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Short communication

Inhibitory effects of *Enteromorpha linza* polysaccharide on micronucleus of *Allium sativum* root cells

Zhongshan Zhang[a,*], Xiaomei Wang[a], Jingfen Li[a], Chongbin Liu[a], Quanbin Zhang[b]

[a] Department of Medicine, Huzhou University, Huzhou 313000, China
[b] Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

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ABSTRACT

In this study, the antimutagenic function of the polysaccharide from *Enteromorpha linza* with the micronucleus test of *Allium sativum* root cells induced by sulfur dioxide and ultraviolet was studied. The concentration-effect relation of the two inducers was firstly evaluated. The results showed that an increase of genotoxicity damage was demonstrated and micronuclei frequency induced by sulfur dioxide and ultraviolet displayed dose dependent increases. All the doses of polysaccharide did affect the micronuclei frequency formation compared with the negative control. And also, the significant increase in inhibition rate of micronuclei frequency was observed with the increase of the dose of polysaccharide. It was showed maximum inhibition of micronuclei frequency cells (71.74% and 66.70%) at a concentration of 200 g/mL in three experiments. The low molecular weight polysaccharide showed higher inhibition rate than raw polysaccharide at the higher concentration (50 g/mL) in the absence of sulfur dioxide and ultraviolet. It was confirmed to be a good mutant inhibitor.

1. Introduction

A micronucleus is the erratic (third) nucleus that is formed during the anaphase of mitosis or meiosis. There is a chance of more than one micronucleus forming when more genetic damage has happened. A micronucleus test is a test used in toxicological screening for potential genotoxic compounds [1]. The assay is now recognized as one of the most successful and reliable assays for genotoxic carcinogens, *i.e.*, carcinogens that act by causing genetic damage and are the OECD guideline for the testing of chemicals.

There are two major versions of this test, one *in vivo* and the other *in vitro*. Micronuclei were first used to quantify chromosomal damage by H.J. Evans et al., in root tips of the Broad Bean, *Vicia faba* [2]. Subsequently the *in vitro* assay was developed in onion (*Alluim cepa*) [3], garlic (*Allium sativum*) [4] and spiderwort (*Tradescantia reflexa*) [5]. The well characterised genotoxic chemicals, etoposide (a topoisomerase inhibitor), colchicine (an aneugen), mitomycin C (a DNA cross linking agent) and cyclophosphamide (an alkylating agent requiring metabolic activation) were tested in the *in vitro* micronucleus assay [6]. Because mutations can lead to genetic diseases and cancer, it is necessary and significant to search drugs with anti-mutation. In recent years, it was found several natural substances showed excellent antimutagenic activity such as peptide, phenolic compounds, flavonoid and polysaccharide [7–10]. Recently much attention has been paid to the polysaccharides from seaweed in this field [11].

Previous study showed the polysaccharide from the green alga *Enteromorpha linza* was evaluated as a novel antioxidant [12]. However, the antimutagenic activity was not studied. In this work, we evaluated the antigenotoxic/antimutagenic activity of crude polysaccharide from *E. linza* in root tips of the garlic in vitro by using micronuclei (MN) assays as experimental endpoints induced by sulfur dioxide and ultraviolet.

2. Materials and methods

2.1. Chemicals and raw material

E. linza was collected on the coast of Qingdao in June 2014. The fresh alga was soon washed; sun dried and kept in plastic bags at room temperature for use.

Sulfur dioxide solution was the mixture of $NaHSO_3$ and Na_2SO_3 (1:3, mM/mM) at various concentrations from 0.01 to 10 mM [13].

2.2. Polysaccharide and degradation

The natural polysaccharide (EP) was extracted from *E. linza* in hot water with the method of previous study. Two reagents, ascor-

* Corresponding author: Fax: +86 572 2321166.
E-mail address: zhzs@hutc.zj.cn (Z. Zhang).

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bic acid (10 mM) and H_2O_2 (10 mM) were added into EP solution, and then stirred for 2 h. The reactor was precipitated to give the degraded product named after LEP [14].

2.3. Root tips preparations

Garlic (*A. sativum*) seeds were selected for this study. The preparation procedure was performed as described in the earlier report [15]. After the old roots were removed, the garlic heads were germinated at 22 °C for 24 h. And then the garlic were removed and allowed to germinate between two layers of moist cotton. When the newly emerged roots were of 1.50–2.00 cm in length, they were used in the test.

2.4. Treatments of samples

To study the concentration-effect relation of invorment pollution reagent, growing roots of 1.50–2.00 cm in length were treated with sulfur dioxide solution (0.01–10 mmol/L) for 4 h and ultraviolet (15–40W) for 5 min, and then maintained in tap water for 24 h recovery.

For the following micronucleus assay, three methods were carried out in the process of the treatment. (1) Sulfur dioxide solution and the polysaccharide were added at the same time into the treated solution for 4 h. (2) Sulfur dioxide solution was firstly added into for 4 h and then maintained in tap water for 4 h recovery. After that, the polysaccharide was added into for 4 h. (3) The sequence of the treatment was opposite to the second method. The ultraviolet test was similar to the above methods treated for 5 min at a distance of 30 cm. The entire treatment category included a negative control group and a positive control group. Each group consisted of at least 6 roots. All the experimental groups were kept in an incubator at 22 ± 1 °C. After treated, the roots were rinsed 3 times with distilled water and then maintained in tap water for 24 h recovery. The root tips of 1.50–2.00 cm in length were cut off and fixed overnight in Carnoy's solution (glacial acetic acid/ethanol = 1:3, v/v) for 24 h. After that, they were transferred in 70% ethanol for storage [16].

2.5. Micronucleus assay

The root tips were rinsed in distilled water and hydrolyzed in 1 M HCl at 60 °C for 8–10 min. Then the mitotic zone of 1.00–2.00 mm in length from root tips were cut off and squashed under a cover glass. After staining with Schiffs' reagent, the cells were scored for micronuclei frequencies under a 1000× magnification. A total of 6000 cells from six separate slides per experimental group were scored to determine the mean micronuclei frequencies (MCN‰). Each experiment was run with three replications.

2.6. Statistical analysis of data

The inhibition rate of polysaccharide was calculated using the following equation: Inhibition rate (%) = $[(n_0 - n_1)/n_0] \times 100\%$. Where n_0 is the MCN number of the control and n_1 is the MCN number of the samples. Sample means and standard deviations were statistically analyzed with the *F*-test for analysis of variance (ANOVA).

3. Results

3.1. The chemical characteristics of EP and LEP

The high and low molecular weight polysaccharide, EP and LEP, were selectively prepared and their chemical analysis results were shown in Table 1. From the table, the total sugars and sulfate content of two samples were similar. It indicated that the degradation could not result in destruction of main chain.

Table 1
The chemical characteristics of EP and LEP.

Sample	Total sugar(%)	Sulfate(%)	Uronic acid(%)	Mw(Da)	Mn(Da)
EP	61.39	21.06	16.45	121745	28523
LEP	60.11	23.43	14.82	16490	7583

Table 2
Effects of different concentration of polysaccharide on micronuclei frequency.

Sample	Treat dose (g/mL)	MCN/1000 cells
EP	10	0.89 ± 0.13
	50	0.74 ± 0.34
	100	0.66 ± 0.22
	200	0.64 ± 0.19
	400	0.64 ± 0.37
LEP	10	0.84 ± 0.43
	50	0.70 ± 0.45
	100	0.52 ± 0.21
	200	0.45 ± 0.09
	400	0.46 ± 0.17
Negative control	–	0.85 ± 0.44

3.2. The MCN‰ of invorment pollution reagent

The genotoxicity of sulfur dioxide and ultraviolet evaluated by the garlic MCN-assays were shown in Figs. 1 and 2. As shown in figures, an increase of genotoxicity damage was demonstrated and micronuclei frequency induced by sulfur dioxide and ultraviolet displayed dose dependent increases. Low concentrations of two inducing agents could induce micronuclei in the roots. However, at the highest concentrations, micronuclei frequency decreased due to higher physiological toxicity and a decline in mitotic activity for the sulfur dioxide test. The similar results were reported in previous study [17]. For the ultraviolet test, the significant increase in MCN frequency was observed exposed to 15–40 W ultraviolet. The high MCN frequency suggested the ultraviolet is greatly harmful to cells.

3.3. The MCN test reduced by polysaccharide

The two polysaccharides EP and LEP were added in the treated group and the changes of MCN frequency in root tips were shown in Table 2. From the table, all the doses of polysaccharide did affect the MCN frequency formation compared with the positive control. However, there was no significant difference for the MCN frequency compared with the negative control group. The results showed that EP and LEP had no effect on the frequency of micronucleus of the root tip cells at the bottom.

3.4. 3.4. The *MCN frequency* inhibition of polysaccharide

The MCN frequency was tested in the absence of pollution reagents and polysaccharide and one concentration was evaluated within the tested dose range of pollution reagents (1.5 mM and 40 W). The inhibition results were shown in Tables 3 and 4 . Three experiments suggested the order of addition of the treated solution took no significant effect on MCN frequency. The dose-response relationship for induced MCN showed an extremely increase as expected. For the sample LEP and EP, it was showed maximum inhibition of MCN cells (71.74% and 66.70%) at a concentration of 200 g/mL in three experiments. As is similar to the results shown in Table 2, LEP showed higher inhibition rate than EP. The reason may be that LEP has lower molecular weight to enter the root cells eas-