNE; water extract of *S neglecta*, SND; *S neglecta* mixed diet; **values are means±SD

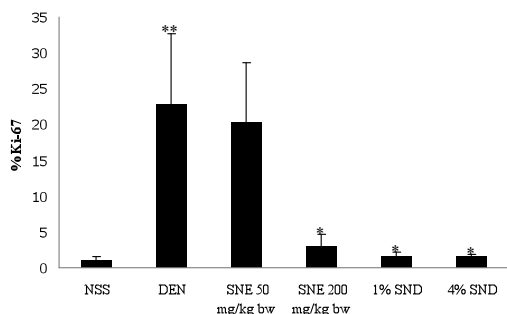


Figure 2. Effect of the Treatment of *Spirogyra Neglecta* (Hassall) Kützing at The Post-Initiation Stage on Ki-67 in Liver of Rats. *p<0.05, significantly different from the DEN group. **p<0.05 significantly different from the NSS group

with *S neglecta* as compared with the positive and negative control groups. Table 1 shows that the AST level in serum of groups treated with *S neglecta* significantly decreased (p<0.05) when compared with those treated with DEN alone (Group 1) with the exception of the group treated with 4% SND for 9 wks (Group 5). The serum ALT level was not significantly changed in the SNE-treated and SND-treated groups compared with the DEN-treated group (Group 1) and the negative control group (Group 10).

The effects of *S neglecta* on DEN-induced glutathione-S-transferase placental (GST-P) positive foci in rat livers are shown in Table 2. GST-P positive foci were not observed in normal saline-treated groups (Groups 10-14), whereas the positive foci were founded in all DEN-injected rats (Groups 1-9). The numbers and areas of GST-P positive foci were significantly diminished in groups treated with SNE and SND at post-initiation, but were not observed in the group treated with SNE at 50mg/kg (Group 2) as compared with the DEN control group. In addition, Ki-67 proliferation marker expression was also significantly (p<0.001) diminished in *S neglecta*-treated groups at post-initiation, with the exception of the group treated with SNE at 50mg/kg (Group 2; see Figure 2).

The number of TUNEL-positive cells in GST-P positive foci and surrounding normal tissue areas in

Table 3. Effects of *Spirogyra neglecta* (Hassall) Kützing on Number of Apoptotic Cells in GST-P-positive Foci and Surrounding Area

Treatment	Administration period (weeks)	Apoptotic cells (No./1000 hepatocytes)	
		GST-P	Surrounding area
DEN	-	16.59±11.31	4.06±3.46
DEN+SNE 50 (mg/kg bw)	9	22.35±8.98	4.92±2.83
DEN+SNE 200	9	21.58±22.98	4.47±2.06
DEN+1% SND	9	21.77±14.88	3.19±2.22
DEN+4% SND	9	12.33±3.46	3.09±1.63
NSS	-	0.00±0	12.00±1.04

*DEN: diethylnitrosamine; NSS: normal saline; SNE: water extract of *S neglecta*; SND: *S neglecta* mixed diet

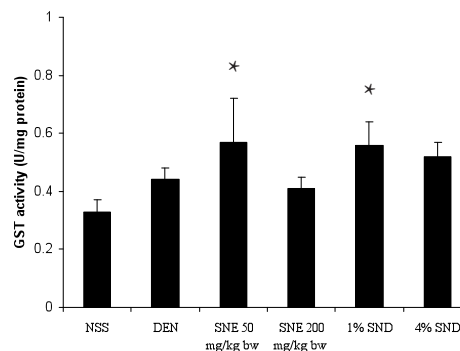


Figure 3. Effect of The Treatment of *Spirogyra Neglecta* (Hassall) Kützing at The Pre-Initiation Stage on Hepatic Glutathione-S-Transferase Activity in Livers of Rats. *p<0.05: Significantly Different From The DEN Group

livers of rats treated with SNE and SND at post-initiation is presented in Table 3. The TUNEL-positive cells in normal tissue in the DEN control group (Group 1) were not significantly different from the negative control group (Group 10). The number of TUNEL-positive cells in GST-P positive foci was significantly higher (p<0.05) than that in the surrounding normal tissue in the DEN-treated and *S neglecta*-treated groups. However, the number of TUNEL-positive cells either within GST-P or the surrounding tissue in the *S neglecta*-treated groups was

not significantly different from the DEN control group. Table 2 also shows that the numbers and areas of GST-P were also significantly diminished in all groups treated with SNE and SND at the pre-initiation stage (Groups 6-9). As shown in Figure 3, cytosolic GST activity was significantly increased only with the administration of SNE at 50 mg/kg bw (Group 6) and the 1% SND-treated group (Group 8).

Discussion

This study has shown that *Spirogyra neglecta*, a freshwater macroalgae, possesses cancer-chemopreventive activity in diethylnitrosamine-initiated preneoplastic lesions in rat liver. The *S neglecta* doses used in this experiment, which ranged from human daily consumption level to 4-fold overdose, were nontoxic and non-carcinogenic to rats. The administration of *S neglecta* in the form of a raw material and a hot water extract reduced the number of glutathione-S-transferase placental form (GST-P) positive foci in rat liver injected with diethylnitrosamine. The appearance of GST-P positive foci has been correlated to the development of hepatocellular carcinoma in rats (Tsuda et al., 2003). These foci have been recognized as reliable and sensitive markers for identifying preneoplastic lesions in the liver. Thus, the number and size of the GST-P positive foci have been widely used as the end-point marker of carcinogenicity screening and chemoprevention studies for hepatocarcinogenesis (Tsuda et al., 2010; Punvittayagul et al., 2012).

Wattenberg L.W. has classified cancer chemopreventive agents by their modes of action into two categories, blocking agents and suppressive agents (Wattenberg, 1997). The active compounds in *S neglecta* might act as both suppressing and blocking agents. Rats fed with *S neglecta* after diethylnitrosamine injection for 9 weeks exhibited a significantly diminished number of GST-P positive foci in the liver. *S neglecta* also led to a reduction in the number of Ki-67 positive hepatocytes in diethylnitrosamine-injected rats. Ki-67 has been used as a proliferation marker for cancer cells (Lin et al., 2000). Treatment with diethylnitrosamine exhibited strong Ki-67 positive nuclear staining in hepatocytes when compared to those of untreated rats, indicating a high rate of proliferation in these cells. These findings suggest that *S neglecta* might inhibit initiated-hepatocyte proliferation in diethylnitrosamine-treated rats leading to a reduction in preneoplastic lesions in rat liver.

The administration of *S neglecta* in the post-initiation stage tended to induce the apoptosis of altered hepatocytes. The number of apoptotic cells within the GST-P positive foci of the diethylnitrosamine control and the *S neglecta*-treated groups was significantly increased as compared with that in the surrounding normal tissue. This result is consistent with previous reports that apoptosis also increases during hepatocarcinogenesis in rats (Bursch et al., 2005; Ong et al., 2006). The apoptosis was shown to constitute an innate tissue defense against carcinogens by preventing the survival of initiated cells. Apoptosis plays an important role in elimination of cancer cells. Thus, the induction of apoptosis was proposed as a mechanism

of cancer prevention and treatment (Sun et al., 2004). Hence, *S neglecta* might suppress the promotion stage of rat hepatocarcinogenesis by inhibiting initiated-cell proliferation.

Furthermore, the administration of *S neglecta* prior to the injection of diethylnitrosamine at the beginning of week 3 attenuated the number of hepatic GST-P positive foci in the rats. *S neglecta* also enhanced the activity of glutathione S-transferase, a detoxifying enzyme of diethylnitrosamine metabolism, in rat liver. Diethylnitrosamine is metabolized by CYP2E1 to form the known carcinogenic species, alkyl diazonium ions (Yamazoe et al., 1983). These electrophiles commonly conjugate with glutathione by glutathione S-transferase, which prevents them from forming DNA adducts. Some publications have reported that the induction of glutathione S-transferase activity is correlated with cancer prevention (Puatanachokchai et al., 2002; Tsuda et al., 2004). Our previous studies also showed that *S neglecta* enhanced some hepatic antioxidant enzymes in rats (Thumvijit et al., 2013b). Based on these observations, it seems possible that *S neglecta* can block and suppress diethylnitrosamine induced-hepatocarcinogenesis in rats.

Several studies have shown that not only medical plants, but also algae contain beneficial non-nutritive agents such as polyphenols, chlorophylls, and sulfated polysaccharides, which possess antimutagenicity, anticarcinogenicity, and antioxidant activity (Cornish and Garbary, 2010; Lee et al., 2013). We previously demonstrated that the amounts of total phenolic compounds, total carbohydrates, and sulfates of the hot water extract were greater than those of raw materials (Thumvijit et al., 2013b). One gram of this hot water extract contained 33.9 ± 1.46 mg of carbohydrates and 1.08 ± 0.01 mg of sulfated polysaccharides. These enhanced concentrations might be one reason why the administration of the hot water extract of *S neglecta* had greater anticarcinogenicity than dried algae.

Several reports have shown that many algae, including seaweed, contain biologically active antitumor and immunostimulative polysaccharides. Sulfated polysaccharides like fucoidan, carrageenan, or algin show antioxidant, antimutagenic, anticoagulant, and antitumor activity (Smit, 2004). Furthermore, sulfated polysaccharides isolated from seaweed, *Monostroma nitidum*, inhibit some cytochrome P450s and enhance some detoxifying enzymes in rats (Charles et al., 2007). Chlorophylls, lipid-soluble pigments abundantly found in algae and higher plants, exert their anti-cancer activity via many mechanisms. Chlorophylls block the absorption of aflatoxin and other carcinogens, thereby inhibiting carcinogenesis (Ferruzzi and Blakeslee, 2007). We have reported that the chlorophyll content in dried *S neglecta* is greater than that in the hot water extract (Thumvijit et al., 2013b). Thus, it seems possible that the cancer chemopreventive agents in *S neglecta* are polysaccharides.

In conclusion, our studies show that *Spirogyra neglecta* (Hassall) Kützing exhibits cancer chemopreventive properties at the early stages of diethylnitrosamine-induced hepatocarcinogenesis in rats. *S neglecta* blocks and suppresses carcinogenesis via the enhancement

of some detoxifying enzymes, thereby suppressing precancerous cell proliferation in rat liver. Our current research seeks to identify the anticarcinogenic compounds in *Spirogyra neglecta* (Hassall) Kützing.

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