

EVALUATION OF GASTROPROTECTIVE EFFECTS OF THE ETHANOLIC EXTRACT OF *PEPEROMIA PELLUCIDA* (L) KUNTH

Roslida AH^{1*} and Noor Aini Z¹

¹Department of Biomedical Sciences, Faculty of Medicine & Health Sciences, University
Putra Malaysia , 43400 Serdang, Selangor, Malaysia.

Summary

This work was carried out to investigate the anti-ulcerogenic activity of *Peperomia pellucida* (L.) Kunth in necrotizing agent ie (ethanol , sodium chloride, sodium hydroxide and hydrochloric acid) and indomethacin-induced models in rats. The 70% of ethanolic extract of aerial part of *Peperomia pellucida* (PPE) was prepared. Four doses ie 10, 30, 100 and 300 mg/kg were selected for further study. Ulcer effects were determined by counting the total surface area of lesion in mm². Results showed that PPE provided significant protection in various experimental models used. Pretreatment with the PPE at all doses (10,30,100 and 300 mg/kg) has produced significant inhibition of gastric mucosal damage induced by 80% EtOH, 25% NaCl, 0.6 M HCl, 0.2 M NaOH and 30 mg/kg indomethacin. The result suggests that PPE possesses anti-ulcer properties.

Key words: *Peperomia pellucida*, anti-ulcer, Ethanol-induced ulcer, Indomethacin – induced ulcer, necrotizing agents

Corresponding author: Dr Roslida AH,
Department of Biomedical Sciences,
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
E-mail: roslida@medic.upm.edu.my

Introduction

Global expansion of consumption of alcohol and non-steroidal anti-inflammatory drugs (NSAID) and inappropriate diets have contributed to growing ulcer etiopathology (1). In this way, the peptic ulcer is considered a disease of modern times, related to the addictions that are increasingly frequent in the society and to its stressful lifestyle. Treatment with natural products presents promise of a cure. Plants have been used as sources of raw material for the synthesis of many drugs and they remain an important source of new therapeutic agents (2). Borrelli and Izzo (3) presented a review that demonstrated the enormous variety of chemical substances isolated from plants that present antiulcerogenic activity, indicating their great potential in the discovery of new therapies for ulcers.

Peperomia pellucida is an annual herb that is widely distributed in many South American and Asian countries (4). The plant grows to a height of 15 to 45 cm, and its shiny light-green leaves are succulent, well spaced, and heart shaped. The species develops during rainy periods (often in the spring) and thrives in loose, humid soils under the shade of trees. (4). The plant can be utilized as a vegetable and in salads, but the shiny bush has also medicinal applications. In Malaysia, the plant is known as ‘ketumpangan air’ and it has been used for treating various ailments such as abdominal pain, indigestion, abscess, acne, boils, colic, fatigue, gout, headache, renal disorders, and rheumatic pain, and to treat breast cancer, impotence, measles, mental disorders, and smallpox (5).

Previous chemical investigation on this plant indicated the existence of flavonoids (6-7), phytosterols, apiols, substituted styrenes, and a dimeric ArC2 compound (4). Carotol (13.41%) was the major hydroxylated sesquiterpene in a chemical analysis of *Peperomia pellucida*. Other compounds, like the peperomins, have cytotoxic or anticancer activity in vitro (8). Isolated flavonoids include acacetin, apigenin, isovitexin, and pellucidatin. Phytosterols such as campesterol and stigmasterol have also been isolated (9). Xu *et al* (8) isolated secolignans, lignans and highly methoxylated dihydronaphthalenone from the whole plant.

The plant has been reported to possess analgesic, antipyretic, anti-inflammatory, anti-bacterial, anti-fungal and CNS depressant effect (10-16), in either leaves or aerial part of the plant. However to the author's knowledge, none has reported on its anti-ulcer activity yet. Therefore, the present study was undertaken with the aim to assess the anti-ulcerogenic properties claimed by the ethnobotanic information and also the traditional system of medicine

Methods

Preparation of Plant Extract

The whole plants were collected from Serdang, Selangor, Malaysia . The botanical identification and authentication of the plant was done by Dr Richard Chung, the Curator of the Herbarium of Forest Research Institute in Kepong, Selangor, Malaysia where a voucher specimen FRI 65401 (KEP) was deposited for future reference.

The aerial parts of *Peperomia pellucida* were dried in an oven at 40 to 42°C for 3 days. Then, they were weighed as dried weight. Later, they were grounded into powdered form and were weighed once again. The grounded form of *Peperomia pellucida* was macerated in 70% ethanol for two days. The extracted solvent was then filtered and was concentrated by using rotary evaporator until the solvent was completely removed and crude extract was obtained. The crude extract of *Peperomia pellucida* was then dissolved with 10% Tween 80 and was prepared into desired dose concentrations for pharmacological testing.

Animals and Experimental Design

Healthy *Sprague dawley* rats of either sex weighing between 170-250 g were obtained from Animal Unit of Faculty of Medicine & Health Sciences, Universiti Putra Malaysia . The animals were kept in metal cages at room temperature under standard environmental condition and were fasted 24 hours before the experiment but were allowed free access of water. All the procedures were conducted in accordance with the guide line for Animal Ethic Committee.

Gastric ulcers induced by necrotizing agents (cytoprotective studies)

Cytoprotective studies were carried out according to the method of Robert *et al* (17) with some modifications. 1 ml of necrotizing agent viz 80% (v/v) aqueous ethanol, 25% NaCl, 0.6 M HCl and 0.2 M NaOH was administered orally to induce the ulcer. SD rats of either sex weighing between 170-200 g were divided into 5 groups of 6 animals each and fasted for 24 hours with water ad libitum prior to experiment. The animal of group 1 were pretreated with vehicle (1% Tween 80) and the animals of group 2, 3, 4 and 5 were pretreated with 70% ethanolic extract at 10, 30, 100 and 300 mg/kg respectively. 1 ml of necrotizing agent (80%, (v/v) aqueous ethanol, 25% NaCl, 0.6 M HCl and 0.2 M NaOH) was administered to all the animals of group 1 -5, 60 minutes after the respective treatments. The animals were sacrificed by cervical dislocation after 8 hours of necrotizing agent administration and stomach was incised along the greater curvature to determine the lesion damage. The percentage protection was calculated based on the total surface area of lesion in treated group compared with the lesions in control group.

NSAIDs (Indomethacin)-induced ulcer

The experiments were performed according to the method of Hayden *et al* (18) with some modifications. SD rats of either sex weighing between 170-200 g were divided into 5 groups of 6 animals each and fasted for 24 hours with water ad libitum prior to experiment. The animal of group 1 were pretreated with vehicle (1% Tween 80) and the animals of group 2 and 3 were treated with standard ie cimetidine 50 mg/kg and 150

mg/kg respectively. Similarly, the animals of group 4, 5, 6 and 7 were pretreated with 70% ethanolic extract at 10, 30, 100 and 300 mg/kg respectively. Indomethacin (30 mg/kg, po) was administered to all the animals of group 1 -7, 60 minutes after the respective treatments. The animals were sacrificed by cervical dislocation after 8 hours of indomethacin administration and stomach was incised along the greater curvature to determine the lesion damage. The percentage protection was calculated based on the total surface area of lesion in treated group compared with the lesions in control group

Statistical analysis

Data was expressed as mean \pm S.E.M. The results of the experiments were expressed as changes of percentage from control values. Data was analyzed by two-way analysis of variance (ANOVA), followed by Duncan's multiple comparison test for post-hoc comparison of group means. Student's *t*-test was used to compare between two groups. For all tests, effects with probability of $p < 0.05$ were considered significant.

Results

Gastric ulcers induced by necrotizing agents (cytoprotective studies)

The anti-ulcer effect of PPE induced by various necrotizing agents are summarized in Table 1. The inhibitory action exerted by PPE on the ulcers induced by 80% ethanol, 25% NaCl, 0.6 M HCl and 0.2 M NaOH was dose dependent and highly significant. The inhibition of ulceration being in the range of 63-98% .

Indomethacin – induced ulcer

NSAIDs ie indomethacin used in the present study resulted in the production of gastric ulcers, mainly in the glandular segment of the stomachs. As shown in Table 1, in indomethacin-induced gastric ulceration model, pretreatment with PPE significantly inhibited gastric ulceration at all doses administered but not in dose dependent manner. Percentage inhibition of gastric erosions was in the range of 71-85%. At lower dose, ie 10 mg/kg, PPE exhibited maximum inhibition of 84.8% and comparable to 50 mg/kg cimetidine.

Table 1 : Effects of 70% ethanolic extract of *Peperomia pellucida* (PPE) on different models of acute gastric lesion induced in rats

Gastric lesion models	Treatment (p.o)	Dose (mg/kg)	Number of animals	Total area of lesions (mm ²)	Inhibition (%)
80% EtOH	Vehicle	-	6	322.67 ± 22.00	-
	PPE	10	6	84.00 ± 15.53**	73.97
		30	6	46.60 ± 28.14**	85.86
		100	6	44.00 ± 11.33**	86.36
		300	6	10.17 ± 3.37**	96.85
25% NaCl	Vehicle	-	6	296.50 ± 23.64	-
	PPE	10	6	110.60 ± 37.82*	62.70
		30	6	29.00 ± 4.02**	90.22
		100	6	1.60 ± 0.40**	99.46
		300	6	4.60 ± 0.75**	98.45
0.6 M HCl	Vehicle	-	6	351.33 ± 12.12	-
	PPE	10	6	87.75 ± 25.11**	75.02
		30	6	5.20 ± 1.77**	98.52
		100	6	2.33 ± 0.84**	99.34
		300	6	4.83 ± 1.76**	98.63
0.2 M NaOH	Vehicle	-	6	270.50 ± 48.04	-
	PPE	10	6	43.80 ± 15.83*	83.80
		30	6	8.00 ± 1.53**	97.04
		100	6	5.50 ± 2.09**	97.97
		300	6	2.80 ± 0.80**	98.96
Indomethacin 30 mg/kg	Vehicle	-	6	152.67 ± 24.46	-
	Cimetidine	50	6	23.56 ± 6.21**	84.57
		150	6	1.00 ± 1.00**	99.34
	PPE	10	6	23.20 ± 4.09**	84.80
		30	6	43.83 ± 15.11*	71.29
		100	6	34.40 ± 7.71**	77.47
		300	6	39.17 ± 11.77	74.34

Data presented as means ± S.E.M. Asterisks indicate significant differences from controls (*P<0.05; **P<0.001 ;Dunnett's test)

Discussion

PPE is effective orally against gastric damage in various experimental models. The result of this study showed that PPE inhibits the formation of gastric ulcer induced by different ulcerogenic drugs by cytodestructive agents. PPE exerts a dose dependent inhibitory action on gastric mucosal lesions caused by various necrotizing agents. A key feature of (gastric) cytoprotection is that a variety of gastric mucosal damage produced by different necrotizing agents (e. g. 0.6 M HCl, 0.2 M NaOH, 96 % ethanol, 25 % NaCl or thermal stress) without affecting gastric acid secretion (17). Such cytoprotection has commonly been referred to as the ability of prostaglandins to reduce the severity of injury of the gastrointestinal tract induced by an array of noxious agents. Cytoprotection may occur as result of the capacity that some compounds have to induce prostaglandin production, fundamental for mucus protection because they stimulate mucus and bicarbonate synthesis (19). Therefore we can postulate that PPE may have cytoprotective factors based due to the reduction of total lesion area when induced with necrotizing agents

Ethanol-induced damages are induced by disturbance of mucosal microcirculation, ischaemia and appearance of free radicals, endothelin release, degranulation of mast cells, and inhibition of prostaglandins and decrease of mucus production (20). Ethanol-induced gastric lesion formation may be due to stress in gastric blood flow that contributes to the development of the hemorrhage and necrotic aspects of tissue injury (21). This chemical agent also increases Na⁺ and K⁺ flux into the lumen and increases pepsin secretion along with histamine release.

Furthermore, it is well known that ethanol-induced ulcers are not inhibited by the anti-secretory agents, but are inhibited by agents that enhance mucosal defensive factor such as prostaglandin (22) The incidence of ethanol-induced ulcer which is predominant in the glandular part of the stomach has also been reported to stimulate the formation of leukotriene C₄ (LTC₄) resulting in the damage of gastric mucosa (23-24). 70% PPE significantly protected the gastric mucosa against ethanol challenge as shown by reduced total surface area of lesion as compared to control group, suggesting its potent gastroprotective effect. Similarly, NSAID's like indomethacin inhibits COX-2 thereby inhibits the prostaglandin synthesis.

Consequently lipoygenase pathway is enhanced liberating leukotrienes and these leukotrienes are reported to have a role in ulcerogenesis. In addition there is some evidence that NSAIDs may induce ulcer by causing the back diffusion of H⁺ ion in to mucosal cells (25). Therefore the gastroprotective effect of PPE may be due to its ability to inhibit prostaglandin/leukotrienes synthesis.

On the other hand, ethanol-induced gastric damage may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhage and necrotic aspects of tissue injury (21). This action is direct on the gastric epithelium also causing perturbation of mast cells and release of a vasoactive mediator such as histamine (26).

Some reports show that changes in gastric circulation after ethanol administration remains unknown, but it has been reported that microcirculation damage can be prevented by prostaglandin administration (21). On the other hand, ethanol-induced gastric lesions are thought to arise as a result of direct damage of gastric mucosal cells, resulting in the development of free radicals (27) and hyperoxidation of lipids (28). These data suggest that antioxidant compounds could be active in this experimental model, producing antiulcerogenic effects.

Numerous studies have demonstrated that the effect of absolute ethanol on the gastric mucosa is related to the production of free radicals, the increase of lipoperoxidation and the decrease of the levels of nonprotein SH compounds in the gastric mucosa (29). Exposition to ethanol increases, in a dose dependent way, the generation of superoxide anions and the extension of cellular damage (30)

The gastrointestinal irritant properties of nonsteroidal anti-inflammatory drugs (NSAIDs) are the major impediment to their use as anti-inflammatory drugs (31). Non-steroidal anti-inflammatory drugs like indomethacin are known to induce gastric damage, particularly due to inhibition of biosynthesis of prostaglandins by inhibition of the cyclooxygenase pathway of arachidonic acid metabolism (32). The present work demonstrated that PPE significantly inhibited ulcer lesion area induced by indomethacin but not in dose dependant manner. The gastrototoxicity of the NSAIDs including indomethacin in animals can be attributed to their ability to induce the reactive oxygen metabolites which may in turn promote lipid peroxidation (33).

Ulceration due to NSAIDs is also believed to occur because of non-selective inhibition of cyclooxygenases that hampers the release of mucus due to reduction in prostaglandin synthesis (34-35). However instead of indomethacin can cause gastric damage by inhibiting cytoprotective prostaglandin, it can also affect enzymatic and non-enzymatic oxidant and anti-oxidant mechanism such as GSH, CAT, SOD, MPO, and MDA (36). Therefore, further works ie biochemical analyses to determine the enzymes activity should be done to confirm its definite mechanism.

In conclusion, ethanolic extract of *Peperomia pellucida* prevented necrotizing agent and indomethacin induced ulcer in rats. The findings has justified the traditional use of the plant to treat abdominal pain. The phytochemical constituent might be contributory to the anti-ulcer effect of *Peperomia pellucida*, and as the plant also exhibited anti-inflammatory and analgesic activities, it is suggested that the constituents of the plant extract might possess COX-2 inhibitory properties as well. However, further investigations are required to identify the active constituents as much as to determine the exact mechanism responsible for the anti-ulcer activity of *Peperomia pellucida*

Acknowledgement

The authors wish to thank the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for the facilities and funding provided for the study.

References

1. Peskar BM, Maricic N. Role of prostaglandin in gastroprotection. *Dig.Dis.Sci* 1998; 10: 43(9): 23S-25S
2. Souza Brito ARM. How to study the pharmacology of medicinal plants in underdeveloped countries? *Journal of Ethnopharmacology* 1996; 54: 131–138
3. Borrelli F, Izzo AA . The plant kingdom as a source of anti-ulcer remedies. *Phytotherapy Research* 2000; 14: 581–591.
4. Bayma JD , Arruda MS , Müller AH , Arruda AC , Canto WC . A dimeric ArC₂ compound from *Peperomia pellucida* . *Phytochemistry* 2000; 55:779-782
5. Burkill IH.. A Dictionary of The Economic Products of The Malay Peninsula, Vol I (A-H), (Ministry of Agriculture and Co-operative, Kuala Lumpur) 1966: 218-223.
6. Aqil M , Khan IZ , Ahmad MB . Flavonoids from *Peperomia pellucida* . *Sci Phys Sci* 1993 ; 5: 213-215
7. Aqil M , Rahman FA , Ahmad MB . A new flavonol glycoside from *Peperomia pellucida* . *Sci Phys Sci* 1994; 6:141-143
8. Xu S , Li N , Ning MM , Zhou CH , Yang QR , Wang MW . Bioactive compounds from *Peperomia pellucida* . *J Nat Prod.* 2006 ; 69: 247-250.
9. Belardo LO. Some constituents of the volatile oil of *Peperomia pellucida*. *Perfumery and Essential Oil Record* 1967; 58:359
10. Aziba PI , Adedeji A , Ekor M , Adeyemi O . Analgesic activity of *Peperomia pellucida* aerial parts in mice . *Fitoterapia* 2001 ; 72:57-58
11. Khan A, Rahman M, Islam MS . Anti-pyretic activity of *Peperomia pellucida* leaves in rabbits. *Turk J.Biol* 2008 ; 37:41
12. Arrigoni-Blank Mde F , Oliveira RL , Mendes SS . Seed germination, phenology, and antiedematogenic activity of *Peperomia pellucida* (L.) H. B. K. *BMC Pharmacol* 2004 ; 2 :12-19
13. Khan MR, Omolosa AD. Antibacterial activity of *Hygrophila stricta* and *Peperomia pellucida*. *Fitoterapia* 2002 ; 73:251-254
14. de Padua LS. Bunyapraphatsara N, Lemmens RHMJ (Editors). *Plant Resources of South East Asia 12(1) Medicinal and Poisonous Plants*. Backhuys Publishers, Leiden, the Netherlands. 1999:771.
15. Ragasa CY , Dumato M , Rideout JA . Antifungal compounds from *Peperomia pellucida* . *ACGC Chem Res Commun* 1998 ; 7:54-61.
16. Khan A, Rahman M, Islam MS (2008). Neuropharmacological effects of *Peperomia pellucida* leaves in mice. *DARU* 2008; 16 :1:35-39
17. Robert A , Nezamis JE, Lancaster C, Hanchar AJ . Cytoprotection in rats: Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. *Gastroenterology* 1979; 77:433-443
18. Hayden LJ, Thomas G, West GB . Inhibitors of gastric lesions in the rat. *Journal of Pharmacy and Pharmacology* 1978; 30: 244–246
19. Robert A, Nezamis JE, Lancaster C, Davis JP, Field SO, Hanchar AJ. Mild irritants prevent gastric necrosis through “adaptative cytoprotection” mediated by prostaglandins. *Am. J. Physiol.* 1983; 245: G113–G121

20. Samonina GE, Kopylova GN, Lukjanzeva GV, Zhuykova SE, Smirnova EA, German SV, Guseva AA . Antiulcer effects of amylin: a review. *Pathophysiology* 2004; 11: 1–6.
21. Guth PH, Paulsen G, Nagata H. Histologic and microcirculatory changes in alcohol-induced gastric lesions in the rat: effect of prostaglandin cytoprotection. *Gastroenterology* 1984; 87:1083–1890
22. Morimoto Y, Shimohara K, Oshima S. and Sukamoto T. Effect of the new antiulcer agent KB-5492 on experimental gastric mucosal lesions and gastric mucosal defensive factors, as compared to those of teprenone and cimetidine. *Japanese Journal of Pharmacology* 1991 ; 57: 495–505
23. Peskar BM, Lange K, Hoppe U and Peskar BA, Ethanol stimulates formation of leukotriene C₄ in rat gastric mucosa. *Prostaglandins* 1986; 31 : 283.
24. Cho CH, Ogle CW, Sevilla EL . The protective effects of sulphasalzine against ethanol-induced gastric damage in rats. *Br. J. Pharmacol.* 1987; 87:228
25. Davenport HW . Gastric mucosal haemorrhage in dose-effect of acid, aspirin and alcohol. *Gastroenterol.* 1969; 56: 439-49
26. Oates PJ, Hakkinen JP. Studies on the mechanisms of ethanol-induced gastric damage in rats. *Gastroenterology* 1998; 94:10–21
27. Pihan G, Regill C, Szabo S . Free radical and lipid peroxidation in ethanol- or aspirin-induced gastric mucosal injury. *Digestive Diseases and Sciences* 1987; 32: 1395–1401.
28. Puurunen J, Huttunen P, Hirvonen J. Is ethanol-induced damage of the gastric mucosa a hyperosmotic effect? Comparative studies on the effects of ethanol, some other hyperosmotic solutions and acetylsalicylic acid on rat gastric mucosa. *Acta Pharmacologica et Toxicologica* 1980; 47: 321–327.
29. Mizui T, Doteuchi M (1983). Effect of polyamines on acidified ethanol-induced gastric lesions in rats. *Japanese Journal of Pharmacology* 33: 939–945.
30. Mutoh H, Hiraishi H, Ota S, Ivey KJ, Terano A, Sugimoto T. Role of oxygen radicals in ethanol-induced damage to cultured gastric mucosal cells. *Am J Physiol* 1990; 258:G603–G609
31. Chiba T, Sato K, Kudara N, Shinozaki H, Ikeda K, Sato K, Endo M, Orii S, Suzuki K. Upper gastrointestinal disorders induced by non-steroidal anti-inflammatory drugs. *Inflammopharmacology* 2008; 16, 16–20
32. Rainsford KD. Gastric ulcerogenicity of non-steroidal anti-inflammatory drugs in mice with mucosa sensitized by cholinomimetic treatment. *Journal of Pharmacy and Pharmacology* 1987; 39:669–672.
33. Vaananenn PM, Medding JB, Wallace JL. Role of oxygen derived free radicals in indomethacin-induced gastric injury. *Am. J. Physiol.* 1991; 261: G470–G475
34. Hawkey CJ, Langman MJ . Non-steroidal anti-inflammatory drugs: overall risks and management. Complementary roles for COX-2 inhibitors and proton pump inhibitors. *Gut* 2003; 5: 600–608
35. Hawkins C, Hanks GW. The gastroduodenal toxicity of nonsteroidal anti inflammatory drugs. A review of the literature. *J. Pain Symp. Manag.* 2000; 20: 140–151.
36. Suleyman H, Cadirci E, Albayrak A, Polat B, Halici Z, Koc F, Hacimuftuoglu A, Bayir Y. Comparative study on the gastroprotective potential of some antidepressants in indomethacin-induced ulcer in rats, *Chem. Biol. Interact* 2009; 180(2):318-324