Pharmacognosy Research [Phcog Res]
Vol 1, Issue 5, Sep-Oct, 2009 Page 250-255
(An Official Publication of Pharmacognosy Network Worldwide)
Received: Jul 10, 2009
Modified: Jul 16, 2009
Accepted: Aug 23, 2009

PHCOG RES.: Research Article

Evaluation of anti-ulcer activity of *Ardisia crispa* Thunb. D.C

Roslida A.H*, Teh Y.H[1], Kim K.H[2]

[1]*Department of Biomedical Sciences, Faculty of Medicine & Health Sciences, University Putra Malaysia , 43400 Serdang, Selangor, Malaysia*
[2]*Department of Physiology, Faculty of Medicine, University Malaya, 50603 Kuala Lumpur, Malaysia*

*** Author for correspondence:** *E-mail : roslida@medic.upm.edu.my*

ABSTRACT

Ardisia crispa Thunb D.C (Myrsinaceae), has long been used in treating various ailments among the local villagers. The objective of this study was to investigate experimentally the possible anti-ulcer activity of *Ardisia crispa*. The effect of hexane fraction of root of *Ardisia crispa* (ACRH) was evaluated in experimental ulcer models with necrotizing agents ie ethanol, NaCl, HCl, NaOH and also COX-1 inhibitor namely indomethacin as inducers. 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 mm^2. Results showed that ACRH provided significant protection in various experimental models used. Pretreatment with ACRH at all doses (10,30,100 and 300 mg/kg) has produced significant inhibition of gastric mucosal damage induced by 80% EtOH and 25% NaCl, whilst at 30, 100 and 300 mg/kg, ACRH significantly reduced the lesion formation in ulcer induced by 0.6 M HCl, 0.2 M NaOH and 30 mg/kg indomethacin. The present study indicates that the hexane fraction of *Ardisia crispa* (ACRH) exhibits significant anti-ulcer effect.

Keywords: *Ardisia crispa*, anti-ulcer, Ethanol-induced ulcer, Indomethacin–induced ulcer, necrotizing agents

INTRODUCTION

Peptic ulcers are illnesses that affect a considerable number of people globally. Stress, smoking, nutritional deficiencies and ingestion of nonsteroidal – anti-inflammatory drugs (NSAIDs) augment gastric ulcer incidences (1). Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers (2–3). In traditional medicine for example, several plants and herbs have been used to treat gastrointestinal disorders, including gastric ulcers (4–6). This is an important reason to investigate anti-ulcer effect of medicinal plants with traditional use in gastric disease.

The plant *Ardisia crispa* Thunb. D.C belongs to the family Myrsinaceae and it is widely distributed in Asia stretching from Japan and the Himalayas to Java and the Philipines. It can be found in the undergrowth and jungle fringes, dappled shades and shady edges in Malaysia (7). The root and leaves has been claimed to have various medicinal properties based on the ethnobotanic information and traditional medicine. Its root is reported to be used as one of the traditional ingredient in post-natal syndromes where the root is boiled and the boiled concoction is used to treat pain in the throat and chest as well as to treat rheumatism. The mixture of its leaves and root is used as skin liniment (8). The root juice is useful for treating earache, cough, fever, diarrhea and also for women after-birth. In Canton, it has been marketed as "sin-lo-san", a herbal decoction drunk for sprains and broken bones. In Thailand, the root will be mixed with other plants to wash "dirty blood' or in women with dysmenorhea (menstrual pain) (9).

Previous chemical and biological investigation on this plant indicated the existence of triterpenoid saponins which gave uterocontraction in mice (9), n-peptide from the whole plant which showed anti-hypertensive and

antiplatelet aggregating properties *in vitro* (10), and a benzoquinone compound which exhibited anti-metastatic effect (11). In previous study, we have reported the anti-inflammatory and anti-hyperalgesic effects on its hexane fraction (12)., therefore, the present study is now conducted to evaluate the anti-ulcer activity of the hexane fraction obtained from *Ardisia crispa* root bark, using different in vivo ulcer models in rat.

MATERIALS AND METHODS

Plant Material and Extraction.

The roots of *Ardisia crispa* (Family: Myrsinaceae) were collected from Tangga Batu, Melaka, Malaysia between July to December 1999 and was deposited as a voucher specimen (no: 20841) in the herbarium of Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia. The samples were cut into small pieces and dried at 60°C for 3 days. The dried root and leaves were then grounded using Wiley laboratory mill. Grounded dried plant materials were macerated in cold aqueous ethanol (70% ethanol) for 48 hours. The extract was concentrated under reduced pressure in a rotary evaporator. The crude aqueous ethanol extract was then fractionated successively with n-hexane, dichloromethane and methanol. The solvents were removed under reduced pressure in a rotary evaporator at 40°C and the concentrates dried at room temperature to yield solid residues; hexane fraction (14.1% w/w), dichloromethane fraction (7.62% w/w) and methanol fraction (57.40% w/w). In this experiment, we only used the hexane fraction which is labelled as ACRH.

Animals

Healthy male *Sprague dawley* rats weighing between 170-250 g were obtained from Animal Unit of Faculty of Medicine, Universiti Malaya with ethics approval from the Animal Ethics Committee of Universiti Malaya (FIS/16/04/02 RAH(R)). 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.

Antiulcer activity

Gastric ulcers induced by necrotizing agents (cytoprotective studies)

Cytoprotective studies were carried out according to the established method (13) 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 animals of group 1 were pretreated with vehicle (1% Tween 80) and the animals of group 2, 3, 4 and 5 were pretreated with ACRH 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* (14) 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 ranitidine 50 mg/kg and 150 mg/kg respectively. Similarly, the animals of group 4, 5, 6 and 7 were pretreated with ACRH 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

The data for each experiment were expressed as the mean value ± S.E.M (standard error of mean) (n=6). Unless otherwise specified, differences between vehicle control and treatment groups were tested using one way Analysis of Variant (ANOVA) followed by suitable multiple comparison of either Dunnett's, Dunn's or Fisher LSD Test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Gastric ulcers induced by necrotizing agents (cytoprotective studies)

After 8 hours, ACRH at 10 mg/kg significantly reduced the total area of lesion from 322.67 ± 22.00 to 193.75 ± 40.92

Table 1: Effects of hexane fraction of *Ardisia crispa* (ACRH) root 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 (mm2)	Inhibition (%)
80% EtOH	Vehicle	-	6	322.67 ± 22.00	-
	ACRH	10	6	193.75 ± 40.92*	39.95
		30	6	59.00 ± 29.59*	81.72
		100	6	55.00 ± 30.77**	82.95
		300	6	24.25 ± 10.14**	92.48
25% NaCl	Vehicle	-	6	296.50 ± 23.64	-
	ACRH	10	6	172.80 ± 42.47*	41.92
		30	6	112.75 ± 35.39**	62.1
		100	6	37.00 ± 30.37**	89.56
		300	6	28.40 ± 11.05**	90.45
0.6 M HCl	Vehicle	-	6	351.33 ± 12.12	-
	ACRH	10	6	238.75 ± 72.67	32.04
		30	6	19.5 ± 11.77**	94.45
		100	6	17.75 ± 5.39**	94.95
		300	6	16.00 ± 2.55**	95.45
0.2 M NaOH	Vehicle	-	6	270.50 ± 48.04	-
	ACRH	10	6	172.25 ± 14.87	36.32
		30	6	15.20 ± 11.12**	94.38
		100	6	7.75 ± 3.04**	97.13
		300	6	3.25 ± 2.63**	98.8
Indomethacin 30 mg/kg	Vehicle	-	6	152.67 ± 24.46	-
	Ranitidine	50	6	23.56 ± 6.21**	84.57
		150	6	1.00 ± 1.00**	99.34
	ACRH	10	6	90.40 ± 23.11	40.79
		30	6	51.20 ± 19.71*	66.46
		100	6	41.00 ± 27.82*	73.14
		300	6	22.25 ± 8.37*	85.43

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

and from 297.50 ± 23.64 to 172.80 ± 42.47 in NaCl - induced ulcer model respectively. On the other hand, it showed no significant differences in groups induced by NaOH and HCl. Whilst at 30 mg/kg, ACRH significantly reduced the total area lesion in all necrotizing agents induced ulcer model. In ethanol induced ulcer, the total area of lesion when pretreated with ACRH has reduced from 322.67 ± 22.00 to 59.00±29.59 mm^2. It has reduced from 351.33 ± 12.12, 297.50 ± 23.64 and 270.50 ± 48.04 to 19.5 ± 11.77, 112.75 ± 35.39 and 15.20 ± 12.12 mm^2 when induced by HCl, NaOH and NaCl respectively. At both 100 and 300 mg/kg, ACRH also significantly reduced the lesion formation in every necrotizing agent viz EtOH, HCl, NaoH and NaCl induced ulcer model.

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 ACRH significantly and dose-dependently inhibited gastric ulceration at 30, 100 and 300 mg/kg . At those doses, ACRH significantly reduced the total area lesion from 152.67 ± 24.46 to 51.20±19.71, 41.00±27.82 and 22.25±8.37 mm^2 respectively. Percentage inhibition of gastric erosions was in the range of 66.5-85.5%.

DISCUSSION

In gastrointestinal disorder, ulcer requires a well targeted therapeutic strategy. A number of drugs including antacids, proton pump inhibitors and histamine H2 receptor antagonists are available for the treatment, but clinical evaluation of these drugs showed an incidence of relapses, side effects, and drug interactions (15). This medication has been the cause for the development of new anti-ulcerogenic drugs and the search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse.

It is generally accepted that ulcer results from an imbalance between aggressive factors and the defence mechanism (16). To regain the balance, drugs of plant origin are investigated to inhibit the gastric acid secretion or to activate the mucosal defence mechanism by increasing mucus production (3). Medicinal plants provide an important source of new chemical substances with potential therapeutic effects. They have been used in

traditional medicine for the treatment of several diseases. *Ardisia crispa* is known for its several medicinal values which include its anti-inflammatory and analgesic effect (12). The present study is to investigate the antiulcerogenic activity of the plant from its hexane fraction of its crude ethanolic extract.

According to the experimental models used in this study, NSAIDs like indomethacin induce ulcer formation by depleting cytoprotective prostaglandin and increase in acid secretion (13). It is known that PGE_2 and PGI_2 (prostaglandin) of gastric and duodenal mucosa is responsible for mucous production and maintaining cellular integrity of the gastric mucosa (17). It has been reported that the NSAID-induced decrease in gastric mucosal blood flow in humans is notable around the gastric antrum (18). Accordingly, the primary pathology of NSAID-induced acute gastric mucosal damage is likely to be mucosal lesions due to ischemia. The main mechanism by which NSAIDs reduce gastric mucosal blood flow is thought to be their inhibition of COX-1. This inhibition leads to a deficiency in endogenous PGE_2, which in turn causes vasodilation resulting in a microcirculatory disturbance in the gastric mucosa (19). However, recent clinical and non-clinical studies have demonstrated that the mechanism of the NSAID induced disturbance can not be fully explained by the inhibition of prostaglandin biosynthesis alone (20).

In our study, Ranitidine is a standard control used here to test on the anti- ulcerogenic effect of ACRH that induced by indomethacin. It is well known that anti-secretory agents like Ranitidine are not effective against HCl or ethanol induced gastric lesions, thus the Ranitidine is only used as a positive control for the ulcer induced by indomethcin, as done by Bayir *et al.* (21).

The results obtained in this study clearly showed that ACRH was able to reduce the ulcer formation induced by indomethacin. At 300 mg/kg, the effect of ACRH (85.43%) is comparable to 50 mg/kg of Ranitidine (84.57%). ACRH has been proved to possess anti-inflammatory effect as well (12). Thus, this indicated that the ACRH is a potential COX-2 inhibitor. COX-2 inhibition is necessary and sufficient for analgesic or anti-inflammatory efficacy and the inhibition is not expected to cause the gastrointestinal complications such as stomach ulcers (22). Moreover, Sun *et al.* (23) also reported that COX-2 inhibitors may also possess mucoprotective effect. In indomethacin induced ulceration model, it is also suggested that the gastroprotective effect of ACRH is more likely mediated by preservation of gastric mucus secretion and at least partially, through a mechanism based on the stimulation of endogenous prostaglandin synthesis. In addition, its antisecretory activity cannot be excluded since its reaction is same with anti-secretory agents like Ranitidine. Therefore, further tests should be carried out to confirm our postulation.

The formation of gastric mucosal lesions by necrotizing agents such as HCl and ethanol has been reported to involve the depression of these gastric defensive mechanisms (24). HCl or ethanol induced gastric ulcers also promote stasis in gastric blood flow that contributes to the development of the hemorrhagic and necrotic aspects of tissue injury (25). Administration of ethanol and HCl can lead to intense damage of the gastric mucosa and it induces multiple hemorrhagic red bands (patches) of different sizes along the long axis of the glandular stomach (26).

In our experiment, oral administration of ethanol alone had induced ulcer lesion area after treatments with different doses of ACRH (10,30,100,300 mg/kg) respectively (Table 1). The results obtained clearly showed the ability of ACRH in preventing the ulcer induced by ethanol. Since vascular changes in the gastric mucosa appeared to be the most pronounced feature of ethanol-induced lesions, maintenance of the mucosal vasculature and normal blood flow may be the major mechanism of cytoprotection. A possible mechanism for the action of ACRH might due to an increased mucosal blood circulation (27).

The pathogenesis of ethanol-induced gastric mucosal damage is still unknown. However, factor like the solubility of mucus constituents, a concomitant fall in the transmucosal potential difference, increases the flows of Na^+ and K^+ into the lumen, pepsin secretion, and the histamine content in the lumen, and depression of tissue levels of DNA, RNA and proteins leading to flow stasis in damaged areas and formation of oxygen-derived free radicals, which are considered the main reasons for mucosa injury (28). In other word, ethanol produces necrotic lesions by direct necrotizing action which in turn reduces defensive factors, the secretion of bicarbonate and production of mucous (29). Besides, ethanol injures the mucosa and initiates the migration of activated leukocytes. As a response to inflammation, leukocytes produce H_2O_2 radicals that cause injury deeper in the mucosa (30).

In the HCl induced gastric ulceration model, HCl causes severe damage to gastric mucosa (31). The synthesis of mucous that strengthens the mucosa barrier against harmful agents has an important function in gastric protection. The continuous adherent mucous layer is also a barrier to luminal pepsin, thereby protecting the underlying mucosa from proteolytic digestion (32). Oral administration of 0.6M HCl to the control group clearly produced a pattern of mucosal damage similar to ethanol that characterized by multiple hemorrhage

red bands of different sizes along the long axis of the glandular stomach (26). Pre-treatment with ACRH, given orally at doses of 30,100,300 mg/kg induced significant gastroprotective effect with its percentage of inhibition within the range of 94-95%. HCl-induced ulcers are not inhibited by anti secretory agents such as ranitidine, but are inhibited by agents that enhance mucosal defensive factors such as prostaglandins .Prostaglandins play a role in HCl-induced ulcer (33). Thus, the gastroprotective effect in HCl–induced ulcer model indicates that the ACRH could enhance cytoprotective mechanism of the gastric mucosa.

The ability of ACRH in preventing the formation of ulcer induced by NaCl and NaOH were possibly due to its preventive action on the destruction of the mucosal layer. Destruction of mucosal layer by NaCl and NaOH is believed to decrease the secretion of mucous that protects the stomach from damage by the stomach acid (34). Once again, it showed that ACRH possessed the cytoprotective effect in strengthening the defensive mechanism of gastric mucosal.

Reactive oxygen species (ROS) also has an important role in the pathogenesis of mucosal damage caused by indomethacin, ethanol and other agents (35). Superoxides produced by peroxidases in the tissues might damage membranes and cause ulcer in the stomach tissues by increasing lipid peroxidation (36). Previously it has been found that NSAIDs inhibit gastric peroxidase and increase mucosal H_2O_2 and . OH level to cause lipid peroxidation and mucosal damage (37). Therefore, this study indicates that ACRH might possibly possess effect on antioxidant defense systems against oxidative damages in tissues although the mechanism of action is not fully understood.

The ED_{50} of ACRH calculated from the log dose-response curve was 12.59mg/kg for the rats treated with EtOH and NaOH, while for the rats treated with indomethacin, HCl and NaCl, the ED_{50} were 14.13 mg/kg, 13.18 mg/kg and 16.22 mg/kg respectively. The results indicated that ACRH at lower dose is enough to exert desired anti- ulcerogenic effect. Moreover, the 50% of the maximal effect can be attained by lower dose of ACRH.

In summary, it is suggested that the mechanisms involved in anti-ulcer effect of ACRH is mediated via COX-2 inhibition and it also gave mucoprotective and cytoprotective effects when it significantly reduced the lesion formations when induced by various necrotizing agents. Phytochemical screening done on the hexane fraction indicated that it contains saponin, triterpenoid, flavonoid and tannins. Therefore, we postulated that flavonoids in the fraction may correlate appropriately for the present activities (38).

CONCLUSION

This study provides evidences that the ACRH produced anti-ulcerogenic effect, which are related to antisecretory, cytoprotective and antioxidant mechanism. It is also suggested that ACRH is a potential COX-2 inhibitor as it was able to inhibit ulcer induced by indomethacin. The ED_{50} of ACRH calculated from the log dose-response curve showed that ACRH can exert desired anti-ulcerogenic effect even at lower dose. As a conclusion, observation made from the study indicates that the ACRH can be a potential source for the treatment of ulcer. However, detailed study like isolation and identification of bioactive compound(s) are required to confirm the bioactive compound(s) responsible for the activity

ACKNOWLEDGEMENT

The authors wish to thank Universiti Putra Malaysia, Serdang for the facilities and funding provided for the study

REFERENCES

1. Belaiche J., Burette A., De Vos M., Louis E., Huybrechts M. and Deltenre M. Study group of NSAID-GI complications. Observational survey of NSAID-related upper gastro-intestinal adverse events. *Belgium. Acta Gastroenterology* Belgiam **65**: 65–73 (2002)
2. Lewis D.A and Hanson P.J. Anti-ulcer drugs of plant origin. *Progress in Medicinal Chemistry* **28**: 201–231 (1991).
3. Borrelli F. and Izzo A.A. The plant kingdom as a source of anti-ulcer remedies. *Phytotherapy Research* **14**: 581–591 (2000).
4. Gracioso J.S., Hiruma-Lima C.A. and Souza Brito A.R. Antiulcerogenic effect of a hydroalcoholic extract and its organic fractions of *Neurolaena lobata* (L.) R.Br. *Phytomedicine* **7**: 283–289 (2000).
5. Hiruma-Lima C.A., Gracioso J.S., Bighetti E.J., Grassi-Kassisse D.M., Nunes D.S. and Brito A.R. Effect of essential oil obtained from *Croton cajucara* Benth. on gastric ulcer healing and protective factors of the gastric mucosa. *Phytomedicine* **9**: 523–529 (2002).
6. Toma W., Gracioso J.S., Hiruma-Lima C.A., Andrade F.D., Vilegas W. and Souza Brito A.R.M. Evaluation of the analgesic and antiedematogenic activities of *Quassia amara* bark extract. *Journal of Ethnopharmacology* **85**: 19–23 (2003).
7. Heyne K. Tumbuhan berguna Indonesia III, translated by Badan Litbang Kehutanan Jakarta (Yayasan Sarana Wana Jaya, Jakarta, 1987). Pp.1554
8. Zakaria Muhammad and Ali Mohd Mustafa. 1994. *Traditional Malay Medicinal Plants*. (Fajar Bakti, Kuala Lumpur 1994). pp 39–41
9. Chaweewan J., Herbert B., Lennart K. and Gunnar S. Ardisiacrispin A and B, Two utero-contracting Saponins from *Ardisia crispa*. *Planta Med.* **53**(5): 405–409 (1987)
10. Yoshida K., Koma Y. and Kikuchi H. 1987. Therapeutic Substance FR-900359 from *Ardisia crispa*. *Jappan Kokkai Tokyo Koho JP* **62** 283 (999): 917–920 (1987).
11. Kang Y.H., Kim W.H., Park M.K. and Han B.H. Antimetastatic and antitumor effects of benzoquinonoid AC7-1 from *Ardisia crispa*. *Intl J. Cancer* **93**: 736–740 (2001)
12. Roslida A.H. and Kim K.H. Anti-inflammatory and anti-hyperalgesic effects of Ardisia crispa Thunb.D.C. *Pharmacognosy Magazine* **4**(16):262–268 (2008)

13. Robert A., Nezamis J.E., Lancaster C. and Hanchar A.J. Cytoprotection in rats: Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. *Gastroenterology* **77**: 433–443 (1979)
14. Hayden L.J., Thomas G. and West G.B. Inhibitors of gastric lesions in the rat. *J. Pharm. Pharmacol.* **30**: 244–246 (1978).
15. McIntosh J.H., Byth K. and Piper D.W. Causes of death amongst a population of gastric ulcer patients in New South Wales, Australia. *Scandinavian J. Gastroenterology* **26**: 806–811 (1991).
16. Charlet N., Gallo-Torres H.E., Bounameaux Y., and Wills R.J. Prostaglandins and the protection of the gastroduodenal mucosa in humans: a critical review. *J. Clinical Pharmacol.* **25**: 564–582 (1985).
17. Konturek S.J., Obtulowiez W., Kwiecieu N. and Oleksy J. Generation of prostaglandin in gastric mucosa of patients with peptic ulcer disease. Effect of non-steroidal anti-inflammatory compounds. *Scandinavian J. Gastroenterology* **19**: 75–77 (1984).
18. Taha A.S., Angerson W., Nakshabendi I., Beekman H., Morran C., Sturrock R.D. and Russell R.I. Gastric and duodenal mucosal blood flow in patients receiving non-steroidal anti-inflammatory drugs-influence of age, smoking, ulceration and *Helicobacter pylori*. *Alimentary Pharmacol. Ther.* **7**: 41–45 (1993).
19. Shorrock C.J. and Rees W.D. Mechanisms of gastric damage by nonsteroidal anti-inflammatory drugs. *Scandinavian J. Rheumatol.* **78**: 5–11 (1989).
20. Frezza M., Gorji N. and Melato M. The histopathology of non-steroidal anti-inflammatory drug induced gastroduodenal damage: correlation with Helicobacter pylori, ulcers, and haemorrhagic events. *J. Clin. Pathol.* **54**:521–525 (2001).
21. Bayir Y., Odabasoglu F., Cakir A., Aslan A., Suleyman H., Halici M. and Kazaz C. The inhibition of gastric mucosal lesion, oxidative stress and neutrophil-infiltration in rats by the lichen constituent diffractaic acid. *Phytomedicine* **13**(8): 584–590 (2005).
22. DeWitt D.L. COX-2-Selective-Inhibitors: The New Super Aspirins. *Mol. Pharmacol.* **55**: 625–631 (2001).
23. Sun W.H., Tsuji S., Tsujii M., Gunawan E.S., Kawai N., Kimura A. Kakiuchi Y., Yasumaru M., Ijima H., Okuda Y., Sasaki Y., Hori M. & Kawano S. Induction of cycoloxygenase-2 in rat gastric mucosa by rebamipide, a mucoprotective agent. *J. Pharmacol. Exp. Ther.* **295**: 447–452 (2000).
24. Kinoshita M., Kume E. and Tamaki H. Roles of prostaglandins, nitric oxide and the capsaicin-sensitive sensory nerves in gastroprotection produced by ecabet sodium. *J. Pharmacol. Exp. Ther.* **275**: 494–501 (1995).
25. Konturek P.C., Brzowski T., Sliwowski Z., Pajido R., Stachura J., Hahn E.G. and Konturek S.J. Involvement of nitric oxide and prostaglandins in gastroprotection induced by bacterial lipopolysaccharide. *Scandinavian J. Gastroenterology* **33**: 691–700 (1998).
26. Mincis M., Chebli J.M.F., Khouri S.T. and Mincis R. Ethanol and the gastrointestinal tract. *Arch. Gastroenterology* **32**: 131–136 (1995).
27. Matsuda H., Li Y. and Yoshikawa M. Gastroprotections of escins Ia, Ib, IIa, and IIb on ethanol-induced gastric mucosal lesions in rats. *Eur. J. Pharmacol.* **373**: 63–70 (1999).
28. Guth P.H., Paulsen G. and Nagata H. Histologic and microcirculatory changes in alcohol-induced gastric lesions in the rats: effects of prostaglandin cytoprotection. *Gastroenterology* **87**: 1083–1090 (1984).
29. Marhuenda E., Martin M.J. and Alarcon de la Lastra C. Antiulcerogenic activity of aescine in different experimental models. *Phytother. Res.* **7**: 13–16 (1993).
30. Itoh M. and Guth P.H. Role of oxygen derived free radicals inhemorrhagic shock-induced gastric lesion in rat. *Gastroenterology* **88**: 1162–1167 (1985).
31. Yamahara J., Mochizuki M., Matsuda H. and Fujimura H. The anti-ulcer effect in rat of ginger constituents. *J. Ethnopharmacol.* **23**: 299–304 (1988).
32. Allen A. and Flemstrom G. Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. *Amer. J. Physiol. Cell* **288**: C1–C19 (2005).
33. Morimoto Y., Shimohara K., Oshima S. and Sukamoto T. Effects of the new anti-ulcer agent KB-5492 on experimental gastric mucosal lesions and gastric mucosal defensive factors, the compared to those of terpenone and cimetidine. *Japanese J. Pharmacol.* **57**: 495–505 (1991).
34. Wallace J.L. and Whittle B.J. Role of mucous in the repair of gastric epithelial damage in rats. Inhibition of epithelial recovery by mucolytic agents *Gastroenterology* **91**(3): 603–611 (1986).
35. Elliot S.N. and Wallace J.L. Neutrophil-mediated gastro-intestinal injury. *Canada J. Gastroenterology* **12**: 559–568 (1998).
36. Miura T., Muraoka S. and Fujimoto Y. Lipid peroxidation induced by indomethacin with horseradish peroxidase and hydrogen peroxide: involvement of indomethacin radicals. *Biochem. Pharmacol.* **63**: 2069–2074 (2002).
37. Banerjee R.K. Non-steroidal anti-inflammatory drugs inhibit gastric peroxidase activity. *J. Biochem. Biophys. Mol. Biol. Acta* **1034**: 275–279 (1990).
38. Kim H.P., Son K.H., Chang H.W. and Kang S.S., Anti-inflammatory plant flavonoids and cellular mechanisms, *Journal of Pharmacological Sciences* **96**: 229–245 (2004).