Protective Effects of *Vaccinium arctostaphylos* L., *Berberis thunbergii* var. atropurpurea Chenault, *Elaeagnus angustifolia* L. and *Launaea acanthodes* (Boiss.) O. Kuntze against Indomethacin-Induced Gastric Ulcers in Rats

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Abstract

Background: Data suggest that *V. arctostaphylos*, *B. thunbergii* var. atropurpurea, *E. angustifolia* and *L. acanthodes* may prevent gastric ulcers induced by Non-steroidal anti-inflammatory drugs (NSAIDs).

Objective: To explore the ulcer-protective potential of these plants in indomethacin-induced gastric ulcers in rats.

Methods: Saline, hydro-alcoholic extract of each plant (100, 400, 800 mg/kg) and omeprazole (30 mg/kg) were gavaged to the groups of animals for 4 consecutive days. Gastric ulcers were induced by the onetime gavage of indomethacin (30 mg/kg, p.o.). On the fifth day, each group was pretreated with physiological saline as control, extract (100, 400 or 800 mg/kg, p.o.) or omeprazole (30 mg/kg, p.o.) 30 min before the indomethacin administration. The animals were killed 6 h after the indomethacin administration. The stomachs were removed, opened along the greater curvature and washed in physiological saline. A person unaware of the type of treatment received by the animals examined the stomachs under a 3-fold magnifier. The areas and lengths of hemorrhagic lesions induced by indomethacin were measured using a dial caliper and the sum of measurements for each animal was referred to as the ulcer index.

Results: All extracts reduced the ulcer index significantly compared to the control group ($p < 0.05$).

Conclusion: These plants prevent NSAID-induced gastric ulcers in rats. The efficacy and potency of the gastro-protective effect of *L. acanthodes* appears to be higher than the other 3 plants.

Keywords: Anti-ulcer, Herbal, Medicine, Rat, Traditional

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase (COX)-2 inhibitors (COXIBs) are perhaps some of the most extensively used medications in the world [1]. Preventable NSAID-related hospital admissions have been reported to range from 7% to 11% [2, 3]. NSAIDs and COXIBs are used for the treatment of fever, inflammation and pain; in addition to these indications, aspirin also is used for the prevention of vascular events. Unfortunately, NSAIDs and COXIBs are not without adverse effects. NSAIDs commonly cause gastrointestinal (GI) side effects which are potentially serious, with as many as 60% of people who use traditional NSAIDs experiencing some type of adverse effect [4, 5]. Per year, upper-GI complications will develop in 1% to 2% of people using these NSAIDs [6 – 8]. This rate is three to five times higher than in people who do not use these NSAIDs [6 – 8]. The risk of severe complications is even higher in individuals with established risk factors, with a potential case-fatality rate of 5% [9].

Various strategies can be employed to help reduce adverse GI outcomes secondary to traditional NSAIDs. Misoprostol, a prostaglandin E analog, can help prevent gastric ulcers [10, 11]. Doses necessary to prevent NSAID-induced gastric ulcers have been associated with intolerable abdominal discomfort and diarrhea, leading to discontinuation of the medication in up to a third of patients [12, 13]. Proton-pump inhibitors (PPIs) are effective for reducing symptomatic and endoscopic ulceration [4]. However, PPIs are associated with noticeable adverse effects such as osteoporosis-related fractures, *Clostridium difficile* associated diarrhea, community and hospital-acquired pneumonia, pharmacologic interaction with clopidogrel and aspirin with subsequent increased rate of cardiovascular events, refractory hypomagnesemia and rebound reflux symptoms etc. The risk-benefit ratio of PPIs is increasingly recognized as being less favorable. This leads to a more critical viewpoint and raises the question whether the side effects of PPIs may outweigh the benefits, especially with long-term use [14]. Although they are safer than nonselective NSAIDs, COXIBs are not without GI risk, and this risk may be increased when COXIBs are used concurrently with aspirin. Even when used at a low dose, aspirin has been shown to block COX-1 sufficiently to minimize any GI protection provided by the COXIB [15, 16]. Considering the remarkable adverse effects of misoprostol and PPIs, safer agents are needed to prevent NSAID-induced gastric lesions. Human being is involved in herbal medicine from the ancient times. Also, there is growing interest in the health benefits of herbs and botanicals [17]. *Vaccinium arctostaphylos* L. is rich of a subclass of flavonoids called bioactive anthocyanins [18, 19] which have antioxidative activity [20]. Additionally, *Berberis thunbergii* var. atropurpurea D.C. has had antioxidative property [21]. *Elaeagnus angustifolia* L. and *Launaea acanthodes* O. Kuntze are used in the Iranian traditional medicine for treatment of gastrointestinal disorders including gastric and duodenal ulcers [22, 23]. Moreover, the other traditional uses of the plants are as follows. *V. arctostaphylos*: hypertension and diabetes mellitus [24]; Plants belonging to the genus *Berberis*: indigestion, ulcer healing, obesity and liver disorders [25]; *E. angustifolia*: asthma and fever [22];

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L. acanthodes: pain and wound healing [23]. In view of the role of oxidative stress in the gastric damage induced by indomethacin [26] and antioxidative effects of *Vaccinium arctostaphylos* and *Berberis thunbergii* var. atropurpurea and the traditional use of the other two plants in treatment of gastrointestinal disorders, we investigated the efficacy of these plants in prevention of indomethacin-induced gastric ulcers in rats.

Material and Methods

Plant materials

The fruits of *V. arctostaphylos* were collected from the lands of the Ardebil province of Iran in October. The aerial parts of *B. thunbergii* var. atropurpurea were collected from the lands of the Tehran province in August. *E. angustifolia* and *L. acanthodes* were collected from the lands of the Yazd province of Iran in August. *V. arctostaphylos* was identified by the botanist Dr. Yousef Ajanii and the other plants were identified by the botanist Dr. Hasan Asghari. The voucher specimens of the plants were deposited in the Tehran University Central Herbarium. The herbarium voucher specimen numbers of *V. arctostaphylos*, *B. thunbergii* var. atropurpurea, *E. angustifolia* and *L. acanthodes* are 15062, 1631, 1652 and 1667 respectively. The fruits and aerial parts were separated from the plants, washed and dried in shade at room temperature. The dried fruits and aerial parts were ground into powder.

Preparation of plant extracts

The dried fruit/aerial parts powder (100 g) was extracted with ethanol/water (70/30) as the solvent in a percolator three times; the solvent was completely removed from the hydro-alcoholic extracts at 42 ºC by Rota-vapor. The extraction yields of *V. arctostaphylos*, *B. thunbergii* var. atropurpurea, *E. angustifolia* and *L. acanthodes* were 20%, 37%, 28% and 20% respectively [27].

Drugs

Indomethacin and omeprazole were purchased from Sigma. For dilution, all drugs and extracts were dissolved in physiological saline. All drugs and extracts were prepared immediately before use.

Animals

Male adult Wistar rats weighing 250 - 300 g from our own breeding colony were used. Animals were maintained under standard environmental conditions and had access to standard rodent feed and water.

Measurement of protection against indomethacin-induced gastric ulcers

Rats were deprived of food for 24 prior to starting the experiments but they had free access to drinking water. The animals were kept in cages with raised floors of wide mesh to prevent coprophagia and they were divided into 15 groups of 10 rats each. Saline, each extract (100, 400, 800 mg/kg) and omeprazole (30 mg/kg) were administered orally to the groups of animals for 4 consecutive days. Gastric ulcers were induced by the onetime oral administration of indomethacin suspended in 0.5% carboxymethylcellulose (CMC) in water (30 mg/kg, p.o.). On the fifth day, each group was pretreated with oral physiological saline as control, extract (100, 400 or 800 mg/kg, p.o.) or omeprazole (30 mg/kg, p.o.) 30 min before the indomethacin administration.

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All drugs and extracts were dissolved and administered in physiological saline in a volume of 5 ml/kg [28, 29] (Table 1).

The animals were killed 6 h after the indomethacin administration by using an overdose of chloroform. The stomachs were removed, opened along the greater curvature and washed in physiological saline. A person unaware of the type of treatment received by the animals examined the stomachs under a 3-fold magnifier. The areas and lengths of hemorrhagic lesions induced by indomethacin were measured using a dial caliper and the sum of measurements for each animal was referred to as the ulcer index [28, 29] (Table 1).

Statistical analysis

The results were expressed as means ± S.D. and analyzed with the One-Way ANOVA followed by the tukey post hoc test. $p < 0.05$ was taken as significant.

Results

Omeprazol, *V. arctostaphylos* (800 mg/kg), *B. thunbergii* var. atropurpurea (400 mg/kg, 800 mg/kg), *E. angustifolia* (400 mg/kg and 800 mg/kg) and *L. acanthodes* (100 mg/kg, 400 mg/kg and 800 mg/kg) decreased significantly the gastric ulcer index compared to the saline + indomethacin group ($p < 0.05$) (Table 1).

Table 1 – Effects of pretreatment with omeprazole and the plant extracts on the gastric ulcer index in rats treated with indomethacin (30 mg/kg). P-values smaller than 0.05 are significant

Treatment (N = 10 in each group)	Dose (mg/kg, p.o.)	Ulcer index	P-value compared to saline + indomethacin
Saline + saline	-	0	
Saline + indomethacin	-	17.4 ± 9.6	
Omeprazole	30	0 ± 0	0.000
V. arctostaphylos	100	8.9 ± 13.4	0.301
V. arctostaphylos	400	6.5 ± 5.8	0.050
V. arctostaphylos	800	4.4 ± 5.8	0.006
B. thunbergii var. atropurpurea	100	7.6 ± 8.6	0.128
B. thunbergii var. atropurpurea	400	4.7 ± 3	0.009
B. thunbergii var. atropurpurea	800	2.9 ± 5.4	0.001
E. angustifolia	100	17 ± 9.5	1.000
E. angustifolia	400	2.4 ± 1.3	0.001
E. angustifolia	800	0.9 ± 0.6	0.000
L. acanthodes	100	1 ± 1.6	0.000
L. acanthodes	400	0.7 ± 1	0.000
L. acanthodes	800	0.2 ± 0.4	0.000

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Discussion

The results indicate that all the 4 plants tested possess promising anti-ulcerogenic activity, probably by potentiating the defensive barriers in the gastric mucosa. V. *arctostaphylos* at the high dose, *B. thunbergii* var. atropurpurea and *E. angustifolia* at the medium and high doses and *L. acanthodes* at all doses have significant protective effects against indomethacin-induced gastric ulcers. The efficacy and potency of the gastroprotective effect of *L. acanthodes* appears to be higher than the other 3 plants. Omeprazole has completely prevented indomethacin-induced gastric ulcers, while the gastro-protective effects of the extracts are partial compared to omeprazole. It seems that the extracts are less effective than omeprazole in protecting against the gastric ulcers. This is the first report about the gastro-protective effects of *V. arctostaphylos, B. thunbergii* var. atropurpurea, *L. acanthodes* and Iranian *E. angustifolia*. It is worth mentioning, however, that in a study, fruits of Turkish *E. angustifolia* had protective effect against ethanol-induced gastric ulcers in mouse [30]. Further, *Vaccinium myrtillus* L. is a plant closely related to *V. arctostaphylos*. *V. myrtillus* anthocyanins had anti-ulcerogenic activity in various murine models of gastric ulcerogenesis possibly through their anti-oxidative effect [31, 32]. This study concurs with the gastrointestinal effects of *E. angustifolia* and *L. acanthodes* noted in the Iranian traditional medicine and the afore-mentioned studies [30 – 32]. The active constituents and mechanisms involved in the gastro-protective effects of the extracts were not determined in the present study. In terms of the mechanism of NSAID-induced gastric damage, prostaglandin deficiency is of prime importance to the gastric ulcerogenic response to NSAIDs, yet it has proven to be more complicated than expected and involves multiple closely interacting elements including hypermotility, neutrophils, free radicals and so on [33]. Of note, indomethacin induced gastric ulcer is a multi-factorial process where reactive oxygen species (ROS) play a vital role in gastric damage either by its direct oxidative action or through apoptotic cell death. Among various ROS, H_2O_2 can act as a signal transduction messenger to activate transcription factors NFκB (Nuclear Factor Kappa B) and AP-1 (Activator protein 1) for gene expression of various inflammatory cytokines and proteases to cause cell damage. In fact, involvement of TNF-α (Tumor Necrosis Factor Alpha) and matrix metalloproteinases has been evident in indomethacin-induced gastric hypermotility and increased microvascular injury also cause ischemia to generate ROS through the mitochondrial electron transport chain. Mitochondria from gastric mucosal cells contain a highly active peroxidase to scavenge H_2O_2 and protect the cells from ROS-mediated oxidative damage. Indomethacin significantly inactivates the gastric peroxidase to generate H_2O_2 and H_2O_2-derived $^{\bullet}OH$. Indomethacin significantly increases endogenous $^{\bullet}OH$ to cause oxidative damage by increased lipid peroxidation and thiol depletion [26]. Moreover, the fact that proton pump inhibitors like omeprazole have pronounced antioxidant properties and scavenge hydroxyl radicals may explain the anti-ulcer effect of omeprazole [34, 35]. As yet the constituents of *B. thunbergii* var. atropurpurea have not been identified in

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any study. Consequently, it can not be stated which constituents of this plant possibly mediate its anti-ulcerogenic effect. It should also be noted that *V. arctostaphylos*, *E. angustifolia* and *L. acanthodes* contain flavonoids, which have anti-oxidative property [18, 19, 36, 37]. Thus, the anti-oxidative property of these plants' flavonoids may have a role in their gastro-protective effects. However, action on any of the parameters underlying the NSAID-induced gastric ulcerogenesis [33] may be responsible for the anti-ulcerogenic effects of the plants in the present study.

Conclusion

Considering the anti-ulcerogenic effect of these plants, further research into the bioactives and mechanisms mediating their gastro-protective effects and conduction of human clinical trials regarding their safety and efficacy in preventing NSAID-induced gastric ulcers are recommended.

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