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 condensed 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).
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) solution using 1 ml 80% aqueous Carrageenan (Fluka, Switzerland). 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 plethesmometer (Ugo, Basel, Switzerland). The displacement 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

Intraplant 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 haemorrhoids

Intra-rectal administration of a mixture of croton oil, pyridine, diethyl ether and water into rats resulted in significant haemorrhage and oedemas that led to significant increases in the recti pieces weights compared with non-treated animals (Table 2). In fact, the haemorrhoids-inducer resulted in more than doubling of the weight. Induction of haemorrhoids in rats that drank Yumberry drink for four weeks revealed the inherent anti-haemorrhoids activity of Yumberry. The treatment suppressed the induced haemorrhoids 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 sulphydryl 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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Net oedema volume (ml) after,</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>0.57±0.1</td>
</tr>
<tr>
<td>Yumberry + carrageenan</td>
<td>0.39±0.03*</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight of 2 cm rectum per 150 g body weight (mg)</th>
<th>Net haemorrhoids weight</th>
<th>Percentage of the 2 cm rectum to the whole body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rectum</td>
<td>178.1±9.7</td>
<td>-</td>
<td>0.071</td>
</tr>
<tr>
<td>Haemorrhoids inducer</td>
<td>362.4±13.9</td>
<td>184.3±2.5</td>
<td>0.145</td>
</tr>
<tr>
<td>Haemorrhoids inducer + yumberry</td>
<td>226.0±8.4*</td>
<td>47.9±1.3*</td>
<td>0.09</td>
</tr>
</tbody>
</table>

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

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 PGE₂ production (Moody, 1976; Arakawa et al., 1981) and disturbed microcirculation 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 myeloperoxidase 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 inflammation 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 (Buksa 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 NFκβ has been previously reported (Yokozawa et al., 2012). In this connection, it is pertinent to recall that NFκβ 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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