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Grape Seed Extract Protects Mice against Disseminated Candidiasis

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Abstract – Effect of grape seed extract (GSE) against *Candida albicans* was examined under in-vitro and in-vivo conditions. The GSE was extracted in ethanol. In-vitro results from an agar diffusion susceptibility assay showed the GSE inhibited *C. albicans* growth. This anticandidal effect was at dose-dependency. In experiments with animals, mice that received the GSE (0.5 mg per mice), intravenously (i.v.), before i.v.-infection with viable *C. albicans* yeast cells survived longer than diluent (buffer)-received control mice. In contrast, when GSE was given to mice after the mice were infected with the yeast cells, these mice showed a similar survival rate as compared to control mice that received no treatment with the GSE. Taken together, these data indicate that GSE has prophylactic effect but not therapeutic effect against disseminated candidiasis.
Key words – grape seed extract, *Candida albicans*, antifungal susceptibility, disseminated candidiasis.

Introduction

Fungal infections are difficult to treat. Treatment for the fungal infections, amphotericin B and the azols are mainly used. However, toxicity and resistance to these antifungal drugs are major problems (Edwards, 1991; Body, 1988), which have led to search for new sources of antifungal agents from natural sources such as plants and herbs. Recent reports (Park *et al*, 1999; Park *et al*, 2001), for example, showed that berberine isolated from *Coptidis Rhizoma* inhibits *Candida* species. In addition, essential oils from medicinal herbs are claimed to have antifungal activity.

Procyanidins, a group of polyphenolic compounds, found in grape (*Vitis vinifera* L.; Vitaceae) seed extract (GSE) have been received attention for their diverse range of biological activities. That is, procyanidins appear to increase resistance of rats against oxidative stress (Koga *et al*, 1999) and serum total antioxidant activity (Nuttall *et al*, 1998), to prevent cataract formation in cataractous rats (Yamakoshi *et al*, 2002), and to have therapeutic effect on ischemia/reperfusion injury in rabbits (Maffei *et al*, 1996). In addition, procyanidins exert antiviral and antitumor effects by activating of T lymphocytes, which induces the Th1-derived gamma interferon (Nair *et al*, 2002). However, the activity of grape procyanidines against a pathogenic fungus such as *Candida albicans* remains unknown. In this study, we examined the effect of GSE against infection due to *C. albicans* that causes blood stream infection and local diseases such as vaginitis. In-vitro data by an agar diffusion susceptibility method resulted in that the GSE blocked *C. albicans* yeast cell growth on agar plate. By further examinations with animals, it was found that GSE enhanced resistance of mice against disseminated candidiasis. These data suggest that this protective effect was prophylactic rather than therapeutic.

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Materials and Methods

Organisms and culture conditions – The previously characterized *C. albicans* strain CA-1 (Han and Cutler, 1995; Han *et al*, 1999; Han *et al*, 2000) was grown in glucose-yeast extract-peptone (GYEP) broth at 37°C. Yeast cells were collected from the broth cultures, washed with cold sterile Dulbecco's phosphate-buffered saline (DPBS; Sigma, St. Louis Mo, USA) solution, and enumerated by hemocytometer to obtain desired numbers of yeast cells.

Mice – CD-1 (ICR) female mice (Bio Genomics, Seoul, Korea) were used at 6 weeks of age. Mice were maintained in the Dongduk Women's University animal facility.

Grape seed extract (GSE) – Seeds from grape were collected, washed with cold sterile distilled water, and dried at room temperature (R.T.) before extraction. The grape seeds were grinded into powder. Ten grams of this powder were put in ethanol (300 ml) in a beaker. After 48 hrs extraction in ethanol, the supernatant part (approximately 250 ml) was collected by centrifugation and concentrated by a rotary vacuum evaporator (Eyela, Japan) until no moisture was observed. This extract of the gape seeds

(GSE) was dissolved in DPBS and sterile-filtered (a pore size = 0.22 μM, Corning, U.S.A.) for a future use. A possible contamination in the final product was checked by inoculating on Ntrient Agar (Difco, U.S.A.) and blood agar (Plates Korean Culture Media, Seoul, Korea). Presence of polyphenols in the GSE was measured by ferrous sulfate-potassium and sodium tartrate colorimetric method.

Antifungal susceptibility test – To determine effect of GSE against *C. albicans*, an agar diffusion susceptibility method was applied. *C. albicans* (5×10^6 yeast cells per ml) was inoculated with sterile swabs on GYEP agar plates. Wells (6 mm in diameter) were dug out by a metallic puncher on the plates. One hundred microliters of GSE prepared in DPBS at 6.25, 12.5, 25, and 50 mg/ml, respectively, were put into designated wells on the plates. A control well received same volume of DPBS, instead of the GSE. The plates were incubated at 37°C. For a positive control, amphotericin B (Sigma) in DPBS at doses of 50 μg/ml, 5 μg/ml, 0.5 μg/ml, and 0.05 μg/ml, respectively, were used. Zones of inhibition on these plates were observed after 48 hrs incubation.

Effect of the GSE against disseminated candidiasis – A mouse model that causes disseminated candidiasis previously characterized (Han and Cutler, 1995; Han *et al*, 1999; Han *et al*, 2000; Han *et al*, 2001) was used to determine GSE effect against diseminated candidiasis. Prior to these experiments, GSE doses at 1 mg, 2 mg, and 4 mg per mouse were tested by intravenous (i.v) route to naive mice. The GSE was dissolved in DPBS. Mice given respective 2 mg and 4 mg of GSE per mouse, respectively, died immediately after the i.v.-injection. In tests, 0.5 mg and 1 mg GSA in a volume of 100 μl per mouse were administered to mice, i.v. One hour later after the administration, the mice were inoculated, i.v., with viable *C. albicans* yeat cells (5×10^5 per mouse). Control mice received 100 μl of DPBS instead of GSE. Their survivability was then measured.

In other experiments, mice were infected, i.v., with *C. albicans* yeast cells one hour before treatment with GSE. The GSE at the same doses as described above was given to the pre-infected mice by the same route.

Results

Analyses of the GSE – The GSE was extracted as shown in the Fig. 1. The Final product of GSE was initially clear without any precipitation after filtering. A microbial contamination in the final product was not detectable (data not shown). The ferrous sulfate-potassium and sodium tartrate test resulted in presence of polyphenols in the final product of the GSE.

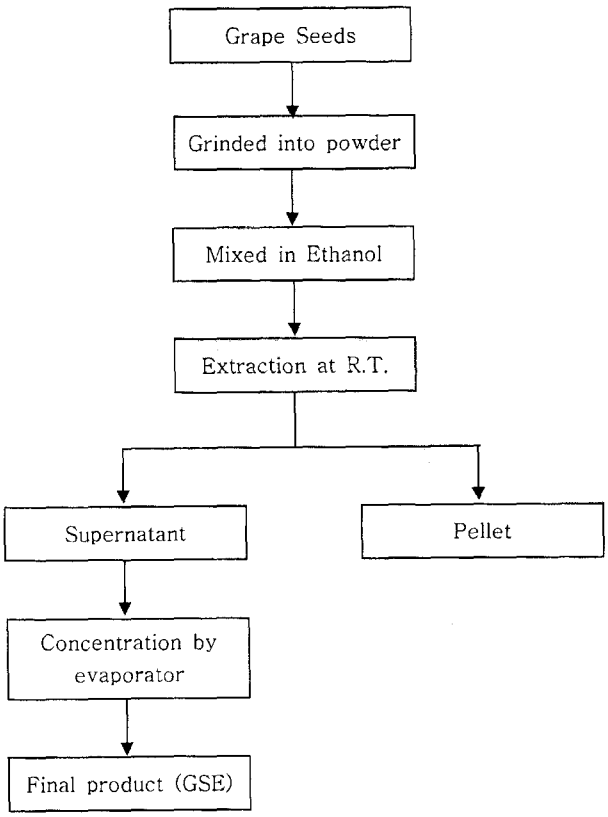

Fig. 1. A scheme of extracting procedure form grape (*Vitis vinifera*) seeds.
Powder form of grape seeds were extracted in ethanol at R.T. for 48 hrs. The supernatant was collected by centrifugation and concentrated. GSE; grape seed extract.

Anticandidal effect of the GSE – Results from the agar diffusion susceptibility assay showed that the GSE at 25 mg/ml and 50 mg/ml produced inhibitory zones corresponding to 10 mm and 16 mm in diameter including size of a well (6 mm), respectively (Table 1). At the lower concentrations, growth of *C. albicans* yeast cells were not inhibited. Amphotericin B used as a positive control inhibited growth of *C. albicans*, and the inhibitory rate was dose-dependent as expected (data not shown).

Effect of the GSE against disseminated candidiasis – The resulting survival curves showed that mice given the GSE before infection with the yeast cells survived longer than control mice that received buffer instead of the GSE (Fig. 2). Mice that received 0.5 mg and 1 mg of the GSE had mean survival times (MST) of 11.2±7.3 days and 11.3±7.0 days (MST±standard error), respectively, resulting in similar MSTs. This measurement was terminated at day 20.

In experiments mice that were pre-infected before treatment with the GSE, these mice died at a similar rates regardless of the GSE-treatment (data not shown).

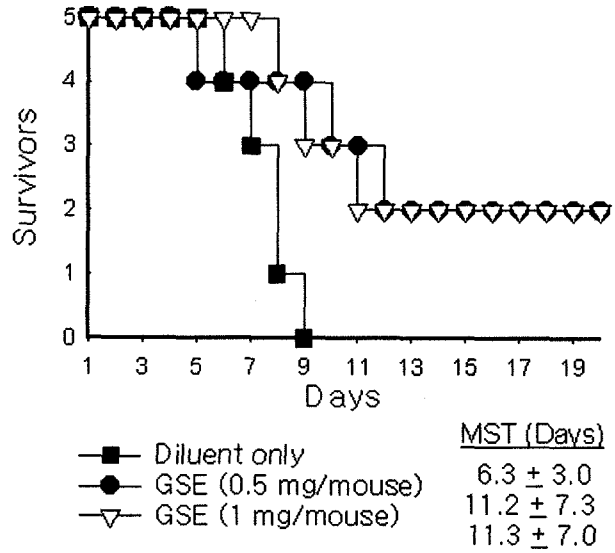

Fig. 2. The GSE (grape seeds extract) enhances resistance of mice against disseminated candidiasis.
ICR female mice were given GSE intravenously and infected with viable 5×10^5 *C. albicans* yeast cells permouse. The resulting survival curves were plotted and found to differ from those of mice given DPBS (buffer) instead of the GSE. Mice that were treated with GSE survived longer than DPBS-received control mice. The measurement was terminated at day 20. MST stands for mean survival times.

Table I. Antifungal activity of grape seed extract (GSE) on *Candida albicans* CA-1

	Concentrations of GSE (mg/ml)				
None	0	6.25	12.5	25	50
Size of inhibition zone[2] (mm)	6[3]	8	18	22	24

[1]GSE stands for grape seeds extract.
[2]Entire size of inhibition zone was measured including the size of a well in center of the zone.
[3]The 6 mm indicates size of a well in diameter.

Discussion

Grape seed extract has shown several pharmacological effects. In this study, effect of the GSE extracted in ethanol against *C. albicans* was examined under in-vitro and in-vivo conditions. The extracting procedure of GSE was simple and straightforward. The colorimetric assay showed the presence of polyphenols in the GSE.

Data from the agar diffusion susceptibility assay indicates that the GSE has growth-inhibitory activity against *C. albicans*. The inhibitory concentrations of the GSE seems to be slightly high to consider for utilization as an anticandidal agent as compared to activity of amphotericin B. These observations suggest a further purification of GSE may be necessary to isolate a major component that is effective against *C. albicans*. However, the data with use of animals demonstrate that GSE-treated mice survived longer than the control mice against disseminated candidiasis. These results indicate that GSE contains antifungal activity against *C. albicans*. Taken together, this protection is considered prophylactic rather than therapeutic. What factor in the GSE is responsible for the protection is under investigation.

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