Full Length Research Paper

Some pharmacological actions of *Myrica rubra* part 1: Effect on experimentally-induced gastric ulcers, inflammation and haemorrhoids in rats

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Chinese arbutus (*Myrica rubra*) fruits or Yumberry, are stony red fruits with berry-like edible portions growing in China, India, Japan and some other south eastern Asian countries. A 50% juice under the trade name Yumberry is available in some countries. It is rich in polyphenols and proanthocyanidins. This study investigated the effect of 4 weeks administration of the juice as a substitute for the normal rats' drinking water on ethanol- and stress-indomethacin-induced ulcer, carrageenan-induced paw inflammation and croton oil-induced hemorrhoids in male Wistar rats. Consumption of Yumberry drink for 4 weeks almost completely protected the rats against the alcohol-induced gastric ulcers ($p < 0.05$) with no significant effect against the combined cold stress-indomethacin-induced ulcers ($p < 0.05$). The treatment significantly suppressed carrageenan-induced oedema in the rat's paw in a time-dependent manner ($p < 0.05$ to $p < 0.001$, $N = 8$). The treatment also significantly suppressed experimentally-induced hemorrhoids by $74 \pm 5.9\%$ ($p < 0.001$, $N = 8$). The different mechanisms of actions of the observed beneficial actions are discussed. The results of this study pointed for the first time the direct protective effects of *M. rubra* beverage on the gastric mucosa and its direct anti-inflammatory effect against skin and rectal inflammations.

Key words: Bayberry, gastric ulcers, inflammation, hemorrhoids.

INTRODUCTION

Myrica rubra sieb et zucc. fruits, family Myricaceae, are stony red fruits with berry-like edible portions. The fruit is grown in China, India, Japan and some other south eastern Asian countries such as, Vietnam, Burma and Thailand. It is known by various other names such as Yangmei, Bayberry and Chinese arbutus. It is also known as Wax-mytle and Yamamoto in Japan.

Phytochemical investigations of the fruit juice revealed the presence of high concentrations of polyphenols and proanthocyanidins (Fang et al., 2006). The latter are con-densed tannins (polymers) composed of various flavan-3-ol or catechin units. The most available are the reddish procyanidins (Saito et al., 1998). In addition to the fruits, both of the leaves and the bark of the tree constituents were analyzed (Tong et al., 2009; Wang et al., 2010; Tao et al., 2002). The proanthocyanidins were reported to possess various actions that included anti-oxidant (Yokozawa et al., 2012; Dixon et al., 2005), anti-viral (Yokozawa et al., 2012; Cheng et al., 2003), hypolipidemic (Lee et al., 2008), anti-cancer (Kuo et al., 2004), depigmenting (Yamakoshi et al., 2003) and anti-inflammatory actions (Lee et al., 2007; Wang, 2010). The flavonoids and polyphenols were proven to express anti-inflammatory properties by which they inhibit the proliferation and activity of lymphocytes (Sima et al., 2012).

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Peptic ulcer is a chronic disease that impairs the quality of life (Sai et al., 2011). It occurs as a result of the imbalance between the defense and the aggressive factors (Sai et al., 2011). Inflammatory processes play a major role in the etiology of ulcer. *M. rubra* juice is now widely distributed worldwide as a 50% refreshing drink and also as a carbonated beverage under the trade name Yumberry. Thus, the objective of this study was to investigate the influence of the non-carbonated juice drink in experimentally-induced gastric ulcers and inflammation in rats, in order to explore its antiulcer activity and the anti-inflammatory involvement of any antiulcer activity that may arise.

MATERIALS AND METHODS

Yumberry juice drink

Yumberry juice bottled drink was purchased from the local market in Riyadh city, Kingdom of Saudi Arabia. The bottled drink is a product of China (Zhejiang Yumberry Juice Co.).

Chemicals used and their sources

Ethanol, diethyl ether, pyridine and sodium carboxymethylcellulose (BDH, UK), Yumberry drink (Zhejiang Yumberry juice Co., Ltd, China), and Carrageenan (Fluka, Switzerland) were used for this study.

Animals

In this study, male Wistar rats (body weight 250 ± 10 g) were used. The animals were provided with standard chow diet supplied by Silo and Flour Mills Organization, Feed Mill, Riyadh, Saudi Arabia. All animals were housed at a temperature of 22 ± 1°C and relative humidity of 50 ± 5%. The light: dark cycle was 12 h each. The animals' treatment was conducted in accordance with the Guide for the Care and Use of Laboratory Animals. The protocol of the current study was approved by the ethics Committee of the College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia (KSA).

Treatment of the animals

Control group was allowed tap water *ad libitum*. All treatment groups (*N* = 8 animals/group) were administered Yumberry drink (50%) as the sole source of drinking water for 4 weeks.

Induction of alcohol gastric ulcers

In both Yumberry-treated and the control rats, gastric ulcers were induced by the oral administration of 1 ml 80% ethanol per rat by gavage. One hour after administration of alcohol, all rats were killed by cervical dislocation. The abdomen of each rat was opened, the stomach was removed and opened along the greater curvature and gently rinsed with isotonic saline. The stomach was pinned out flat on a flat surface with the mucosal surface uppermost. The induced gastric ulcers were assessed by calculating an ulcer index using a modification of the scoring system used by Stroff et al. (1993), Gretzer et al. (1998), Lambrecht et al. (1993) and Schmassmann et

al. (1998). The severity factor of the ulcer was defined according to the length of the ulcer (lesion) as follows:

1. Severity factor 1 = no longitudinal ulcers but only hyperemia;
2. Severity factor 2 = lesion < 2 mm in length;
3. Severity factor 3 = lesion 2 to 4 mm in length;
4. Severity factor 4 = lesion 4.1 to 10 mm;
5. Severity factor 5 = lesion 10.1 to 15 mm.

The ulcer index was calculated as the total number of ulcers per stomach multiplied by their respective severity factor.

Induction of stress-indomethacin ulcers

Stress-indomethacin ulcers were induced in male Wistar rats by a modification of the methods described by Senay and Levine (1967), Levine (1971), Bhargava et al. (1975), Beck et al. (1990) and Schmassmann et al. (1998). In brief, all animals were fasted for 48 h from food only with the respective drinking liquids *ad libitum*. Each animal in the control and the treated group was treated with indomethacin in a dose of 20 mg/kg by gavage. Indomethacin was suspended in a 0.25 % sodium carboxymethylcellulose (w/v). The volume of liquid administered was 4 ml/kg body weight. Then all of the animals were immobilized in restrained cages and placed inside a ventilated refrigerator maintained at a temperature of 4°C. Three hours later, the rats were killed by cervical dislocation, stomachs removed and the ulcer index was calculated following the scoring system that depends upon the approximate diameter of the induced ulcer. The scoring system was 1, 2, 3, 4, 5 and 6 for ulcers with diameters of 1, 2, 3, 4, 5, and 6 mm. the ulcer index was calculated by multiplying the number of ulcers by their respective scores and summing up.

Induction of paw inflammation

In both control and treated rats, paw inflammation was induced by the modification of the method described by Winter et al. 1962 and Sima et al. 2012). In brief, initially, each animal was marked around its right ankle in a circular manner using a non-erasable blue ink. The volume of each paw up to the ankle mark was then measured using a plythesmometer (Ugo, Basil, Switzerland). The dis-placement fluid used was 0.45% sodium chloride solution. The volume of the displaced paw was then read in the digital display of the instrument. The paw was then injected intraplantarly with 0.2 ml of 2% aqueous Carrageenan solution using 1 ml syringe fitted with a fine hypodermic needle. Thereafter, the paw volume was measured after 1, 2 and 3 h. The net volumes of oedemas formed were then calculated. The percentage decrease in oedemas in the treated animals was calculated relative to the oedemas in the control group.

Induction of hemorrhoids

Hemorrhoids were induced in both control and treated animals by a modification of the methods described by Nishiki et al. (1988) and Okumura et al. (1995). In brief, male Wistar rats (body weight 250 ± 10 g) were used in this study. To induce hemorrhoids, a mixture of 6% croton oil in diethyl ether, pyridine, diethyl ether and water in a ratio of 10:4:5:1 (v/v) was used. All rats were fasted from food for 3 days but water and Yumberry drink were allowed *ad libitum*.

On the day of experiment, each rat was immobilized in a restrainer cage. The tampon of an ear cotton bud (produced by Septona SA, Greece and purchased from the local market in Riyadh, Kingdom of Saudi Arabia) was dipped in 10 ml of the above inducer contained in a 10 ml vial for 60 s. Then, it was inserted into

the rectum and allowed to contact the mucosa to a depth of 2 cm for 60 s and then removed smoothly. The animals were freed from their restrainer and placed in cages with no food or water for complete 4 h. The volume of the inducer absorbed by each tampon was 333 μl. The animals were then sacrificed by an overdose of diethyl ether. The lower abdomen was opened, all of the reproductive organs removed, the rectum bone cut and the lower rectum cleared from any adhering tissue. Then a rectum-anus portion measuring 2 cm was cut from each rat starting from the circular hairline on the anus epithelium using a pair of compass. The cut piece was then opened longitudinally, blotted on a piece of tissue-paper and weighed immediately. The weight of tissue corresponding to 150 g body weight was then calculated in mg. Similarly, the percentage ratio of the whole 2 cm piece relative to the total body weight of the animal was also calculated. The percentage reductions in the weights of the recti of the treated animals in relation to the control weights were then calculated. A third group of animals was administered cotton buds immersed in de-ionized water only as described above.

Statistics

The values reported in this study were mean ± standard error of mean, with N = number of animals used. Statistical significant differences were calculated using non-paired Student's 't' test. Differences with p values less than 0.05 were considered significant. Normality test was done using Kolmogorove Smirnov test.

RESULTS

Effect of yumberry on gastric ulcers

Effect on alcohol-induced ulcers

Administration of 80% alcohol by gavage into rats induced excessive hemorrhages and ulcers. The ulcer index was 18.6 ± 1.4 (N = 8). Continuous treatment of the rats with Yumberry drink for 4 weeks almost completely prevented the induction of ulcers. The maximum effect observed in the eight rats was only occasional hyperemia. The ulcer index was only 1.0 ± 0.5. Yumberry drink significantly blunted alcohol-induced ulcers.

Effect on stress-Indomethacin-induced ulcers

Exposure of rats to the combined hypothermic stress (4°C) and oral indomethacin induced excessive number of small ulcers. The ulcer index was 46.4 ± 4.1. Treatment of rats with Yumberry for 4 weeks continuously resulted in slight protection of the animals against the induced ulcers. The ulcer index was 41.6 ± 5.9 (p > 0.05, N = 8).

Effect of yumberry on carrageenan-induced paw oedema

Intraplantar administration of carrageenan into the rats

paw resulted in time-dependent inflammation as reflected by the paw volume (Table 1). Similar intraplantar administration of carrageenan into Yumberry drink-treated rats resulted in highly significant and time-dependent reduction in the paw oedema (P < 0.05 to < 0.001), N = 8) (Table 1). The percentage decreases in the induced oedemas were 31.5 ± 1.7, 65.3 ± 4.9 and 78.0 ± 3.4%, 1, 2, and 3 h, following administration of carrageenan.

Effect of yumberry on experimentally-induced haemorroids

Intra-rectal administration of a mixture of croton oil, pyridine, diethyl ether and water into rats resulted in significant haemorrage and oedemas that led to significant increases in the recti pieces weights compared with non-treated animals (Table 2). In fact, the haemorroids-inducer resulted in more than doubling of the weight. Induction of haemorroids in rats that drank Yumberry drink for four weeks revealed the inherent anti-haemorroids activity of Yumberry. The treatment suppressed the induced haemorroids by 74.0 ± 5.9% (p < 0.001, N = 8) (Table 2).

DISCUSSION

The results of this study clearly demonstrated that consumption of Yumberry drink protects against alcohol-induced ulcers and suppresses inflammations, whether skin or rectal types (haemorrhoids). Such actions seemed to be due to some Yumberry drink constituents. The juice of *M. rubra*, from which Yumberry drink is made, is known to contain proanthocyanidins such as cyanidin-3-glucoside, flavonoids such as quercetin-3-o-glucoside and various polyphenols such as gallic acid, and various vitamins of the B, C, and E groups (Zhongxiang et al., 2007; Fang et al., 2006).

Alcohol-induced ulcers are known to be due to stimulation of production of oxygen-derived free radicals (Wallace and Whittle, 1985; Oates and Hakkinen, 1988) and a decrease in mucosal level of non-protein sulfhydryl compounds (Szabo et al., 1981). Previous studies using pure proanthocyanidins revealed the ability of these compounds to inhibit production of oxygen free radicals (Dixon et al., 2005; Xie and Dixon, 2005; Yokozawa et al., 2012) and to elevate reduced glutathione level (Yokozawa et al., 2012).

Flavonoids (Kim and Uyamay, 2005) and polyphenols (No et al., 1999) were also reported to be anti-oxidants. Thus, the observed ability of Yumberry drink to protect against alcohol-induced ulcers seemed to be due to the presence of anthocyanidins, flavonoids and polyphenols in the drink. Furthermore, an additional physical mechanism such as binding of the proanthocyanidins to the gastric mucosa proteins cannot be ruled out since procyanidins can bind to bovine serum albumin (Saito et

Table 1. Effect of yumberry on carrageenan-induced paw oedema in rats.

Treatment	Net oedema volume (ml) after,		
	1 h	2 h	3 h
Carrageenan	0.57±0.1	1.45±0.13	2.1±0.2
Yumberry + carrageenan	0.39±0.03*	0.50±0.05**	0.46±0.1*

*$p < 0.05$, N = 8, **$p < 0.001$, $N = 8$.

Table 2. Effect of yumberry drink on experimentally-induced haemorrhoids in rats.

Treatment	Weight of 2 cm rectum per 150 g body weight (mg)	Net haemorroids weight	Percentage of the 2 cm rectum to the whole body weight
Normal rectum	178.1±9.7	-	0.071
Haemorroids inducer	362.4±13.9	184.3±2.5	0.145
Haemorroids inducer + yumberry	226.0±8.4*	47.9±1.3*	0.09

*Compared with haemorroids inducer ($N = 8$) ($p < 0.001$).

al., 1998). On the other hand, indomethacin-induced ulcers seemed to involve inhibition of gastric prostaglandins derived mainly via COX-1, with some involvement of COX-2 (El-Bayer et al., 1985; Beck et al., 1990; Iseki, 1995; Wallace, 1997; Schmassmann et al., 1998).

Stress-induced ulcers are believed to result from an increase in gastric acid secretion (Kitagawa et al., 1979; Marrone, 1984), a decrease in gastric PGE_2 production (Moody, 1976; Arakawa et al., 1981) and disturbed micro-circulation in the gastric mucosa (Murakami et al., 1985). Proanthocyanidins are shown to suppress COX-2 enzyme (Yokozawa et al., 2012). Thus, the success of Yumberry drink to selectively suppress alcohol-induced ulcers but not the indomethacin cold stress-induced ulcers seemed to be due to the difference in the mechanisms of the induced ulcers. Previous reports have shown the ability of some plants containing proanthocyanidins, for example, grape seed extract to suppress gastric ulcers (Saito et al., 1998). Furthermore, Grisham et al. (1986) related the gastric protective effect of proanthocyanidins to their ability to decrease mycloperoxidase activity (Grisham et al., 1986).

The results of this study revealed another inherent property for *M. rubra* drink (Yumberry), an anti-inflammatory and antihaemorrhoidal action as shown by the inhibition of carrageenan-induced paw oedema (Winter et al., 1962) and croton oil mixture-induced inflammatory haemorrhoids (Nishiki et al., 1988; Okumura et al., 1995). Carrageenan-induced inflamma-tion is shown to be due to release of various mediators, majorities of which are TNFα (Gong et al., 2009; Bhavin et al., 2010), IL-1β (Chou, 2003; Loram et al., 2007), nitrogen oxide (NO) (Bilici et al., 2002), prostaglandins, kinins, histamine and serotonin (Bhukya et al., 2009). The time-dependent inhibitory effect of Yumberry drink on the

induced inflammation may be due to its constituent proanthocyanidins. Indeed, these are shown to inhibit NO and the PGs via inhibition of their producing enzymes NO synthase and COX-2 (Yokozawa et al., 2012). An additional inhibitory effect on TNFα cannot be ruled out since a proanthocyanidin induced inhibition of their inducer NFkβ has been previously reported (Yokozawa et al., 2012). In this connection, it is pertinent to recall that NFkβ stimulates the transcription of the genes that encode the enzymes NO synthase and COX-2 (Touyza and Schiffrin, 2004). Furthermore, proanthocyanidins do indeed inhibit TNFα in the rat's paw (Li et al., 2001). Croton oil mixture-induced inflammation and haemorrhoids seemed to involve mainly prostaglandins and IL-6, with no effect on NO or TNFα (Shin et al., 2010). Thus, proanthocyanidins contained in Yumberry drink may also be involved in the observed inhibition of croton oil mixture-induced inflammation (Yokozawa *et al.* 2012).

On a broad basis, the results of this study pointed for the first time the direct protective effect of *M. rubra* juice beverage on the gastric mucosa and its direct anti-inflammatory effect against skin and rectal inflammations. Thus, *M. rubra* juice drink can be considered as a useful functional food.

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REFERENCES

Arakawa T, Kobayashi N, Nakamura H, Chono S, Yamada H, Ono T (1981). Effect of water immersion stress on prostaglandin E_2 in rat

gastric mucosa. Gastroenterol. Jpn. 16(3):236-241.

Beck WS, Schneider HT, dietzel K, Nuernberg B, Brune K (1990). Gastrointestinal ulceration induced by anti-inflammatory drugs in rats. Physicochemical and biochemical factors involved. Arch. Toxicol. 64(3):210-217.

Bhukya B, Anreddy RN, William CM, Gottumukkala KM (2009). Analgesic and anti-inflammatory activity of leaf extract of *Kydia calcina* Roxb. Bangladesh J. Pharmacol. 4:101-104.

Bhargava KP, Gupta MB, Tangri KK (1973). Mechanism of Ulcerogenic Activity of Indomethacin and Oxyphenbutazone. Eur. J. Pharmacol. 22(2):191-195.

Bhavin VA, Ruchi VB, Shrikant JV, Dev SD (2010). Evaluation of anti-inflammatory activity of n-butanol extract from *Pergularia daemia* in experimental animal model. J. Pharm. Res. 3(12):3039-3044.

Bilici D, Akpinar E, Tunc AK (2002). Protective effect of melatonin in carrageenan-induced acute local inflammation. Pharmacol. Res. 46(2):133-190.

Cheng HY, Ln TC, Ishimaru KK, Yang CM, Wang KC, Lin CC (2003). *In vitro* anti-viral activity of prodelphinidin B-2 3, 3'-di-O-gallate from *Myrica rubra*. Planta Med. 69(10):953-956.

Schmassmann R, Peskar M, Selteler C, Netzer P, Straff T, Flogerzi B, Fred HZ (1988). Effect of inhibition of PG endoperoxide synthase1 on chronic gastro-intestinal ulcer models in rats. Br. J. Pharmacol. 123:795-804.

Chou TZC (2003). Anti-inflammatory and analgesic effects of paeonol in carrageenan-evoked thermal hyperalgesia. Br. J. Pharmacol. 139(6):1146-1152.

Dixon RA, Xie DY, Sharma SB (2005). Proanthocyanidins: A final frontier in flavonoids research? New Phytol. 165(1):9-28.

Fang Z, Zhang M, Tao G, Sun Y, Sun J (2006). Chemical composition of clarified bay berry (*Myrica rubra* Sieb et Zucc.) Juice sediment. J. Agric. Food Chem. 54(20):7710-6.

Gretzer B, Ehrlich K, Maricic N, Lambrecht N, Respondek M, Peskar BM (1998). Selective cyclooxygenase inhibitors and their influence on protective effect of a mild irritant in the rat stomach. Br. J. Pharmacol. 123(5):927-935.

Gong D, Geng C, Jiang L, Cao H, Yoshimura H, Zhong L (2009). Effect of hydroxytyros 1-20 on carrageenan-induced acute inflammation and hyperalgesia in rats. Phytother. Res. 23(5):646-650.

Grisham MB, Hernandez LA, Granger DN (1986). Xanthine oxidase and neutrophil infiltration in intestinal ischemia. Am. J. Physiol. 251(4 Pt. 1):G567-74.

Iseki S (1995). Immunocytochemical localization of cyclooxygenase-1 and cyclooxygenase-2 in the rat stomach. Histochem J. 27:323-328.

Kim YJ, Uyama H (2005). Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition, mechanism and perspective for the future. Cell Mol. Life Sci. 62(15):1707-1723.

Kitagawa H, Fujiwara M, Osumi Y (1979). Effects of water-immersion stress on gastric secretion and mucosal blood flow in rats. Gastroenterology 77(2):298-302.

Kuo PL, Hsu YL, Lin TG, Lin LT, Lin CC (2004). Induction of apoptosis in human breast adenocarcinoma MCF-7 cells by prodelphinidin B-2, 3,3-di-o-gallate from Myrica rubra via fas-mediated pathway. J. Pharm. Pharmacol. 56(11):1399-1406.

Lambrecht N, Burchert M, Respondek M, Mueller KM, Peskar BM (1993). Role of calcitonin-gene related peptide and nitric oxide in the gastro protective effect of capsaicin. Gastroenterology 104:1371-1380.

Lee YA, Kia YJ, Cho EJ, Yokozawa T (2007). Ameliorative effects of proanthocyanidin on oxidative stress and inflammation in streptozotocin-induced diabetic rats. J. Agric. Food Chem. 55(23):9395-9400.

Lee YA, Cho EJ, Yokozawa T (2008). Effects of proanthocyanidin preparations on hyperlipidemia and other biomarkers in mouse model type 2 diabetes. J. Agric. Food Chem. 56(17):7781-7789.

Levine RJ (1971). In: Pfeiffer CJ (ed). "Peptic Ulcer". Munksgaard, Copenhagen pp 92-97.

Li WG, Zhang XY, Wu XJ, Tian X (2001). Anti-inflammatory effect and mechanism of proanthocyanidins from grape seeds. Acta Pharmacol. Sin. 22(12):117-120.

Loram LC, Fuller A, Fick LG, Cartmell T, Poole S, Mitchell D (2007). Cytokine profiles during carrageenan-induced inflammatory hyperal-

gesia in rat muscle and hind paw. J. Pain 8:127-136.

Marrone PO (1984). Pathogensis, diagnosis and treatment of acute gastric mucosal lesions. Clin. Gastroenerol. 13(2):633-650.

Moody FG (1976). Stress ulcers, their diagnosis and treatment. Surg. Clin. North Am. 56(6):1469-1478.

Murakami M, Lam SK, Inada N, Miyake T (1985). Pathophysiology and pathogenesis of acute gastro mucosal lesions after hypothermic restraint stress in rats. Gastroenterology 880(3):660-665.

Nishiki K, Nishinaga K, Kudoh D, Iwai K (1988). Croton oil-induced hemorrhoid model in rat: comparison of anti-inflammatory activity of diflucortolone valerate with other glucocorticoids. Nippon Xakurigaku Zasshi 92(4):215-225.

No JK, Soung DY, Kim YS, Shim KH, Juan YS, Rhee SH, Yokozawa T, Chung HY (1999). Inhibition of Tyrosinase by green tea components. Life Sci. 65(21):241-246.

Oates PJ, Hakkinen JP (1988). Studies on the mechanism of ethanol-induced gastric damage in rats. Gastroenterology 94(1):10-21.

Okumura M, Okude T, Nakamura T, Yajima M (1995). Preparation for treating wounds or hemorrhoids. US. Patent 5403867.

Saito M, Hosoyama H, Ariga T, Kataoka S, Yamaje N (1998). Antiulcer activity of grape seed extract and procyanidins. J. Agric. Food Chem. 46(4):1460-1464.

Sai KB, Radha KL, Gowrinath RM (2011). Anti-ulcer effect of *Aloe vera* in non-steroidal anti-inflammatory drug induced peptic ulcers in rats. Afr. J. Pharm. Pharmacol. 5(16):1867-1871.

Senay EC, Ievine RI (1967). Synergism between cold and restraint for rapid production of Stress Ulcers in rats. Proc. Soc. Exp. Biol. Med. 124(4):1221-1231.

Sima N, Anoush M, Khatmi N (2012). Evaluation of analgesic and anti-inflammatory effects of fresh onion juice in experimental animals. Afr. J. Pharm. Pharmacol. 6(23):1679-1684.

Stroff J, Plate S, Seyed Ebrahim J, Ehrlich K, Respondek M, Peskar BM (1996). Tachykinin-induced increase in gastric mucosal resistance: Role of afferent neurons, CGRP and nitric oxide. Am. J. Physiol. 271(6 Pt. 1):G1017-1027.

Szabo S (1981). Sulfhydyl compounds may mediate gastric cytoprotection. Science 214(4517):201-202.

Tao J, Morikawa T, Toguchida I, Ando SS, Matsuda HH, Yoshikawa MM (2002). Inhibitors of nitric oxide production from the bark of *Myrica rubra*: Structures of new biphenyl type diarylheptanoid glycosides and taraxerane type tripterpane. Bioorg. Med. Chem. 10(12):4005-4012.

Tong Y, Zhou XM, Wang SJ, Yung X, Cao YL (2009). Analgesic activity of myricetin isolated from *Myrica rubra* Sieb et Zucc leaves. Arch. Pharm. Res. 32(4):527-533.

Touyza RM, Schiffrin EL (2004). Reactive O_2 species in vascular biology: Implication in hypertension. Histochem. Cell Biol. 122(4):339-352.

Wallace JL (1997). Nonsteroidal anti-inflammatory drugs and gastroenteropathy, the second hundred years. Gastroenterology 112(3):1000-1016.

Wallace JL, Whittle BJR (1986). Role of mucus in the repair of gastric epithelial damage in rat. Gastroenterol. 91(3):611-630.

Wang SJ, Tong Y, Lu S, Yang R, Liao X, Xu YF, Li X (2010). Anti-inflammatory activity of myricetin isolated from *Myrica rubra* Sieb Zucc. Leaves. Planta Med. 76(14):1492-1496.

Winter CA, Risley EA, Nuss GW (1962). Carrageenan-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 111:544-547.

Xie DY, Dixon RA (2005). Proanthocyanidin biosynthesis - still more questions than answers? Phytochemistry 66(18):2127-2144.

Yamakoshi J, Otsuka F, Sano A, Tokutake S, Saito M, Kikuchi M, Kubeta Y (2003). Lightening effect on UV-induced pigmentation of guinea pig skin by oral administration of a proanthocyanidin extract from grape seeds. Pigment Cell Res. 16(6):629-638.

Yokozawa I, Cho EJ, Park CH, Kim JH (2012). Protective effect of proanthocyanidin against diabetic oxidative stress. Evid. Based Complement. Alternat. Med. Epub 2011 Sep 7.

Zhongxiang Fang, Min zhang and Linxiang Wang (2007). HPLC-DAD-ES, MS analysis of phenolic compounds in baberries (*Myrica rubra* Sieb et Zucc). Food Chem. 100:845-852.