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Full Length Research Paper

In vitro antigenotoxic and anti-oxidative capacity of *Hypnea musciformis* (Wulfen) Lamouroux extract in human lymphocytes

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Several hazardous substances, can damage our DNA in various ways. Defining these substances and the protective ones is very important. Therefore, researches have increased on various compounds based on natural sources which keep the harmful effects of these various agents at the minimum level. In the present study, we evaluated the potential genotoxic/ antigenotoxic/ antimutagenic activity of the crude chloroform: methanol (2:1) extracts of *Hypnea musciformis* (Wulfen) Lamouroux (HME), in human lymphocytes culture *in vitro* against genotoxic/mutagenic agents mitomycin C (MMC) and ethyl methanesulfonate (EMS) by using chromosome aberration (CA), sister chromatid exchange (SCE) and micronuclei (MN) assays, and also determined total antioxidant capacity (in soluble lipid and water), phenolic compound, protein, carbohydrate, vitamins (A, C and E) contents. The frequency of chromosome aberrations and SCE increased by MMC, were significantly decreased by HME ($p < 0.05$ for CA, $p < 0.001$ for SCE). The MN frequencies of the cells were significantly decreased by the treatment with HME plus MMC or EMS when compared with the positive controls (MMC or EMS) ($p < 0.05$). In conclusion, HME itself is not a clastogenic or cytotoxic substance. On the other hand, HME possesses a strong antigenotoxic, anti-clastogenic and protective effects against MMC *in vitro.*

Key words: *Hypnea musciformis*, anti-oxidative and antigenotoxic effect, chromosome aberration, sister chromatid exchange, micronuclei.

INTRODUCTION

The cellular macromolecules of humans, such as DNA, proteins and lipids, are continuously at risk for endogenous and environmentally induced structural alterations. Several man-made chemicals find their way into the environment and pose health risks to the human population. Acute and chronic exposure to these environmental chemicals, such as pesticides, metals, polycyclic aromatic hydrocarbons (PAHs), solvents, and alkylating agents, has been shown to produce marked toxicity at their target sites. Some of these chemicals are used as therapeutic agents by virtue of their antitumor antibiotic activity, such as mitomycin C (MMC). MMC is a potent DNA cross-linker, and alkylates DNA by this means, giving rise to oxidative DNA damage (Lee and Kohn, 2009). Another important alkylating agent is ethyl methanesulfonate (EMS), used experimentally as a mutagen, teratogen and brain carcinogen (IARC, 1987).

Reactive oxygen species (ROS) are another important class of damage agents for cellular macromolecules. ROS, such as O_2^-, OH^- and H_2O_2, are highly genotoxic/ mutagenic and harmful to cellular macromolecules such as DNA, proteins and lipids (Mantena et al., 2008). The

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Abreviations: HME, *Hypnea musciformis* (Wulfen) Lamouroux; **MMC,** genotoxic/mutagenic agents mitomycin C; **EMS,** ethyl methanesulfonate; **CA,** chromosome aberration; **SCE,** sister chromatid exchange; **MN,** micronuclei; **ROS,** reactive oxygen species; **PAHs,** polycyclic aromatic hydrocarbons; **NDI,** nuclear division index; **CAHT,** water-soluble antioxidant capacity; **CALT,** lipid-soluble antioxidant capacity.

adverse effect is represented by the oxidative stress that can arise from a lack of antioxidant defence or by an increase of oxidative processes in the body (Cornelli, 2009). Many different diseases have been related to oxidative stress (Roy et al., 2009). For this reason, it has been proposed that dietary antioxidants in food significantly decrease the adverse effects of ROS, reactive nitrogen species, or both on normal physiological function in human (Cornelli, 2009).

There is an increasing demand for natural antioxidant molecules to replace the synthetic additives used in the food industry. The preservative effect of many plant species and herbs suggests the presence of anti-oxidative and anti-genotoxic constituents in their tissues (Hirasa and Takemasa, 1998; Ebeed et al., 2010). For this reason, interest in marine organisms as a promising potential source of pharmaceutical agents has increased during the last few years (Mayer and Hamann, 2002; Newmann et al., 2003). Also, seaweeds and their extracts have been demonstrated to have strong antioxidant activity (Yuan and Walsh, 2006; Hwang et al., 2010). Proteins with anti-oxidative properties; phenolic compounds such as flavonoids, coumarins and tocopherols; nitrogen-containing compounds including alkaloids, chlorophyll derivates, amino acids and amines; as well as other compounds such as carotenoids, ascorbic acid, glutathione and uric acid are powerful antioxidant molecules in macroalgae (Potterat, 1997). Also, there are some reports on the biological activities of several seaweed species, specifically the antiproliferative activity of ethanolic and aqueous extracts from green algae (Wu et al., 2005) and from *Turbinaria orrata* (Deslandes et al., 2000) against human cancer cells. In two previous *in vitro* studies, Celikler et al. have noted the anti-genotoxic activity of *Ulva rigida* (2008) and the DNA damage-protecting activity and antioxidant potential of *Codium tomentosum* Stackhouse crude ethanolic extract in human lymphocytes (2009).

In the present study, we investigated the possibly chemotherapeutic, oxidative DNA damage-protecting activity and antioxidant capacity of *Hypnea musciformis* (Wulfen) Lamouroux crude chloroform: methanol (2:1) extract (HME) in relation to its total polyphenol and other anti-oxidative molecular contents *in vitro*. To the best of our knowledge, this is the first report of antioxidant capacity and DNA damage-protecting activities of *H. musciformis* (Wulfen) Lamouroux collected from the coast of the Southeast Marmara Sea.

MATERIALS AND METHODS

Sampling and identification

H. musciformis was collected by scuba diving at depths of 5 - 10 m from Southern Marmara Sea shores (40° 22.8'N, 28° 48.0' E) in the Bursa-Zeytinbagi region. Epiphytes were cleaned and necrotic parts were removed. The surface microflora was removed by washing algal samples for 10 min with 30% ethanol. The samples were successively rinsed with sea water and distilled water, then dried in the shade and finely powdered. For the chemical analysis, the clean fresh algae pieces were frozen and stored at - 20°C.

Extraction

The dry powdered algal material (7 g) was extracted with 100 ml of chloroform: methanol (2:1) (Merck, Darmstadt, Germany) for 24 h using Soxhlet equipment. The extract was filtered by syringe (0.22 µm), then cooled and concentrated using a rotary evaporator at 30 - 45° C. This was stored in labelled sterile screw-capped bottles at - 20° C until use.

Chemical

5-Bromdeoxyuridine (BrdUrd, CAS: 59-14-3), gallic acid (CAS: 149-91-7), α- tocopherol (CAS: 10191-41-0) and cytochalasin B (C29H37NO5, CAS: 14930-96-2) were purchased from Sigma-Aldrich, and mitomycin-C (MMC) was purchased from Kyowa Hakko. All other solvents and chemicals were of analytical grade.

Genotoxicity/ antigenotoxicity assays

Heparinised peripheral blood obtained from four healthy, non-smoking donors (two males and two females, ages 18 to 23) were used in all experiments. The cultures were set up by adding 0.3 ml of whole blood to RPMI 1640 medium (1X, Sigma) supplemented with 20% foetal calf serum (Biochrom AG), 100 IU/ml penicillin- 100 µg/ml streptomycin (Biological Industries), 0.5 mg/ml L-glutamine and 6 µg/ml phytohemagglutinin (PHA-L, Biochrom AG). Lymphocytes were incubated at 37°C for 72 h. For induction of oxidative and chromosomal damage, the lymphocytes were treated with MMC (0.25 ppm) and EMS (ethyl methane sulfonate, 1250 ppm), respectively, as positive controls. All mutagenic solutions were prepared immediately before use, to avoid degradation of the agents. As a negative control, distilled water was also used. The cells were treated separately with mutagenic agents and diluted sterile HME (200, 400 and 800 ppm), which were chosen after a preliminary study (50, 100, 200, 400, 800, 1600 and 2500 ppm), for 24 h prior to harvest. To determine the antigenotoxic effect of the HME, the dose of 400 ppm HME was chosen and co-cultured with each mutagenic agent.

For SCE analyses, cultures were incubated in the dark in the presence of 10 µg/ml of 5-bromodeoxyuridine (BrdUrd, Sigma). Metaphases were obtained by adding colcemid (0.2 µg/ml final concentrations, Sigma) 2 h prior to harvest.

For micronuclei assay, cytochalasin B (6 µg/ml) was added at 44 h to block cytokinesis, and lymphocyte cultures were harvested after 72 h (Fenech, 2000).

Chromosomes were prepared using standard procedures (Benn and Perle, 1992). For analysis of SCE, the chromosome slides were stained according to the fluorescence plus Giemsa (FPG) technique (Parry and Wolff, 1974). Staining of the micronuclei was performed by immersing the air-dried slides in a 2% Giemsa solution.

Microscopic evaluation

Chromosomal aberrations (CA)

The analysis of chromosomal aberrations (CAs) was performed in

50 metaphases for each culture. Both gaps and pulverisations were included and both gaps and pulverisations were excluded from total chromosome aberrations. The CAs was classified according to the Environmental Health Criteria (EHC) 51 for short-term tests for mutagenic and carcinogenic chemicals (IPCS, 1985).

Sister chromatid exchange (SCE)

Scoring criteria for SCE were based on the work of Carrano and Natarajan (1988). A total of 30 well-spread, complete (2n = 46) second-division metaphases were scored for SCE, and the frequency of SCE per cell was recorded.

Micronuclei (MN)

Two thousand binucleated cells for each experimental point were examined, following the scoring criteria adopted by the Human Micronucleus Project (Bonassi et al., 2001). We evaluated the binucleated micronucleated lymphocyte (BNMN) frequency as the number of binucleated lymphocytes containing one or more MN per 1000 binucleated cells. In the anti-clastogenicity experiments, the present reduction by treatment with HME in the number of cells with micronuclei that showed protective activity was calculated according to Manoharan and Banerjee (1985) and Waters et al. (1990) using the following formula:

$$\text{Reduction (\%)} = \frac{\text{Number of cells with MN in A} - \text{Number of cells with MN in B}}{\text{Number of cells with MN in A} - \text{Number of cells with MN in C}} \times 100$$

where A was the group of cells treated with the corresponding positive control (MMC, EMS), B was the group of cells treated with HME plus the positive control and C was the negative control group.

Mitotic index (MI), proliferative index (PRI) and nuclear division index (NDI)

The mitotic index was calculated from the number of metaphases in 2000 cells, analysed per culture for each dose group and donor in CA and SCE assays. In the SCE assay, the proliferative rate index (PRI) was calculated using the following:

$$PRI = (M_1 + 2M_2 + 3M_3)/ N$$

where M_1, M_2 and M_3 indicate those metaphases corresponding to first, second and third divisions, and N is the total number of metaphases scored (Lamberti et al., 1983).

Moreover, in the micronuclei assay, 500 lymphocytes were scored to evaluate the percentage of binucleated cells, and the nuclear division index (NDI) was calculated according to the following formula:

$$NDI = [MONO + 2BN + 3TRI + 4TETRA]/ 500$$

where MONO, BN, TRI and TETRA are mononucleated, binucleated, trinucleated and tetranucleated lymphocytes, respectively.

Biochemical analyses

Determination of lipid-soluble antioxidant capacity (CALT)

Samples were homogenised with hexane and shaken for 1 h at 4°C in the dark. After centrifugation at 6000 g for 10 min, the supernatant was transferred to new tubes.

Samples of hexanic extracts (200 µl) were placed in Eppendorf tubes, dried out and re-dissolved in the same volume of ethanol. These ethanolic solutions were supplemented with 1 ml phosphomolybdenum reagent (32 mM sodium phosphate, 4 mM ammonium molybdate, 0.6 M sulfuric acid) and were incubated at 95°C for 90 min. Finally, the absorbance at 695 nm was measured. Lipid-soluble antioxidant capacity is expressed as equivalents of α-tocopherol (Prieto et al., 1999).

Determination of water-soluble antioxidant capacity (CAHT)

Samples of water extracts (200 µL) were supplemented with 1 ml phosphomolybdenum reagent and incubated at 95°C for 90 min. Finally, the absorbance at 695 nm was measured. Water-soluble antioxidant capacity is expressed as equivalents of L-ascorbic acid (Prieto et al., 1999).

Total phenolic content

Phenolic contents of crude methanol extract were measured using Folin Ciocalteu's method as described by Taga et al. (1984). Absorbance was measured at 720 nm and total phenolic content was calculated with a gallic acid standard and expressed as gallic acid equivalent per gram.

Determination of vitamin E

Vitamin E content was determined by using a method described by Prieto et al. (1999); 0.1 ml hexanic extract of algae was mixed with 1 ml phosphomolybdenum reagent solution and incubated at 37°C for 90 min with vigorous shaking. The absorbance was measured at 695 nm. Vitamin E content was expressed as α-tocopherol equivalents per gram of extract.

Determination of vitamin C

Ascorbic acid concentrations were determined by the titrimetric Association of Official Analytical Chemists (AOAC) method No. 967.21 using 2,6-dichlorophenol indophenol as a titrant (AOAC, 1990).

Determination of vitamin A

The samples were extracted with hexane. Vitamin A content was determined and calculated by using a method described by Rutkowski et al. (2006).

Total protein

Total protein content was determined spectrophotometrically at 595 nm and concentrations were calculated by comparison to a calibration curve of bovine serum albumin (Bradford, 1976).

Determination of total soluble carbohydrate

Total soluble carbohydrate was assayed by the anthrone-sulfuric acid method (Laurentin and Edwards, 2003), involving extraction

Table 1. Results of frequencies of chromosome aberrations tests (mean ± SD).

Treatment		Number of chromosome aberration[a]							TA (G+P) ± SD	TA (G-P) ± SD
	MI ± SD	G	CB	ICB	EX	SE	PL	DM		
DW	14.41 ± 2.38	7	1	0	0	0	10	0	0.13 ± 0.09	0.02± 0.02
200 ppm HME	15.76 ± 3.69	6	0	0	2	0	9	0	0.11 ± 0.11	0.01 ± 0.01
400 ppm HME	11.13 ± 2.11	2	2	0	1	0	11	0	0.11 ± 0.08	0.02± 0.02
800 ppm HME	8.00 ± 1.16	3	1	0	2	0	16	0	0.15± 0.14	0.03 ± 0.02
MMC	13.20 ± 2.81	7	9	9	19	0	5	1	0.38 ± 0.07	0.30 ± 0.04
EMS	1.82 ± 1.24	8	6	4	8	1	7	0	0.54 ± 0.35	0.28 ± 0.12
[b]MMC + 400 ppm HME	10.49 ± 1.22	5	3	1	6	0	8	0	0.16± 0.07*	0.07 ± 0.05**
[c]EMS + 400 ppm HME	2.14 ± 1.25	4	3	0	8	0	5	0	0.26 ± 0.17	0.10 ± 0.06

SD, standard deviation; D.W., distilled water; G, gap; CB, chromatid break; ICB, iso-chromatid break, EX, exchange figure; SE, spiralization error; PL, pulverization; DM, double minute. MI, mitotic index; MMC, mitomycin; EMS, ethyl methanesulfonate; CTA (G + P), total chromosome aberration including gaps and pulverizations; TA (G - P), total chromosome aberration excluding gaps and pulverizations; HME, *Hypnea musciformis* (Wulfen) Lamouroux crude chloroform / methanol (2/1) extract.
[a] Total 1000 cells were evaluated;[b] Significancy MMC + 400 ppm HME compared with that of MMC (0.25 ppm); [c] Significancy EMS + 400 ppm HME compared with that of EMS (1250 ppm); * $p < 0.05$; ** $p < 0.001$.

with 15% trichloroacetic acid. The absorbance was measured at 620 nm.

Statistical analysis

The frequencies of CAs, SCE and MN and other biochemical parameters in treated cultures were compared by using one-way analysis of variance (ANOVA) and Tukey honest significant difference (HSD) tests with 95% confidence intervals. These analyses were carried out with commercial software programs SPSS 13.0.

RESULTS

The results of chromosomal aberrations (CAs) tests are shown in Table 1. Mitotic index and the types of structural chromosome aberrations and their frequency in human lymphocytes are also presented in Table 1. Numbers of chromosome aberrations of the cells treated with three different doses (200, 400 and 800 ppm) of HME were not statistically different from the negative control. The positive controls MMC and EMS at concentrations of 0.25 and 1250 ppm were significantly increased in the number of abnormal metaphases and the total number of structural chromosome aberrations, respectively, when compared with the negative control ($p < 0.001$).

The frequency of chromosome aberrations was significantly decreased by the treatment of HME (400 ppm) plus MMC relative to the positive control of MMC alone ($p < 0.05$ for the frequency of total CAs including gaps and pulverizations, $p < 0.001$ for the frequency of total CAs excluding gaps and pulverizations). The frequency of chromosome aberrations of the cells treated with HME plus EMS also decreased relative to the respective positive controls, but this reduction was not statistically significant (Table 1). Moreover, MI decreased in cultures treated with 400 and 800 ppm HME (Table 1).

The results of sister chromatid exchange (SCE) tests are shown in Table 2. The SCE frequencies of the cells treated with three different doses (200, 400, 800 ppm) of HME were not statistically different from negative control values. The SCE frequencies per cell significantly increased in human lymphocyte cultures treated with MMC or EMS as positive controls relative to negative control ($p < 0.001$). The SCE frequencies of the cells treated with HME plus MMC significantly decreased relative to the MMC positive control ($p < 0.001$). The SCE frequencies of the cells treated with HME plus EMS did not decrease relative to the positive controls (EMS) (Table 2). Moreover, PRI (proliferative index) decreased in the cultures treated with 400 and 800 ppm HME relative to negative control.

The results of the micronuclei (MN) assays are shown in Table 3. The MN frequencies of the cells treated with three different doses of HME are decreased relative to the negative controls, but not with statistical significance. The MN frequencies were significantly increased in human lymphocyte culture treated with MMC or EMS as positive controls compared with negative control ($p < 0.01$). The MN frequencies of the cells were significantly decreased by the treatment with HME plus MMC and EMS when compared with the positive controls (MMC or EMS) ($p < 0.05$).

The determined bioactive compound contents of *H. musciformis* are the lipid-soluble antioxidant capacity (CALT), water-soluble antioxidant capacity (CAHT), vitamins A, C, and E, total phenol, protein and carbohydrate. Amounts of these contents are shown in Table 4. The total phenolic compound of *H. musciformis* was 0.58 ± 0.03 mg gallic acid equivalent per gram dry seaweed. Furthermore, lipid soluble and water soluble

Table 2. Results of sister chromatid exchange (SCE) test (mean ± SD).

Treatments	SCE/ Cell ± SD	PRI ± SD	MI % ± SD
DW	2.91 ± 0.09	2.17± 0.14	8.13 ± 3.08
200 ppm HME	2.47 ± 0.21	2.24 ± 0.20	10.86 ± 4.89
400 ppm HME	2.52 ± 0.64	2.11± 0.19	9.57 ± 3.21
800 ppm HME	2.95± 0.59	2.06 ± 0.01	9.18 ± 2.35
MMC	15.80 ± 1.72	2.07 ± 0.05	6.85 ± 2.17
EMS	25.37 ± 10.25	1.82 ± 0.22	1.84 ± 0.62
[a]MMC+ 400 ppm HME	5.48± 0.81**	1.99 ± 0.20	5.10 ± 1.93
[b]EMS+ 400 ppm HME	26.64± 10.86	1.57 ± 0.16	1.57 ± 0.73

SD, standard deviation; DW., distilled water; SCE, sister chromatid exchange; MMC, mitomycin C; EMS, ethyl methanesulfonate; PRI, proliferative index; MI, mitotic index; HME, *Hypnea musciformis* (Wulfen) Lamouroux crude chloroform / methanol (2/1) extract.
[a] Significancy MMC + 400 ppm HME compared with that of MMC (0.25 ppm); [b] Significancy EMS + 400 ppm HME compared with that of EMS (1250 ppm); ** $p < 0.001$.

Table 3. Results of Micronuclei (MN) Tests (Mean ± SD).

Treatments	MN ± SD	NDI ± SD	Reduction (%)
DW	1.00 ± 1.41	1.22 ± 0.20	
200 ppm HME	0.00 ± 0.00	1.35 ± 0.47	
400 ppm HME	0.50 ± 0.71	1.36 ± 0.47	
800 ppm HME	1.00± 0.00	1.10 ± 0.08	
MMC	7.5 ± 2.12	1.26 ± 0.34	
EMS	4.50 ± 0.71	1.15 ± 0.18	
[a]MMC+ 400 ppm HME	0.50 ± 0.71*	1.21 ± 0.28	108
[b]EMS+ 400 ppm HME	0.00 ± 0.00*	1.06 ± 0.07	129

SD, standard deviation; DW, distilled water; MMC, mitomycin C; EMS, ethyl methanesulfonate; MN, micronuclei frequency; NDI, nuclear division index; HME, *Hypnea musciformis* (Wulfen) Lamouroux crude chloroform/ methanol (2/1) extract.
[a]Significancy MMC + 400 ppm HME compared with that of MMC (0.25 ppm); [b] Significancy EMS + 400 ppm HME compared with that of EMS (1250 ppm); * $p < 0.05$.

antioxidant capacities were found to be 252.80 ± 90.26 µmol α-tocopherol/g and 995.31 ± 67.94 µmol L-ascorbic acid/g, respectively.

DISCUSSION

The genome is constantly exposed to agents, both exogenous and endogenous that damage DNA. Consequently, it is very important to determine these agents and other agents that protect against them. The alkylating agents MMC and EMS cross-link DNA, leading to mutations involving different base substitutions, chromosome breaks, deletions, etc (Poersch et al., 2007). Therefore, they are routinely used for evaluating increased sensitivity to DNA cross-linking agents in studies using a variety of cytogenetic endpoints (Liou et al., 2002). Moreover, many antitumor agents, such as MMC, exhibit antitumor activity via ROS-dependent activation of apoptotic cell death, suggesting the possible use of ROS as an antitumor treatment (Fang et al, 2007). In the present study, we investigated potential genotoxic/ antigenotoxic, chemo-protective and cytotoxic effect and anti-oxidative capacity of HME against MMC and EMS by CA, SCE and MN assays. To the best our knowledge, there are no published data on genotoxicity/ antigenotoxicity and anti-oxidative capacity of *H. musciformis*.

EMS and MMC are mutagenic agents capable of inducing chromosome aberrations, micronuclei, and sister chromatid exchange (SCE) in lymphocytes (Matsuoka et al., 2004). Because of CA, SCE and MN values significantly increased in culture treated with only MMC or EMS, our results agree with available data (Tables 1 to 3). For the determination of cytotoxicity, the

Table 4. The contents of bioactive molecules and antioxidant capacity of *H. musciformis* (mean ± SD)

Antioxidant capacity/ bioactive molecules	*Hypnea musciformis* (Wulfen) Lamour ± SD
CALT (µmol a-tocopherol/g)	252.80± 90.26
CAHT (µmol L-ascorbic acid/g)	995.31 ± 67.94
Vitamin E (mg/g)	0.38 ± 0.16
Vitamin A (µM)	1.33 ± 0.53
Vitamin C (mg/g)	0. 67 ± 0.09
Total phenol (mg/g)	0.58 ± 0.03
Total protein (%)	41.33 ± 8.08
Total carbohydrate (%)	24.88 ± 19.36

CALT, lipid-soluble antioxidant capacity; CAHT, water-soluble antioxidant capacity; SD, standard deviation.

MI, PRI and NDI ratios were also evaluated. Only EMS or MMC treatment decreased MI, PRI and NDI ratios relative to DW, as expected (Tables 1 to 3). Moreover, in these tests HME did not show any genotoxic effect as the sole treatment for human lymphocytes in all three genotoxicity assays (Tables 1 to 3). MI, PRI and NDI ratios were decreased with high dose HME treatment, especially 800 ppm. Consequently, we suggest that HME has a relatively low cytotoxic effect on human lymphocytes *in vitro,* even in high doses. Selected HME doses (400 ppm) significantly decrease MMC induced DNA damage compared with positive control in all three tests ($p < 0.001$ or $p < 0.05$, Tables 1 to 3). While HME decreased DNA damage induced by EMS in the CA and MN tests (Tables 1 and 3), the SCE frequency did not decrease by treatment with HME in the SCE assay (Table 2). The increase of SCE frequency in the treat-ment of EMS plus HME may have been a synergistic effect of HME and EMS.

Consequently, the our results of CA, SCE and MN tests shown that HME has a strong antigenotoxic and chemo-protective effect against MMC induced DNA damage and a moderate antigenotoxic and chemo-protective effect against EMS induced DNA damage.

In our opinion, the antigenotoxic and chemo-protective effect may have originated from the anti-oxidative molecules of HME *in vitro*. Plants contain different antioxidants, and considering that vitamin E biosynthesis occurs only in plants, it would be useful to know the total concentration of antioxidants in individual species. Phenolic compounds and total antioxidant capacity have a major effect on antioxidative activities (Cao et al., 1997). Therefore, a number of studies have focused on the biological activities of phenolic compounds. Several authors have reported increased antioxidant and antimutagenic/anticarcinogenic activities due to these compounds (Aaronson, 2000; Siriwardhana et al., 2003; Karawita et al., 2005). In the present study, *H. musciformis* is found to have rather higher total phenol and water-soluble (CAHT) and lipid-soluble antioxidant capacities (CALT) than many plant species (Table 4) (Zhang et al., 2007, Mohamed et al., 2007). Such data might be useful in the identification of the most beneficial dietary plants and in finding new sources of natural antioxidants, such as vitamins E and C and phenolic compounds. In recent years, several algal species have also been reported to prevent oxidative damage by scavenging free radicals and active oxygen, hence possibly preventing cancer cell formation. Therefore, algal species, as alternative sources of natural antioxidative compounds, have attracted much attention from biomedical scientists. There is some evidence that seaweeds contain com-pounds with a relatively high antioxidant and antigenotoxic activity. Corroborating this, Celikler et al. (2009) have found lower total phenol and water-soluble antioxidant capacity (CAHT) in *C. tomentosum* than in *H. musciformis*. On the other hand, *C. tomentosum* has shown higher lipid-soluble antioxidant capacity (CALT) and vitamin E content.

Anticancer agents such as MMC are commonly used as a therapy against different kinds of cancer. These agents usually demolish the physiological homoeostasis in various organs during cancer treatment. Physiological side effects can be induced in non-tumour cells mostly by radical formation and oxidative damage. Thus com-pounds such as HME that have high anti-oxidative content and antigenotoxic effect can be used as protect-tions against these side effects. The present study has clearly demonstrated that HME has significant anti-oxidative and antigenotoxic activities.

In conclusion, HME should be isolated, purified and characterised in order to understand the mechanisms underlying its chemo-protective effect and antigenotoxic effects. The antioxidant function of bioactive molecules of HME in reducing clastogenicity may also be due to the induction of various biochemical pathways or interaction of bio-molecules with DNA and mutagenic agents, which need to be confirmed by additional *in vivo* and *in vitro* studies. Furthermore, the chemo-protective and antigenotoxic potential and anti-clastogenic effect of HME as observed in the present study are of great significance in radioprotection, and may be useful for human pathological conditions.

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