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Effects of Burdock Extract Preparation on Gastric Mucosal Protection

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The root of burdock (*Arctium lappa* L.) has long been cultivated as a popular vegetable in Taiwan and Japan for dietary use and folk medicine. The present study investigates the effects of Bao-Jian burdock extract powders (BJBEP), a commercialized burdock extract preparation, on gastric mucosal protection. *In vitro* study, BJBEP revealed significant protection on rat gastric mucosal cells, RGM1, from indomethacin (INDO)-caused cytotoxicity at the concentration of 125-1000 ppm, whose efficacy was comparable with that of sucralfate from a concentration point of view. *In vivo* study, ethanol-induced acute gastric mucosal lesions (AGML) were applied to assess the gastroprotective activity of BJBEP in rats. BJBEP was able to decrease significantly the AGML areas caused by ethanol at the dose of 260 to 2600 mg/kg/bw. Total polyphenols in BJBEP was in proportion to its free radical scavenging activity which could be involved in biological function of gastric mucosa protective activity. The overall results indicate that BJBEP has protective effect on INDO-induced gastric mucosal cytotoxicity *in vitro* and ethanol-induced AGML in rats.

Key Words: *Arctium lappa*, Burdock, Gastric mucosal protection, RGM1.

INTRODUCTION

A considerable number of people in the world have suffered from peptic ulcers which is caused by several factors such as emotional stress, heavy drinking, smoking, caffeinated drinks, infection of Helicobacter pylori and ingestion of non-steroidal antiinflammatory drugs[1,2]. The incidence of peptic ulcer in USA is about 10 %

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similar to that in Taiwan[3,4]. There has been some evidence that gastric ulcer involved in peptic ulcer disease is associated with an increased risk for gastric cancer[5]. In addition to neutralization of acid and interference of acid secretion, reinforcement of gastric mucosal protection is one of the effective ways for therapy and decreasing incidence of peptic ulcers[2]. Due to most drugs with several adverse effects, plant extracts have been applied as one of the most attractive sources for medicinal purposes[6]. A snumber of plant extracts have been reported as having promising results on gastroprotective effects, which is beneficial for prevention and/or treatment of peptic ulcers[7-9].

Burdock (*Arctium lappa* L.) was introduced from Japan into Taiwan about 80 years ago and has long been cultivated as a vegetable in Taiwan for dietary use[10]. Burdock is also used as a folk medicine as a diuretic and antipyretic[11]. It has become a popular health drink in Taiwan in the last decade. Several studies have reported that the root of burdock possesses various pharmaceutical activities including antibacterial activity[12], desmutagenic activity[13], antioxidant activity[14-16], hepatoprotective efficacy[17,18] and antiinflammatory activity[14], among which the hepatoprotective efficacy, antiinflammatory activity and antioxidant activity are associated with the free radical scavenging activity. Natural plant extracts with gastroprotective activity has been associated with their free radical scavenging activity[19-22]. The present study investigates the effects of Bao-Jian burdock extract powders (BJBEP), a commercialized burdock extract preparation on gastric mucosal protection.

EXPERIMENTAL

The root of burdock (*Arctium lappa* L.) satisfied Good Agriculture Practice (GAP) certification was provided by Gueilai Community Developmental Institute in Pingtung County, Taiwan. Fetal bovine serum (FBS), trypsin-EDTA (0.5 %-5.3 mM), antibiotic-antimycotic (penicillin G sodium 10,000 units/mL/streptomycin sulfate 10,000 µg/mL/amphotericin B 25 µg/mL)and L-glutamine (200 mM) were purchased from Gibco (USA). Dulbecco's modified Eagle medium (DMEM, HyQ®DME/High) and nutrient mixture F-12 Ham's (HamF12) were from Hyclone (Logan, Utah, USA). Methylthiazole tetrazolium (MTT), dimethyl sulfoxide (DMSO) and indomethacin (INDO) were from Sigma Chemicals (St. Louis, MO, USA). The rat gastric mucosal cell line, RGM1 (RCB 0876), was obtained from RIKEN Cell Bank (Tsukuba, Japan). All other chemicals were of analytical reagent grade.

Production of Bao-Jian burdock extract powders (BJBEP), a commercialized burdock extract preparation: Bao-Jian burdock extract powders (BJBEP) kindly supplied by Bao-Jian Tech. Co., Ltd. was prepared under standardized conditions by a GMP certified KO-DA Pharmaceutical Co., Ltd., Taoyuan, Taiwan using the root of burdock with GAP certification cultivated at Gueilai area, Pingtung County, Taiwan. The preparation procedures were explained briefly as follows: root of burdock was extracted with distilled water and then was granulated to obtain BJBEP after mixed with starch as excipient.

Effect of BJBEP on gastric mucosal cytoprotection *in vitro*: Effect of BJBEP on gastric mucosal cytoprotection *in vitro* was conducted by the method reported by Furukawa *et al.*[23] with some modifications. For routine maintenance, rat gastric mucosal cells, RGM1, were grown in a 75 cm^2 cell culture flask in a 1:1 mixture of DMEM and HamF12 medium supplemented with 20 % FBS, 2 mM glutamine and 1 % antibiotic-antimycotic in humidified incubator with 5 % CO_2 at 37 °C.

RGM1 cells were harvested with 0.25 % trypsin-EDTA and were further seeded into a 96-well microplate (NUNC, Roskilde, Denmark) at a density of 2×10^4 cells/200 µL/well. After incubation for 24 h, cells were subjected to exposure of BJBEP or sucrafate (a positive control) in various concentrations (125, 250, 500 and 1000 ppm) at 37 °C for 2 h. Each concentration was tested in 8 replicates. After incubation, the cells were then treated with 250 ppm INDO at 37 °C for 5 h. The cell viability was performed by MTT assay using the following procedure: medium were removed and then 50 µL of MTT reagent was added at a concentration of 2 mg/mL in medium for 3 h. Medium were removed and then 100 µL DMSO was added with gentle shaking for 10 min. The optical density at 560 nm was estimated by an ELISA reader (ThermoLabsystems, Cheshire, UK).

Effect of BJBEP on ethanol-induced acute gastric mucosal lesions (AGML) *in vivo*: Male rats (strain Wistar) were bought from the National Laboratory Animal Center, Taipei, Taiwan. The animals were then kept in experimental animal center, Tajen University, Pingtung, Taiwan under standard laboratory conditions in a 12 h light and dark cycle at an ambient temperature of 22 ± 2 °C. The animals were fasted for 24 h before initiation of experiment and allowed free access to drinking water.

The ethanol-induced AGML was carried out according to the method described by Cho *et al.*[24] with some modifications. The male rats (strain Wistar, 6-8 weeks old) were randomly allotted into 5 groups (sham; control; BJBEP-260; BJBEP-1300; BJBEP-2600) of 8 animals each. Three doses of BJBEP at 260 mg/kg/bw (BJBEP-260), 1300 mg/kg/bw (BJBEP-1300), 2600 mg/kg/bw (BJBEP-2600) was administered orally to three of the test groups, while animals in control group were given vehicle solution only (distilled water). After 4 h, 4 groups of animals were then subjected to 70 % ethanol (10 mL/kg) treatment with exception of sham group. All groups of animals were sacrificed with CO_2 and the stomachs removed after 4 h.

The removed rat stomachs were washed with normal saline and the morphology of each stomach was photographed by a digital camera (Nikon, Tokyo, Japan). The AGML areas of each rat were calculated based on a software of USB Digital Scale 1.0E (Myguard, Taiwan).

Determination of total polyphenols in BJBEP: Total polyphenols in BJEP were measured spectrophotometrically using the Folin-Ciocalteu reagent based on a colorimetric oxidation/reduction reaction[25]. To 0.2 mL of diluted aqueous acetone sample, 1 mL of Folin-Ciocalteu reagent (Merck, diluted 10 times with water) was added. After that, 0.8 mL of 7.5 % Na_2CO_3 was added and mixed thoroughly. After

0.5 h of standing, the absorbance was measured at 765 nm (Hitachi, Tokyo, Japan). The amount of total polyphenols was calculated as a gallic acid equivalent from the calibration curve of gallic acid standard solutions and expressed as mg gallic acid/g BJEP. All measurements were done in triplicate.

Evaluation of free radical-scavenging activity of BJBEP: The free radical-scavenging activity of BJBEP was evaluated using DPPH free radical-scavenging assay as described previously[26]. A stock solution (1 mg/mL) of each extract was prepared and diluted with methanol into various concentrations. An aliquot of 50 µL of each dilution was transferred into a 96-well microplate (NUNC, Roskilde, Denmark). A working solution of DPPH (250 µM) in methanol was freshly prepared and then an aliquot of 150 µL was added to each well. After incubation for 0.5 h, the DPPH scavenging percentage was measured at 490 nm on an ELISA reader (Thermo Labsystems, Cheshire, UK). Each dilution was performed at least in triplicate.

Statistical analysis: Data are presented as the mean ± SD. The statistical significance between groups was analyzed by Tukey-HSD test using a SPSS statistic software, version 10.0 (Chicago, Illinois, USA), a p value of less than 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Effect of BJBEP on gastric mucosal cytoprotection *in vitro*: In order to determine the activities of burdock on gastric mucosal cytoprotection, the effect of BJBEP on INDO-induced cytotoxicity in rat gastric mucosal cells, RGM1, was carried out. The protection effect of BJBEP on INDO-induced cytotoxicity in rat gastric mucosal cells was shown in Fig. 1. The cell viability of the group without INDO treatment was expressed as 100 %. The cell viability was abruptly decreased to be 24.6 % after treatment with INDO at the concentration of 250 ppm. Results indicated that INDO was harmful to rat gastric mucosal cells. After the treatment of BJBEP at various concentrations ranging from 125 to 1000 ppm, the cell viability was increased significantly in a concentration-response manner and the inhibition rate was 10.5~50.1 %. The results indicated that BJBEP was able to protect the rat gastric mucosal cell from INDO-caused damage.

INDO, a non-steroidal antiinflammatory drug, capable of causing a peptic ulcer involved oxidative mechanism of free radical production resulted in damage of gastric mucosal cell[27,28]. Phenolic compounds with antioxidant activity exist ubiquitously in plant materials including herbs, fruits and vegetables. It has been reported free radical scavenging activity of burdock was associated with its phenolic compounds[16,29]. The content of total polyphenols in BJBEP was determined to be about 12.8 mg/g. The free radical scavenging activity of BJBEP was conducted using DPPH free radical scavenging activity. As shown in Table-1, BJBEP was able to scavenge significantly DPPH radical with concentration-dependant manner which was proportion to relative total polyphenols. Several studies have reported that polyphenols in nature plant with free radical scavenging activity possessed gastric

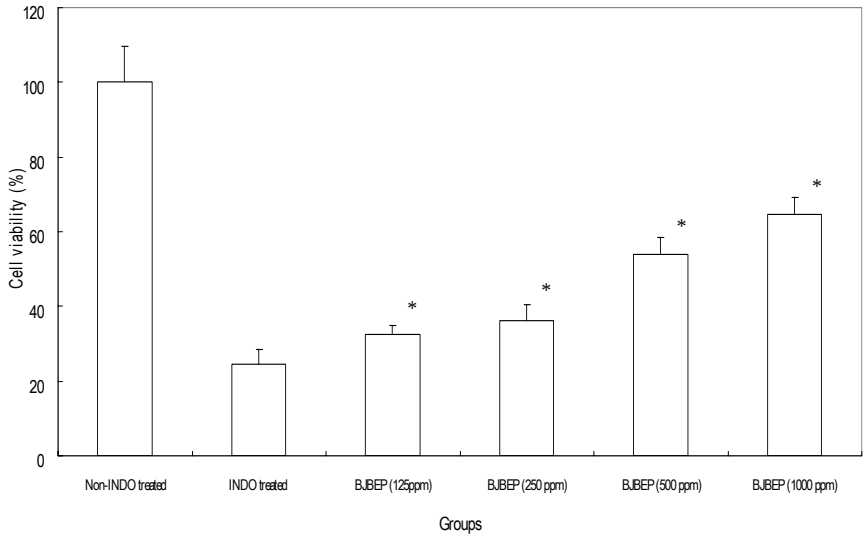

Fig. 1. Protection effect of BJBEP on indomethacin (INDO)-induced cytotoxicity in rat gastric mucosal cells, RGM1. The error bar represents the standard deviation (n = 8). There is a statistic difference between test group and INDO-treated group by Tukey-HSD test (*$p < 0.05$)

TABLE-1
TOTAL POLYPHENOLS IN BJBEP AND ITS FREE RADICAL SCAVENGING ACTIVITY

BJBEP concentration (ppm)	Control	125	250	500	1000
Total polyphenols (mg/g)[a]	-	2.4 ± 0.5	3.4 ± 0.2	6.6 ± 0.5	12.8 ±0.1
% DPPH scavenging[b]	0.9 ± 0.5	2.8 ± 0.7	5.1 ± 1.1*	11.5 ± 2.0*	20.7 ± 1.3*

[a]Total polyphenols were expressed as mg gallic acid /g BJBEP, which was calculated using prepared BJBEP solutions at the concentration of 125 to 1000 ppm.
[b]The free radical scavenging activity was evaluated as the DPPH scavenging percentage based on the reduction of the absorbance at 490 nm in the presence of BJBEP for 0.5 h.
*There was a significant difference between the % DPPH scavenging of the test group and the control according to Tukey-HSD test ($p < 0.05$). Data are presented as the mean ± standard deviation (n = 3).

mucosal protective activity[30-34]. The results suggested that the free radical scavenging activity and polyphenols of BJBEP were involved in the protection effect on INDO-induced gastric mucosal cytotoxicity. An authentic antiulcerogenic drug, sucrafate, was applied to compare the efficacy with that of BJBEP. As shown in Fig. 2, sucrafate possessed significant gastric mucosal cell protection from INDO-caused damage at a concentration range of 125 to 1000 ppm with inhibition rate of 15.8~64.7 %. In comparison of their activities on gastric mucosal cytoprotection, the concentration of BJBEP required to achieve a significant effect on gastric mucosal cytoprotection was 125 g/mL with similar to that of sucrafate. It indicated that the potency of BJBEP on gastric mucosal cytoprotection was comparable to that of sucrafate.

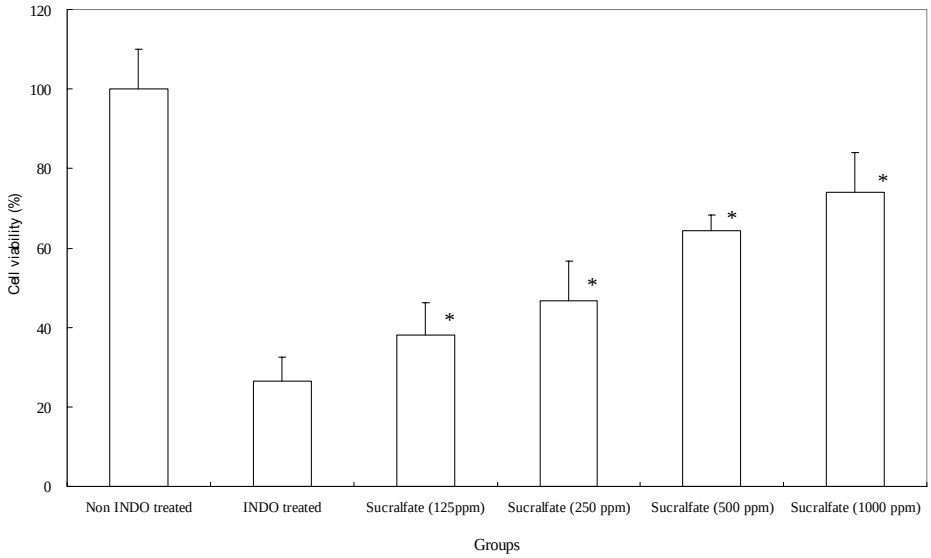

Fig. 2. Protection effect of sucralfate on indomethacin (INDO)-induced cytotoxicity in rat gastric mucosal cells, RGM1. The error bar represents the standard deviation (n = 8). There is a statistic difference between test group and INDO-treated group by Tukey-HSD test (*$p < 0.05$)

Effect of BJBEP on ethanol-induced acute gastric mucosal lesions (AGML) *in vivo*: Due to BJBEP with significant activity on gastric mucosal cytoprotection *in vitro*, ethanol-induced AGML *in vivo* were further investigated. The result of BJBEP on ethanol-induced AGML in rat was shown in Fig. 3. The ethanol-induced AGML areas without BJBEP treatment were 132.95 mm^2 indicating that ethanol was capable of inducing AGML in rat in comparison with that of sham group. After the treatment of BJBEP at three doses at 260 mg/kg/bw, 1300 mg/kg/bw and 2600 mg/kg/bw, the AGML areas were 16.67, 57.22 and 48.72 mm^2, respectively. These results revealed that BJBEP was able to protect rat stomach from ethanol-induced AGML with inhibition rate of 87.5, 57.0 and 63.4 %, respectively.

AGML is one of the major side effects associated with alcohol consumption[35]. Although the mechanism of ethanol-induced AGML is still not clear, production of oxygen radicals with oxidative stress implicated in the damage of gastric mucosal cell membranes through lipid peroxidation could play a significant role in the pathogenesis of ethanol-induced AGML[36,37]. Several studies[30-32] have demonstrated that polyphenols in nature plant extracts with free radical scavenging activity are associated with preventing ethanol-induced AGML. Due to *in vivo* model with more complicated biosystem than *in vitro* model, protection of test agent on ethanol-induced AGML could be resulted from a combination of different mechanisms. The presence of polyphenols in BJBEP with potent free radical scavenging activity may be involved in protective effect on ethanol-induced AGML in rat.

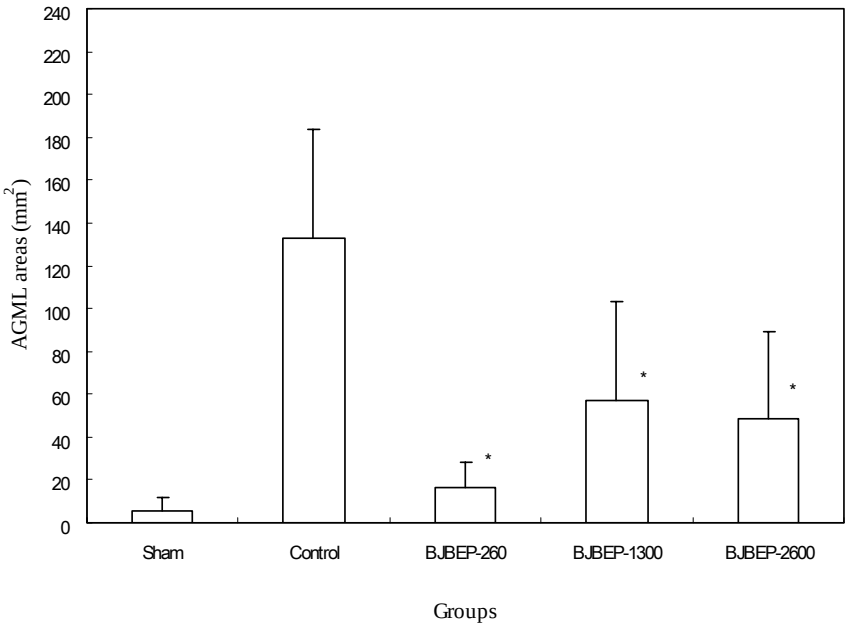

Fig. 3. Effect of BJBEP on ethanol-induced AGML in rat. The error bar represents the standard deviation (n = 8). There is a statistic difference between test group and ethanol-treated group by Tukey-HSD test (*$p < 0.05$)

In conclusion, we have demonstrated that BJBEP possesses protective effects on INDO-induced gastric mucosal cytotoxicity *in vitro* and ethanol-induced AGML in rats, which were associated with total polyphenols in burdock with free radical scavenging activity. The efficacy of BJBEP was comparable with that of sucralfate in INDO-induced gastric mucosal cytotoxicity. Burdock applied as material for preparation of BJBEP is beneficial to gastric ulcer prevention and/or treatment due to burdock with potent free radical scavenging activity regarding to its total polyphenols.

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