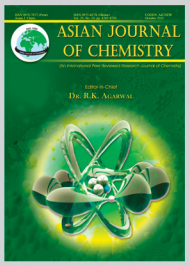
Asian Journal of Chemistry; Vol. 24, No. 2 (2012), 765-768

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Gastro Protective Effect of Madeira Vine Against Ethanol-Induced Gastric Mucosal Lesion in Rat

CHI-TING HORNG[1,2], HOW-RAN CHAO[3], CHIU-FANG LEE[4], CHAO-WEN HSUEH[5] and FU-AN CHEN[2,*]

[1]Department of Ophthalmology, Kaohsiung Armed Force General Hospital, Kaohsiung, Taiwan
[2]Department of Pharmacy & Graduate Institute of Pharmaceutical Technology, Tajen University, Yanpu, Pingtung, Taiwan
[3]Department of Environmental Science and Engineering, National Pingtung University of Science and Technology, Neipu, Pingtung, Taiwan
[4]Kaohsiung Veterans General Hospital, Pingtung Branch, Pingtung, Taiwan
[5]Department of Medicine, Kaohsiung Armed Force General Hospital, Kaohsiung, Taiwan

*Corresponding author: E-mail: fachen@mail.tajen.edu.tw

(*Received*: 18 March 2011; *Accepted*: 14 October 2011) AJC-10524

Madeira vine (*Anredera cordifolia* (Ten.) Steenis or the synonymous name of *Boussingaultia gracilis* Miers var. pseudobaselloides Bailey) belongs to the family Basellaceae and has long been cultivated as a vegetable in Taiwan for dietary use. The present study investigates the effect of madeira vine on gastric mucosal protection. Ethanol-induced gastric mucosal lesions were applied to assess the gastroprotective activity of madeira vine extract powders (MVEP) in rats. Madeira vine extract powders was able to decrease significantly ulcer index of gastric mucosal lesions caused by ethanol at the dose of 250 to 1250 mg/kg. In histopathological evaluation, the ethanol-induced lesions caused moderate to severe/high acute degeneration/ necrosis with deeply ulceration and hemorrhage on the mucosal layer and submucosal edema of stomach. The amelioration of gastric mucosal lesions in histopathological observation was also found in pretreatment of madeira vine extract powders. Madeira vine extract powders possessed significant free radical scavenging activity, which could be involved in biological function of gastric mucosa protective activity. The results indicate that the madeira vine extract powders has protective effect on ethanol-induced gastric mucosal lesions in rats.

Key Words: Madeira vine, Anredera cordifolia, Boussingaultia gracilis, Ethanol, Gastric mucosal protection.

INTRODUCTION

Madeira vine (*Anredera cordifolia* (Ten.) steenis or the synonymous name of Boussingaultia gracilis Miers var. pseudobaselloides Bailey) was introduced from Brazil into Taiwan about 35 years ago and has been cultivated as a vegetable in Taiwan for dietary use[1,2]. Madeira vine is also used as a folk medicine for treatment of diabetes, liver disease and traumatic injuries[2,3]. Several studies have reported that madeira vine possesses various pharmaceutical activities including antimicrobial activity, wound-healing activity, anti-obesity, hypolipidemic effect, hypoglycemic effect, antiinflammatory, hepatoprotective and antimutagenicity[4-7]. 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[8,9]. Peptic ulcer occurs due to an imbalance between the offensive (acid, pepsin and *Helicobacter pylori*) and defensive (mucin, prostaglandin and bicarbonate) factors[10]. In addition to inhibition of offensive factors, reinforcement of gastric mucosal protection is one of the effective ways for therapy and decreasing incidence of peptic ulcers[9]. Due to most drugs' several adverse effects, plant extracts have been applied as one of the most attractive sources for medicinal purposes[11]. A number of plant extracts have been reported as having promising results in gastroprotective effects, which is beneficial for prevention and/or treatment of peptic ulcers[12-14]. Little information of madeira vine has been obtained on gastroprotective effects. The present study investigates gastroprotective effect of madeira vine against ethanol-induced gastric mucosal lesions.

EXPERIMENTAL

Madeira vine was kindly provided by Gueilai Community Developmental Institute in Pingtung County, Southern Taiwan. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased form Sigma Chemicals (St. Louis, MO, USA). Folin-Ciocalteu reagent and ethanol was from Merck (Darmstadt, Germany). All other chemicals were of analytical reagent grade.

Preparation of madeira vine extract powders (MVEP): The leaves of madeira vine were lyophilized and then ground to powders using a electrical blender (Rong Tsong, Taipei, Taiwan). Five hundred g of the ground powders were added

to 1500 mL of distilled water and then refluxed for 3 h in a reflux extraction apparatus (Angu, Kaoshiung, Taiwan). After that, the aqueous extract solution was filtered using filter paper and filter funnel. The filtered extract mixed with excipient was completely dried by a rotary evaporator (Buchi, Flawil, Switzerland). The obtained madeira vine extract powders was stored in an electronic dry cabinet (Komry, Taipei, Taiwan) for following study.

Determination of total polyphenols in madeira vine extract powders: Total polyphenols in madeira vine extract powders were measured spectrophotometrically using the Folin-Ciocalteu reagent based on a colour imetric oxidation/reduction reaction[15,16]. To 0.2 mL of diluted aqueous acetone sample, 1 mL of Folin-Ciocalteu reagent (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 madeira vine extract powders. All measurements were done in triplicate.

Evaluation of free radical-scavenging activity of madeira vine extract powders: The free radical-scavenging activity of madeira vine extract powders was evaluated using DPPH free radical-scavenging assay as described previously[17]. 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 (ThermoLabsystems, Cheshire, UK). Each dilution was performed at least in triplicate.

Effect of madeira vine extract powders on ethanol-induced gastric mucosal lesions: Male rats (strain Wistar) were bought from BioLASCO Taiwan Co. Ltd. Animals were maintained under standard laboratory conditions (12 h light/dark cycle, temperature 22 ± 2 ºC. Standard chow and water were available *at libitum*. Animals (6-8 weeks old) were randomly divided into four groups of 8 rats each and fasted for 24 h before the experiment, but had free access to water. This study was approved by the appropriate animal care and use committees at Tajen University (Pingtung, Taiwan). The ethanol-induced gastric mucosal lesions were carried out according to the method as described previously[17]. Groups of animals received madeira vine extract powders (250 or 500 or 1250 mg/kg) or vehicle, the tap water (10 mL/kg, control) as gastric gavages. After 1 h, 70 % ethanol was given orally to each animal at a dose of 10 mL/kg to induce gastric ulceration. 4 h after ethanol administration, the animals were sacrificed with CO_2 and the stomachs were removed, opened along the greater curvature and rinsed with saline to remove gastric contents and blood clots. The morphology of each stomach was photographed by a digital camera (Nikon, Tokyo, Japan). The gastric mucosal lesion areas of each rat were calculated based on a software of USB Digital Scale 1.0 E (Myguard, Taipei, Taiwan). The ulcer index (UI) is percentage of lesion area in relation to total stomach area. Gastroprotection (%) was calculated according to: % gastroprotection = (UIC-UIT) × 100/UIC, where UIC is ulcer index in control and UIT is ulcer index in test[18].

Histopathological evaluation of gastric mucosal lesions: Stomachs were fixed in a 10 % buffered formalin solution. The fixed stomached were embedded in paraffin wax and processed in a paraffin tissue processing machine (Leica, Nussloch, Germany). Sections of the stomach were made at a thickness of 5 µm and stained with hematoxylin and eosin (H & E) for microscopically examination (Nikon, Tokyo, Japan).

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

Ethanol-induced gastric mucosal lesions were applied to assess the gastroprotective activity of madeira vine extract powders in rats. The result of gross finding was shown in Fig. 1. Severity of lesions was graded according to the methods described by Shackelford *et al.*[19]. Degree of lesions was graded from one to five depending on severity: (1) minimal (< 1 %); (2) slight (1-25 %); (3) moderate (26-50 %); (4) moderate/severe (51-75 %); (5) severe/high (76-100 %). The 70 % ethanol caused acute, moderate/severe (4) to severe/high (5) with deeply hemorrhage of gastric mucosa (Fig.1A). The gross lesions of low-dosed group (70 % ethanol + madeira vine extract powders 250 mg/kg) showed slight (2) to severe/high (5) (Fig.1B), median-dosed group (70 % ethanol + madeira vine extract powders 500 mg/kg) had moderate (3) to severe/high (5) (Fig.1C), high-dosed group (70 % ethanol + madeira vine extract powders 1250 mg/kg) presented slight (2) to moderate (3) (Fig.1D), erotic to deeply hemorrhages of gastric mucosa (arrow). The gross appearance of rat stomach was shown in Fig. 2. As shown in Fig.2, gastric injuries were clearly inhibited

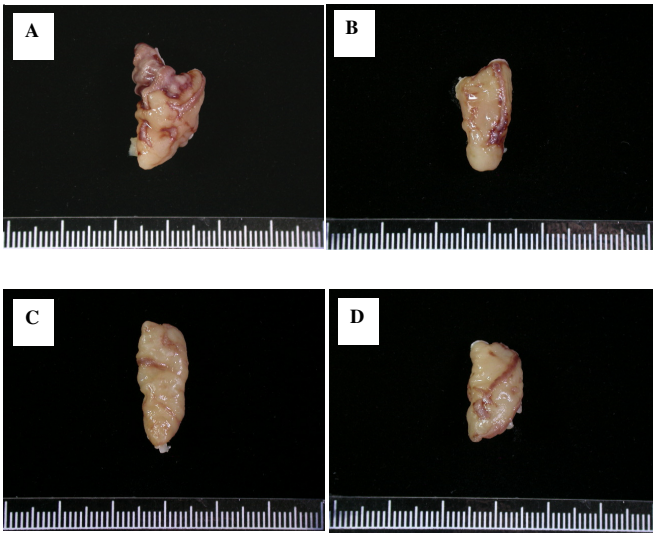


Fig. 1. Gross finding of MVEP on ethanol-induced gastric lesions in rats. A. Control; B. Low-dosed group (70 % ethanol + MVEP 250 mg/kg); C. Median-dosed group (70 % ethanol + MVEP 500 mg/kg); D. High-dosed group (70 % ethanol + MVEP 1250 mg/kg). MVEP, Madeira vine extract powders

by pretreatment with madeira vine extract powders. The ulcer index (UI) of each rat were further calculated and shown in Fig. 3. The ethanol-induced ulcer index without madeira vine extract powders treatment were 31.1 % indicating that ethanol was capable of inducing gastric mucosal lesions in rat. After the treatment of madeira vine extract powders at three doses at 250, 500 and 1250 mg/kg, the ulcer index were 16.0, 12.6, 16.2 %, respectively. These results revealed that madeira vine extract powders was able to protect rat stomach from ethanol-induced gastric mucosal lesions with inhibition rate of 48.6, 59.6, 47.7 %, respectively.

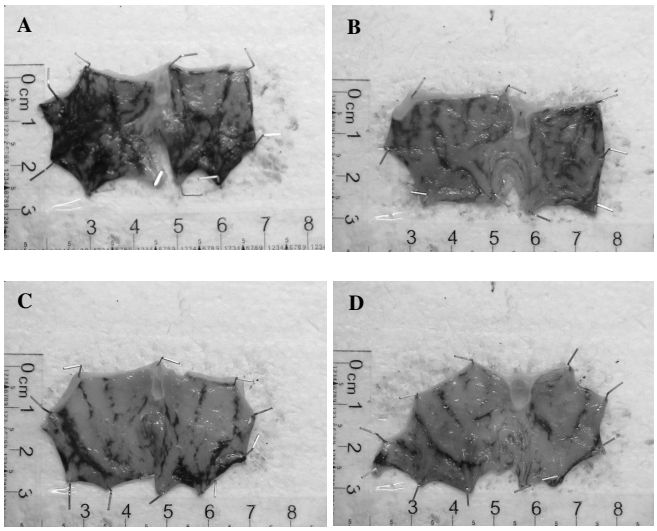


Fig. 2. Macroscopic findings of MVEP on ethanol-induced gastric lesions in rats. A. Control; B. Low-dosed group (70 % ethanol + MVEP 250 mg/kg); C. Median-dosed group (70 % ethanol + MVEP 500 mg/kg); D. High-dosed group (70 % ethanol + MVEP 1250 mg/kg). MVEP, Madeira vine extract powders.

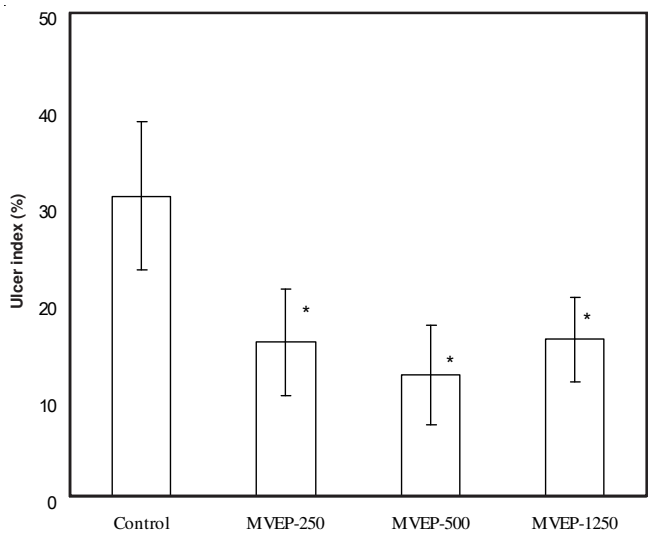


Fig. 3. Effect of MVEP on ethanol-induced gastric lesions in rat. The error bar represents the standard deviation (n=8). There is a statistic difference between test group and ethanol-treated group (control) by Tukey-HSD test (*p <0.05).

Histopathological evaluation of gastric lesions was shown in Fig. 4. As shown in Fig. 4, the 70 % ethanol caused moderate (3) to severe/high (5) acute degeneration/necrosis with deeply ulceration and hemorrhage on the mucosal layer and submucosal edema of stomach (Fig. 4A) with histoscore of 4.4 ± 0.9. The 70 % ethanol + 250 mg/kg group presented moderate (3) to severe/high (5), acute degeneration/necrosis with deeply ulceration and hemorrhage on the mucosal layer and submucosal edema of stomach (Fig. 4B) with histoscore of 3.6 ± 1.0. The 70 % ethanol + 500 mg/kg group caused moderate (3) to severe/high (5) acute degeneration/necrosis with deeply ulceration and hemorrhage on the mucosal layer and submucosal edema of stomach (Fig. 4C) with histoscore of 3.5 ± 0.7. The 70 % ethanol + 1250 mg/kg group presented minimal (1) to moderate (3), acute degeneration/necrosis with erosion and hemorrhage on the mucosal layer and submucosal edema of stomach (Fig. 4D) with histoscore of 2.6 ± 0.5. These results revealed that the histoscore on histopathological evaluation of gastric mucosal lesions can be decreased in the presence of madeira vine extract powders.

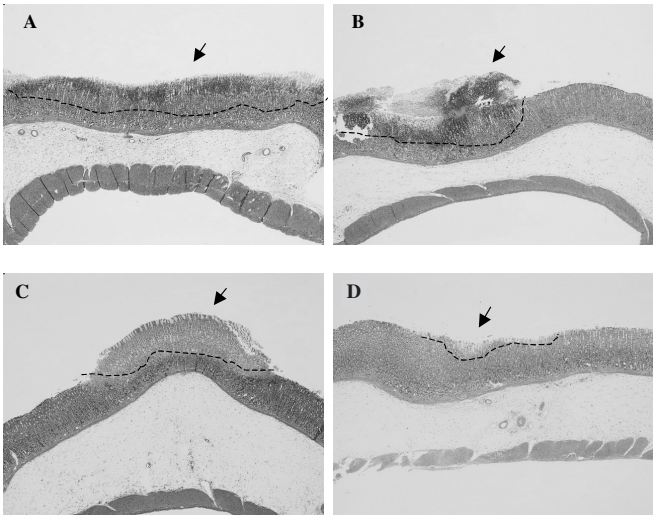


Fig. 4. Histopathological evaluation of MVEP on ethanol-induced gastric lesions in rats. A. Control; B. Low-dosed group (70 % ethanol + MVEP 250 mg/kg); C. Median-dosed group (70 % ethanol + MVEP 500 mg/kg); D. High-dosed group (70 % ethanol + MVEP 1250 mg/kg). MVEP, Madeira vine extract powders. (40 X, H&E stain)

Gastric mucosal lesion is one of the major side effects associated with alcohol consumption[20]. Although the mechanism of ethanol-induced gastric mucosal lesion is still unclear. It is suggestive that the 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 gastric mucosal lesions[21,22]. Phenolic compounds with antioxidant activity exist ubiquitously in plant materials including herbs, fruits and vegetables. The content of total polyphenols in madeira vine extract powders was determined to be about 27.90 mg/g (mg gallic acid/g madeira vine extract powders). The free radical scavenging activity of madeira vine extract powders was conducted using DPPH free radical scavenging activity. As shown in Table-1, madeira vine extract powders was able to scavenge significantly DPPH radical with concentration-dependant manner. Several studies have demonstrated that polyphenols in nature plant extracts with free radical scavenging activity are associated with preventing ethanol-induced gastric mucosal lesions[23-26]. The presence of polyphenols

TABLE-1
FREE RADICAL SCAVENGING ACTIVITY OF MVEP

MVEP concentration (ppm)	Control	250	500	1000	2000
% DPPH scavenging [a]	0.8 ± 0.4	7.9 ± 0.9*	12.1 ± 1.6*	16.1 ± 1.3*	25.8 ± 1.6*

[a] 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 MVEP for 30 min; * 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).

in madeira vine extract powders with potent free radical scavenging activity may be involved in protective effect on ethanol-induced gastric mucosal lesions in rat.

In conclusion, we have demonstrated that madeira vine extract powders possesses protective effects on ethanol-induced gastric mucosal lesions in rats, which may be associated with total polyphenols in madeira vine extract powders with free radical scavenging activity. Madeira vine applied as material for preparation of madeira vine extract powders is beneficial to gastric ulcer prevention and/or treatment.

ACKNOWLEDGEMENTS

This study was financially supported by Clinical Research grants from Kaohsiung Armed Force General Hospital, Kaohsiung, Taiwan (No. 9814).

REFERENCES

1. J.W. Chen, S.S. Wang and M.Y. Chou, *Tainan Dist. Agric. News Lett.*, **1**, 13 (1992).
2. N.Y. Chiu and K.H. Chang, The Illustrated Medicinal Plants of Taiwan, p. 49 (1992).
3. L. Wang, C.Y. Bang and S.Y. Choung, *J. Med. Food*, **14**, 17 (2011).
4. T.E. Tshikalange, J.J. Meyer and A.A. Hussein, *J. Ethnopharmacol.*, **96**, 515 (2005).
5. L.F. Villegas, I.D. Fernández, H. Maldonado, R. Torres, A. Zavaleta, A.J. Vaisberg and G.B. Hammond, *J. Ethnopharmacol.*, **55**, 193 (1997).
6. G.C. Yen, H.Y. Chen and H.H. Peng, *Food Chem. Toxicol.*, **39**, 1045 (2001).
7. C.C. Lin, D.C. Sung and M.H. Yen, *Phytother. Res.*, **8**, 201 (1993).
8. L.M. Perera, D. Ruedas and B.C. Gomez, *J. Ethnopharmacol.*, **77**, 1 (2001).
9. P. Dharmani, V.K. Kuchibhotla, R. Maurya, S. Srivastava, S. Sharma and G. Palit, *J. Ethnopharmacol.*, **93**, 197 (2004).
10. V. Lakshmi, N. Singh, S. Shrivastva, S.K. Mishra, P. Dharmani, V. Mishra and G. Palit, *Phytomed.*, **17**, 569 (2010).
11. M. Sannomiya, V.B. Fonseca, M.A. da Silva, L.R. Rocha, L.C. Dos Santos, C.A. Hiruma-Lima, A.R. Souza Brito and W. Vilegas, *J. Ethnopharmacol.*, **97**, 1 (2005).
12. A. Shirwaikar, P.M. Bhilegaonkar, S. Malini and J.S. Kumar, *J. Ethnopharmacol.*, **86**, 117 (2003).
13. C.H. Baggio, C.S. Freitas, L. Rieck and M.C. Marques, *Pharmacol. Res.*, **47**, 93 (2003).
14. L.H. Zhang, C.B. Yao, M.Q. Gao and H.Q. Li, *World J. Gastroenterol.*, **11**, 2830 (2005).
15. P.S. Negi, G.K. Jayaprakasha and B.S. Jena, *Food Chem.*, **80**, 393 (2003).
16. F.A. Chen, S.C. Lee, H.R. Chao, W.C. Fu, M.C. Hsu, C.T. Horng, C.C. Wang, H. Matsui and G. Agoramoorthy, *Asian J. Chem.*, **21**, 3015 (2009).
17. F.A. Chen, A.B. Wu, P. Shieh, D.H. Kuo and C.Y. Hsieh, *Food Chem.*, **94**, 14 (2006).
18. A. Navarrete, J. Arrieta, L. Terrones, H. Abou-Gazar and I. Calis, *J. Pharm. Pharmacol.*, **57**, 1059 (2005).
19. C. Shackelford, G. Long, J. Wolf, C. Okerberg and R. Herbert, *Toxicol. Pathol.*, **30**, 93 (2002).
20. M. Khazei and H. Salehi, *Iranian J. Pharmacol. Ther.*, **5**, 43 (2006).
21. R. Shetty, K.V. Kumar, M.U.R. Naidu and K.S. Ratnakar, *Indian J. Pharmacol.*, **32**, 313 (2000).
22. R. Hernández-Muñoz, C. Montiel-Ruíz and O. Vázquez-Martínez, *Lab. Invest.*, **80**, 1161 (2000).
23. N. Osakabe, C. Sanbongi, M. Yamagishi, T. Takizawa and T. Osawa, *Biosci. Biotechnol. Biochem.*, **62**, 1535 (1998).
24. S. Khennouf, K. Gharzouli, S. Amira and A. Gharzouli, *Pharmazie*, **54**, 75 (1999).
25. J.S. Lee, T.Y. Oh, Y.K. Kim, J.H. Baik, S. So, K.B. Hahm and Y.J. Surh, *Mutat. Res.*, **579**, 214 (2005).
26. G. Graziani, G. D'Argenio, C. Tuccillo, C. Loguercio, A. Ritieni, F. Morisco, C. Del Vecchio Blanco, V. Fogliano and M. Romano, *Gut*, **54**, 193 (2005).