Effects of *Aloe vera, Camellia sinensis, Hibiscus sabdariffa* and *Sophora alopecuroides* in Rat Model of Indomethacin-Induced Gastric Ulcer

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Abstract

Background: Evidences show that *Aloe vera* (*A. vera*), *Camellia sinensis, Hibiscus sabdariffa* and *Sophora alopecuroides* may prevent gastric ulcer induced by non-steroidal anti-inflammatory drugs (NSAIDs).

Objective: To examine the protective potential of these plants against indomethacin-induced gastric ulcer in the rat.

Methods: Saline, *A. vera* leaf gel powder and 70% alcoholic extracts of the other 3 plants (100, 400, 800 mg/kg) and omeprazole (30 mg/kg) were gavaged to the groups of 10 animals for 4 consecutive days. Gastric ulcers were induced by the onetime gavage of indomethacin (30 mg/kg). On the fifth day, each group was pretreated with physiological saline as control, extract (100, 400 or 800 mg/kg) or omeprazole (30 mg/kg) 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 doses of the *A. vera* leaf gel powder and extracts reduced the ulcer index significantly compared to the control group ($p < 0.05$).

Conclusion: These plants have protective effect against NSAID-induced gastric ulcer in the rat. The *A. vera* leaf gel seems noticeably more effective than the other 3 plants in this respect.

Keywords: Anti-ulcer, Non-steroidal anti-inflammatory drug, Rat

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are an extremely widely prescribed group of drugs that are mainly used for their analgesic effect [1]. On average, 20% of patients on long-term NSAIDs will have a gastric ulcer [2]. Double-dose (e.g. famotidine 40 mg twice daily) but not single-dose H_2 receptor antagonists are effective in reducing the risk of NSAID-induced gastric ulcers. However, they are inferior to proton pump inhibitors (PPIs) (e.g. omeprazole) in this respect [3]. Misoprostol, a prostaglandin analogue, does provide some protection against the development of NSAID-related gastric ulcer. Misoprostol may reduce by half the incidence of NSAID-related gastric ulcer. The misoprostol adverse effects (stomach cramping: 7-20%; diarrhea: 13-40%) limit its use [4]. PPIs are more effective than the other classes of gastroprotective agents. It is now recommended that all patients over 45 years prescribed an NSAID, whether COX-2 selective or not, also receive a PPI [5]. Notably, taking PPIs for more than 14 days can cause adverse effects including osteoporosis, fracture, hypomagnesemia, *Clostridium difficile* infections and pneumonia [6]. Thus, more effective and safer agents are needed for prevention of NSAID-related gastric ulcers [7].

Plant kingdom is a rich source of novel gastroprotective agents (8-10). *Aloe vera* (*A. vera*) leaf gel has antioxidant, gastric acid secretion inhibitory, cytoprotective and gastric mucus secretion increasing effects [11]. Epigallocatechin-3-gallate is the major active ingredient of *Camellia sinensis* (*C. sinensis*) (green tea) leaf which has gastric acid secretion inhibitory, anti-inflammatory and antioxidant effects [12]. *Hibiscus sabdariffa* (*H. sabdariffa*) calyces have large amounts of anthocyanins with antioxidant and cytoprotective effects [13]. The *S. alopecuroides* (*S. alopecuroides*) seeds apart from being antioxidant and anti-inflammatory are used for the prevention and treatment of gastrointestinal diseases [14]. NSAIDs may cause gastric ulcers through several mechanisms including increased free radicals and as a result oxidative stress, decreased synthesis of prostaglandins, gastric hypermotility and increased neutrophil infiltration [15]. Thus, these plants may theoretically be able to prevent NSAID-induced gastric ulcer. The plants' capacity to prevent NSAID-induced gastric ulcer has not been evaluated so far. In the present study, the protective effects of the plants against indomethacin-induced gastric ulcers in rat and some of the plants phytochemical features were examined.

Materials and methods

Plant materials

The *A. vera* leaf gel powder, *C. sinensis* leaves and *H. sabdariffa* calyces were obtained from the Institute of Medicinal Plants (ACECR). The seeds of *S. alopecuroides* were collected at fruiting stages from the Kerman province of Iran. The plants except *A. vera* leaf gel powder were air-dried at room temperature. Samples were authenticated by a botanist and voucher specimens were preserved in the Central Herbarium of the Institute of Medicinal Plants.

Phytochemical studies of the *A. vera* leaf gel powder and extracts

Following preparation of the extracts for spectrophotometric analyses [16], 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay using DPPH from Fluka (USA) [17] and determination of total flavonoid (as milligrams of rutin equivalents per gram of the extract) [18] and phenolic (as milligrams of gallic acid per gram of the extract) [19] contents were performed. Total anthocyanin content of the *H. sabdariffa* was also determined [20]. DPPH radical scavenging assay was performed for the *A. vera* leaf gel powder. Further, aloin was quantified in the *A. vera* leaf gel powder by HPLC [21].

Preparation of plant extracts

The dried plant powders (100 g) except *A. vera* leaf gel powder were 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 [22]. The extraction yields of *S. alopecuroides, C. sinensis* and *H. sabdariffa* were 25%, 20% and 17% respectively.

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 200-250 g from our own breeding colony were used. Animals were maintained under standard environmental conditions and had access to standard rodent feed and water.

Animal Experiment protocol:

Rats were deprived of food for 24 h 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, *A. vera* leaf gel powder and 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, *A. vera* leaf gel powder or each extract (100, 400 or 800 mg/kg, p.o.) or omeprazole (30 mg/kg, p.o.) 30 min before the indomethacin administration. All drugs and extracts were dissolved and administered in physiological saline in a volume of 5 ml/kg [23, 24] (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 [23, 24] (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

Phytochemical studies of the *A. vera* leaf gel powder and extracts

The DPPH scavenging IC_{50} of ascorbic acid was 5.819 ± 0.009 µg/mL. The aloin content and DPPH scavenging IC50 of the *A. vera* leaf gel powder were undetectable and 485 ± 0.006 µg/mL respectively. The total flavonoid and phenolic contents and DPPH scavenging IC_{50} of *C. sinensis* were 12.92 ± 1.3, 526.71 ± 18.05 and 360.75 ± 0.07 µg/mL respectively. The total flavonoid, phenolic and anthocyanin contents and DPPH scavenging IC_{50} of *H. sabdariffa* were 3.61 ± 0.84, 110.48 ± 3.89, 1.3% and 172.30 ± 0.03 µg/mL respectively. The total flavonoid and phenolic contents and DPPH scavenging IC_{50} of *S. alopecuroides* were 5.22 ± 0.67, 217.48 ± 12.44 and 472.28 ± 0.09 µg/mL respectively. The values represent mean of three measurements ± standard deviation.

Animal Experiment protocol:

A. vera leaf gel powder and each extract at all doses and omeprazole (30 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 the *A. vera* leaf gel powder, plant extracts and omeprazole on the gastric ulcer index in rats treated with indomethacin (30 mg/kg). *P*-values below 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	-	22.9 ± 4.9	
Omeprazole	30	0 ± 0	<0.001
A. vera	100	13.0 ± 3.5	<0.001
A. vera	400	7.7 ± 2.7	<0.001
A. vera	800	2.6 ± 1.9	<0.001
C. sinensis	100	17.4 ± 2.6	0.011
C. sinensis	400	17.2 ± 3.1	0.008
C. sinensis	800	10.3 ± 3.9	<0.001
H. sabdariffa	100	15.5 ± 4.5	0.001
H. sabdariffa	400	12.7 ± 3.3	<0.001
H. sabdariffa	800	8.2 ± 3.4	<0.001
S. alopecuroides	100	16.3 ± 3.2	0.002
S. alopecuroides	400	11.1 ± 3.9	<0.001
S. alopecuroides	800	10.7 ± 2.8	<0.001

Discussion

Considering the results, *A. vera* leaf gel had antioxidant activity, but it did not contain aloin. *A. vera* leaf gel should not contain aloin, as it is exclusively concentrated in the leaf skin [25]. Moreover, the extracts flavonoid and phenol contents and antioxidant activity as well as the anthocyanin content of *H. sabdariffa* were determined. The results also suggest that *A. vera*, *C. sinensis*, *H. sabdariffa* and *S. alopecuroides* prevent NSAID-induced gastric ulcer in the rat. Notably, the *A. vera* leaf gel seems considerably more effective than the other 3 plants in this respect. The gastroprotective potential of *S. alopecuroides* has not been examined prior to the present study. The results concur with the previous reports regarding the gastroprotective effects of *A. vera*, tea catechin and anthocyanins. *Aloe vera* gel protected against ethanol-induced gastric damage at least partly by decreasing mRNA expression of inducible nitric oxide synthase, neuronal nitric oxide synthase and matrix metalloproteinase-9 [26]. Tea catechin prevented ethanol-induced gastric ulcer by antioxidant and gastric mucus-increasing actions [27]. The antioxidant effect of an anthocyanin called cyanidin 3-glucoside was involved in its protective effect against ethanol-induced gastric ulcer in rat [28]. The protective effect of *Vaccinium myrtillus* anthocyanins against hydrochloric acid/ethanol-induced gastric ulcer may be partially through the antiperoxidative effect of anthocyanins [29]. The *Rubus coreanus* fruit anthocyanins prevented naproxen-induced gastric ulcer in rat by activation of matrix metalloproteinase-2 and attenuation of the activity of the proinflammatory molecules such as tumor necrosis factor-α and interleukin-1β [30]. Gastric ulceration has a multifactorial pathogenesis. Ulcers may occur with hypersecretion of hydrochloric acid and pepsin causing an imbalance between gastric luminal factors and degradation in the defensive function of the gastric mucosal barrier. Mucosal defenses include mucus, secretion of bicarbonate, mucus blood flow and epithelial cell defense. When acid and pepsin invade a weakened area of the mucosal barrier, histamine is released. Histamine will stimulate parietal cells to secrete more acid. With the continuation of this vicious cycle, erosion occurs to form the ulcer [31]. Thus, *A. vera*, *C. sinensis*, *H. sabdariffa* and *S. alopecuroides* may prevent NSAID-induced gastric ulcer by enhancement of the gastric mucosal defenses or inhibition of the gastric acid secretion. The antiulcer activity of the *A. vera* leaf gel has been attributed to several possible mechanisms including anti-inflammatory, healing and mucus stimulatory effects and regulation of gastric secretions [32]. The components of the *A. vera* gel mediating its gastroprotective effect are unknown. The *A. vera* gel consists of carbohydrates, glycoproteins, enzymes, amino acids, vitamins and minerals. It is believed that the constituents of the *A. vera* gel act synergistically in its beneficial effects [25]. Phenols and flavonoids (e.g. anthocyanins in *H. sabdariffa* and catechin in *C. sinensis*), and antioxidant activity may be involved in the plants antiulcer effect [8-10, 15]. The antioxidant and cytoprotective effects of *H. sabdariffa* anthocyanins may be implicated in its gastroprotective effect [13]. It is also noteworthy that the principal constituent of *C. sinensis* epigallocatechin-3-gallate with its gastric acid inhibitory, anti-inflammatory and antioxidant effects may be involved in the gastroprotective effect of *C. sinensis* [12]. Our unpublished data shows that matrine is the main component of the *S. alopecuroides* growing in Iran. Matrine may have antiulcer activity through increasing gastric mucin (the chief constituent of mucus) secretion [10].

Moreover, antioxidant activity of the *S. alopecuroides* phenols and flavonoids may also have some role in its antiulcer effect. Considering the remarkable antiulcer effect of the *A. vera* leaf gel in the current study, conduction of clinical trials testing its efficacy in the prevention of NSAID-induced gastric ulcer is recommended. Moreover, further research to determine the anti-ulcer mechanisms and constituents of the plants is justified.

Conclusion

A. vera, *C. sinensis*, *H. sabdariffa* and *S. alopecuroides* have protective effect against NSAID-induced gastric ulcer in the rat. *A. vera* leaf gel seems noticeably more effective than the other 3 plants.

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