MEDWELL PUBLISHING
Research Journal of Medical Sciences 3 (2): 42-46, 2009
ISSN: 1815-9346

Anti-ulcerogenic Activity of Aqueous Extract of *Ficus deltoidea* Against Ethanol-induced Gastric Mucosal Injury in Rats

[1]M.A. Siti Fatimah Zahra, [1]A.A. Mahmood, [2]M.A. Hapipah, [1]M.N. Suzita and [1]I. Salmah
[1]Department of Molecular Medicine, Faculty of Medicine,
[2]Department of Chemistry, Faculty of Science, University of Malaya, Malaya, Malaysia

Abstract: The ulcer healing activity of whole-plant extract of *Ficus deltoidea* was studied in gastric ulcer induced by ethanol in rats. Four groups of adult male *Sprague dawley* rats were pre-treated respectively with: distilled water (negative control), 250 and 500 mg kg^{-1} *F. deltoidea* extract (experimental) and omeprazole (positive control) 30 min before oral administration with absolute ethanol to generate gastric mucosal injury. After 1 h later, the rats were sacrificed and the ulcer areas and histological sections of gastric walls were determined. Grossly, the negative control rats exhibited 7 mucosal injury whereas pre-treatment with *F. deltoidea* or omeprazole resulted in significantly less gastric mucosal lesions produced by ulcerogens. The gastric protection was more prominent in 500 mg kg^{-1} *F. deltoidea* extract than 250 mg kg^{-1}. Histological studies confirmed the results wherein compared to the pre-treated and thus cytoprotected groups of rats, the negative control rats showed very severe and deep gastric mucosal necrotic damage, along with edema and leucocytes infiltration of the submucosal layer. In conclusion, the present finding suggests that *F. deltoidea* extract promotes ulcer protection as ascertained by the comparative significant decreases in ulcer areas and inhibition of submucosal edema and leucocytes infiltration of submucosal layer.

Key words: *Ficus deltoidea*, plant extract, peptic ulcer, gastric ulcer healing, gross lesion, histology

INTRODUCTION

In current clinical practice, peptic ulcer has become one of the most common gastrointestinal disorders to occur among developed countries which caused by the disruptions of the gastric mucosal defence and repair systems (Grossman, 1981). Previous studies has discovered a large number of medicinal plants and dietary nutrients which posses gastro-protective activity (Kath and Gupta, 2006; Malairajan *et al*., 2007). *Ficus deltoidea* is a native ephyphytic shrub widely distributed in several countries of the Southeast Asia. In Malaysia, it is commonly known as *Mas cotek*, *serapat angin*, *telinga beruk* and other few names. Different parts of the plant are used traditionally to treat various kinds of ailments. The fruits are chewed to relieve headache, toothache and cold; powdered root and leaves of the plant has been applied externally to wounds and sores and around the joints for relief of rheumatism and traditionally consume as herbal drink for women after childbirth to help in strengthen up the uterus (Sulaiman *et al*., 2008). Besides being one of the popular herbs used in Malay traditional medicine, its pharmacological properties have not yet been studied. Very less publications indicating scientific findings of this plant has been published; only 2 studies on *F. deltoidea* have been reported; fruit extract of *F. deltoidea* demonstrate inhibitory effects against Angiotensin-I Converting Enzyme (ACE) enzyme, suggesting it posses anti-hypertensive properties (Abdullah *et al*., 2008) and report by Sulaiman *et al*. (2008) stated that an aqueous extract of *F. deltoidea* leaves contains pharmacologically active constituents which possess antinociceptive activity in laboratory animals and this might give a supportive fact towards the traditional use of this plant in overcome painful condition. There is no scientific documentation yet about the anti-ulcerogenic activity of *F. deltoidea* whole-plant extract in rats, therefore, the current study was undertaken to evaluate the anti-ulcer activity of this plant extract against ethanol-induced gastric ulcers in rats.

MATERIALS AND METHODS

Omeprazole: Omeprazole is in a class of drugs called proton pump inhibitors used for the treatment of

Corresponding Author: M.A. Siti Fatimah Zahra, Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Malaya, Malaysia

conditions such as peptic ulcers. Omeprazole blocks the enzymes in the wall of the stomach from producing acid. By blocking the enzyme, the production of stomach acid is decreased, thus allowing the stomach to heal. In this study, omeprazole was used as the reference anti-ulcer drug and was obtained from the University Malaya Medical Centre (UMMC) Pharmacy. The drug was administered orally to the rats in concentrations of 20 mg kg^{-1} suspended in distilled water (5 mL kg^{-1}) (Pedernera *et al*., 2006).

***Ficus deltoidea* whole-plant extract:** Whole-plant of *Ficus deltoidea* was purchased from Chemical Engineering Pilot Plant (CEPP), University of Malaysia Technology (UTM), Skudai. The dried sample was then ground into powder using Wiley mill (40-60 mesh). The dried powdered plants were successively extracted with water. The extract was then dissolved in distilled water and administered orally to 2 groups of rats in concentrations of 250 and 500 mg kg^{-1}, body weight (5 mL kg^{-1}), respectively.

Experimental animals: Adult male *Sprague-dawley* rats were obtained from the Animal House, Faculty of Medicine, University of Malaya, Kuala Lumpur (Ethics No. PM 28/9/2006 MAA (R)). The rats weighed between 180-200 g. They were fasted for 48 h before the experiment (Garg *et al*., 1993), but were allowed free access to tap water up till 2 h before the experiment. During the fasting period, the rats were placed individually in separate cages with wide-mesh wire bottoms to prevent coprophagy. On the day of experiment, the rats were randomly divided into 4 groups of 6 rats each. The groups were numbered 1-4.

Group 1: Rats were negative controls and each received distilled water only orally (5 mL kg^{-1}).

Groups 2 and 3: Received 250 and 500 mg kg^{-1} *F. deltoidea* whole-plant extract by the same route, respectively.

Group 4: Rats received 20 mg kg^{-1} omeprazole as positive control. Thirty minutes after pre-treatment, all rats were administered orally with absolute ethanol 5 mL kg^{-1}. After 1 h later, all animals were sacrificed by overdoses of diethyl ether and their stomachs were rapidly removed (Paiva *et al*., 1998).

Gross evaluation of gastric lesions: Any ulcers would be found in the gastric mucosa, appearing as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach. Each gastric mucosa was examined for damage. The length (mm) and width (mm) of the ulcer on the gastric mucosa was measured by a planimeter (10×10 mm^2 = ulcer area) to assess the ulcer areas under dissecting microscope ($\times1.8$). The area of each ulcer lesion was measured by counting the number of small squares, 2×2 mm, covering the length and width of each ulcer band. The sum of the areas of all lesions for each stomach was applied in the calculation of the Ulcer Are (UA) wherein the sum of small squares 4×1.8 = UA mm^2) (Kauffman and Grossman, 1987). The inhibition percentage (I%) was calculated by the following formula:

$$I\% = \frac{\text{UAcontrol-UAtreated}}{\text{UAcontrol}} \times 100\%$$

Histological evaluation of gastric lesions: Specimens of the gastric walls of each rat were fixed in 10% buffered formalin and processed in a paraffin tissue processing machine. Sections of the stomach were made at a thickness of 5 μm, stained with hematoxylin and eosin and analyzed microscopically.

Statistical analysis: All values were reported as mean±SEM. The statistical significance of differences between groups was assessed using one-way ANOVA. A value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Gross evaluation of gastric lesions: Rats orally administered with omeprazole or *F.deltoidea* whole-plant extracts 30 min before administration of absolute alcohol showed significantly ($p<0.001$) reduction of the mean gastric ulcer area compared to rats pre-treated with only distilled water (Table 1, Fig. 1 and 2). Also rats pre-treated with omeprazole or 500 mg kg^{-1} *F. deltoidea* extracts significantly ($p<0.001$) reduced the formation of gastric ulcers area induced by ethanol compared to rats pre-treated with 250 mg kg^{-1} *F. deltoidea* extracts (Table 1). The *F. deltoidea* whole-plant extract exerted the cytoprotective effects in a dose-dependent manner (Table 1).

Histological evaluation of gastric lesions: The rats pre-treated with only distilled water before administration of ulcer-inducing absolute ethanol showed markedly extensive damage to gastric mucosa, the lesions extend deeply to mucosal layer, oedema and leucocytes infiltration of submucosal layer. Rats pre-treated with omeprazole or *F. deltoidea* extracts had comparatively better protection of the gastric mucosa as seen by marked reduction in ulcer area and absence of oedema and leukocyte infiltration of submucosa (Fig. 3 and 4).

Table 1: Observed ulcer area and inhibition percentage in rats

Animal groups	Animals no.	Pre-treatment (5 mL kg^{-1} dose)	Ulcer area $(mm)^2$ (Mean± SEM)	Inhibition (%)
1	6	Distilled water (Control)	975.0±50.00	0.000
2	6	*F. deltoidea* (250 mg kg^{-1})	255.0±14.90*	73.85
3	6	*F. deltoidea* (500 mg kg^{-1})	115.0±9.97*	88.21
4	6	Omeprazole (20 mg kg^{-1})	102.0±9.00*	89.54

All values are expressed as mean±standard error mean. Means with different superscripts are significantly different, $p<0.001$

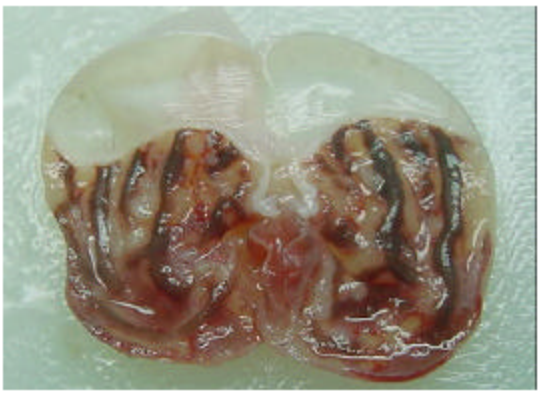

Fig. 1: Macroscopic appearance of the gastric mucosa in a rat pre-treated with only distilled water (negative control). Severe macroscopic hemorrhagic necroses of the gastric mucosa are visible following induction by absolute alcohol

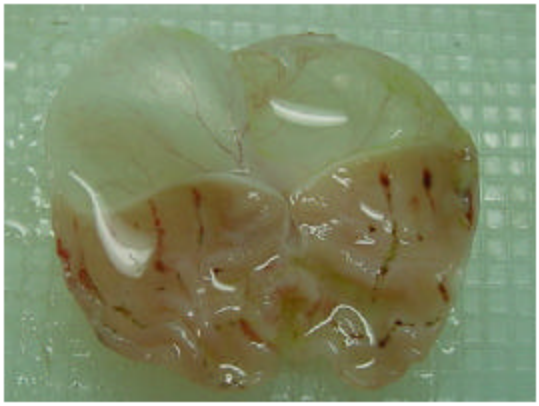

Fig. 2: Macroscopic appearance of the gastric mucosa in a rat pre-treated with *F.deltoidea* whole plant extract (500 mg kg^{-1}). Compared to the negative control, the gastric mucosal injuries are visibly milder following induction by absolute alcohol

Results from this study showed that oral administration of rats with *F. deltoidea* extracts significantly protect the gastric mucosa from ulcer induction by absolute ethanol compared to negative control rats which were treated with distilled water. The cytoprotective effect was confirmed through histological

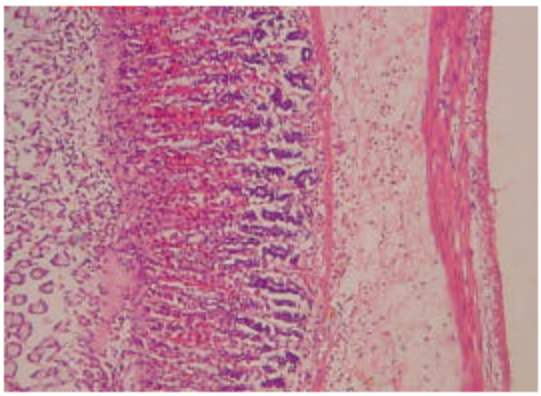

Fig. 3: Histological section of the gastric mucosa in a rat pre-treated with only distilled water (negative control). There is severe disruption of the surface epithelium, deep penetration of necrotic lesions into mucosa and edema of the submucosa layer with leukocyte infiltration of ulcerative tissues (H&E stain, 40x)

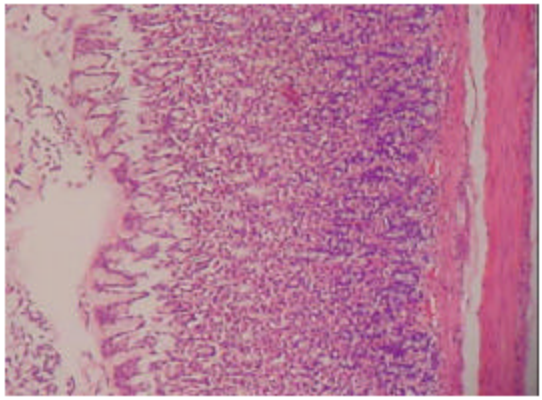

Fig. 4: Histological section of the gastric mucosa in a rat pre-treated with *F.deltoidea* whole plant extract (500 mg kg^{-1}). Compared to the negative control, the disruption to the surface epithelium is very mild and there is no submucosal edema and no leucocytes infiltration (H&E stain, 40x)

examinations of the gastric tissues of the animals that shows marked prevention of mucosal lesions and inhibition of edema and leucocytes infiltration of

submucosa. Using absolute to induce formation of gastric lesions is a well-known rapid and convenient method of screening plant extracts for anti-ulcer potency and cytoprotection in terms of the absence or reduction in macroscopically and microscopically visible lesions. Ethanol induced necrotic lesions in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and production of mucus (Marhuenda *et al*., 1993), stasis in gastric blood flow, which contributes to the development of the hemorrhagic lesions of gastric mucosa (Holzer *et al*., 1991). Bicarbonate ion is produced and trapped by mucus, creating a gradient of pH from 1-2 in the lumen to 6-7 at the mucosal surface, thus preventing gastric acid and pepsin from digesting the mucosa. The pathogenic effects of ethanol could be due to disturbances in gastric secretion, alterations in permeability, gastric mucus depletion and free-radical production (Salim, 1990). Lipid peroxidation is one of the important factor involves in ulcerogenesis which results in the production and release of chemical substance that recruits and activates polymorphnuclear leucocytes (Sairam *et al*., 2002). Previous study done by Kobayashi *et al*. (2001) showed that teprenone exerts a protective effect against mucosal lesions through preservation of gastric mucous synthesis and secretion and inhibition of neutrophil infiltration and enhanced lipid peroxidation in the ulcerated gastric tissue. Increase in neutrophil infiltration into ulcerated gastric tissue delays the healing of gastric ulcers in rats as reported by Fujita *et al*. (1998) and Shimizu *et al*. (2000) whereby neutrophils mediate lipid peroxidation through the production of superoxide anions (Zimmerman *et al*., 1997). Oxygen free radicals derived from infiltrated neutrophils in ulcerated gastric tissues have inhibitory effect on gastric ulcers in rats (Suzuki *et al*., 1998).

CONCLUSION

Our study indicate that pretreatment of *F. deltoidea* extract had significantly reduced ulcer area against absolute ethanol-lesion induction and this mechanism probably be due to stimulation of gastric mucous secretion and inhibition of edema and leucocytes infiltration in submucosal gastric tissue.

ACKNOWLEDGEMENT

The authors express gratitude to the staff of the Animal House, Faculty of Medicine for the care and supply of rats and to the University of Malaya for the financial support (Science fund grant 12-02-03-2051).

REFERENCES

Abdullah, N.A.H., S.A. Karsani and N. Aminudin, 2008. Effects of *F. deltoidea* extract on the serum protein profile of Simultaneously Hypertensive Rats (SHR). JPB., 2 (2): 143-143. http://www.omicsonline.com/JPBAbstractsSpecial/JPBS201.html.

Fujita, H., S. Takahashi and S. Okabe, 1998. Mechanism by which indomethacin delays the healing of acetic acid-induced ulcers in rats. Role of neutrophil antichemotactic and chemotactic activities. J. Physiol. Pharmacol., 49: 71-82. PMID: 9594412. http://www.ncbi.nlm.nih.gov/pubmed/9594412.

Garg, G.P., S.K. Nigam and C.W. Ogle, 1993. The gastric antiulcer effects of the leaves of the neem tree. Planta Medica, 59: 215-217. PMID: 8316589. http://www.ncbi.nlm.nih.gov/pubmed/8316589.

Grossman, M.I., 1981. Peptic ulcer: A guide for the practicing physician. Chicago: Year Book Medical Publishers, pp: 1-35. ISBN: 0815140096.

Holzer, P., E.H. Livingston and P.H. Guth, 1991. Sensory neurons signal for an increase in rat gastric mucosal blood flow in the face of pending acid injury. Gastroenterology, 101: 416-423. PMID: 2065919. http://www.ncbi.nlm.nih.gov/pubmed/2065919.

Kath, R.K. and R.K. Gupta, 2006. Antioxidant activity of hydroalcoholic leaf extract of ocimum sanctum in animal models of peptic ulcer. Indian J. Physiol. Pharmacol., 50: 391-396. PMID: 17402269. http://www.ncbi.nlm.nih.gov/pubmed/17402269.

Kauffman, G.L. Jr. and M.I. Grossman, 1987. Prostaglandin and cimetidine inhibit the formation of ulcers produced by parenteral salicylates. Gastroenterology, 75: 1099-2102. PMID: 361490. http://www.ncbi.nlm.nih.gov/pubmed/2065919.

Kobayashi, T., Y. Ohta, J. Yoshino and S. Nakazawa, 2001. Teprenone promotes the healing of acetic acid-induced chronic gastric ulcers in rats by inhibiting neutrophil infiltration and lipid peroxidation in ulcerated gastric tissues. Pharmacol. Res., 43: 23-30. DOI: 10.1006/phrs.2000.0748. PMID: 11207062. http://www.ncbi.nlm.nih.gov/pubmed/11207062.

Malairajan, P., G. Gopalakrishnan, S. Narasimhan, K.J. Veni and S. Kavimani, 2007. Anti-ulcer activity of crude alcoholic extract of *Toona ciliata Roemer* (heart wood). J Ethnopharmacol., 110: 348-351. DOI: 10.1016/j.jep.2006.10.018. PMID: 17134860. http://www.ncbi.nlm.nih.gov/pubmed/17134860.

Marhuenda, E., M.J. Martin and C. Alaracon de la Lastra, 1993. Antiulcerogenic activity of aescine in different experimental models. Phytother. Res., 7: 13-16. DOI: 10.1002/ptr.2650070105. http://www3.interscience.wiley.com/journal/112262406/abstract.

Paiva, L.A., V.S. Rao, N.V. Gramosa and E.R. Silveira, 1998. Gastroprotective effect of *Copaifea langsdarffii* oleo-resin on experimental gastric ulcer models in rats. J. Ethnopharmacol., 62: 73-78. DOI: 10.1016/S0378-8741(98)00058-0. PMID: 9720615. http://www.ncbi.nlm.nih.gov/pubmed/9720615.

Pedemera, A.M., T. Guardia, C.G. Calderon, A.E. Rotelli, N.E. de la Rocha, S.D. Genaro and L.E. Pelze, 2006. Anti-ulcerogenic and anti-inflammatory activity of the methanolic extract of *Larrea divaricata* Cav, in rat. J. Ethnopharmacol., 105: 415-420. DOI: 10.1016/j.jep.2005.11.016. PMID: 16406415. http://www.ncbi.nlm.nih.gov/pubmed/16406415.

Salim, A.S., 1990. Removing oxygen-derived free radicals stimulates healing of ethanol induced erosive gastritis in the rat. Digestion, 47: 24-28. PMID: 2292345. http://www.ncbi.nlm.nih.gov/pubmed/2292345.

Sairam, K., C.V. Rao, M.D. Babu, K.V. Kumar, V.K. Agrawal and R.K. Goel, 2002. Antiulcerogenic effect of methanolic extract of *Embilica officinalis*: An experimental study. J. Ethnopharmacol., 82: 1-9. DOI:10.1016/S0378-8741(02)00041-7. PMID:12169398. http://www.ncbi.nlm.nih.gov/pubmed/12169398.

Shimizu, N., T. Watanabe, T. Arakawa, Y. Fujiwara, K. Higuchi and T. Kuroki, 2000. Pentoxifylline accelerates gastric ulcer healing in rats: Roles of tumor necrosis factor alpha and neutrophils during the early phase of ulcer healing. Digestion, 6: 157-164. DOI: 10.1159/000007752. PMID:10773720. http://www.ncbi.nlm.nih.gov/pubmed/10773720.

Sulaiman, M.R., M.K. Hussain, Z.A. Zakaria, M.N. Somchit, S. Moin, A.S. Mohamad and D.A. Israf, 2008. Evaluation of the antinociceptive activity of *Ficus deltoidea* aqueous extract. Fitoterapia, 79: 557-561. DOI: 10.1016/j.fitote.2008.06.005. PMID: 18672036. http://www.ncbi.nlm.nih.gov/pubmed/18672036.

Suzuki, Y., M. Ishihara and M. Ito, 1998. Antiulcer effects of antioxidants, quercetin, α-tocopherol, nifedipine and tetracycline in rats. Jpn. J. Pharmacol., 78: 435-441. DOI: 10.1254/jjp.78.435. PMID: 9920200. http://www.ncbi.nlm.nih.gov/pubmed/9920200.

Zimmerman, J.J., W. Ciesielski and J. Lewandoski, 1997. Neutrophil-mediated phospholipids peroxidation assessed by gas chromatography-mass spectroscopy. Am. J. Physiol., 273: C653-661. PMID: 9277363. http://ajpcell.physiology.org/cgi/content/abstract/273/2/C653.