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Protective effects of *Artemisia campestris* extract against gastric acid reflux-induced esophageal mucosa injuries

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ABSTRACT

Artemisia campestris L. has been widely used in alternative medicine to treat digestive system diseases, particularly gastroesophageal disorders. In the present investigation, we studied the putative protective effect of *Artemisia campestris* aqueous extract (ACAE) against gastro-esophageal reflux (GER)-induced esophagitis in rats. The experimental ophagitis was induced by the ligation of the pylorus as well as the junction between the forestomach and the corpus. We firstly found that ACAE administration at 100, 200 and 400 mg/kg, *b.w., p.o.* significantly protected GER-induced macroscopic and histological injuries in the esophagus tissue. Our extract also counteracted GER-induced esophagus lipoperoxidation, restored the depletion of antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) as well as thiol groups levels. Furthermore, we showed that acute GER provoked an increase in esophagus mucosa hydrogen peroxide (H_2O_2), free iron and calcium levels, whereas ACAE treatment reversed all GER-induced intracellular mediators' disturbances. In conclusion, we suggested that ACAE had potent protective effects against esophagitis due, in part, to its antioxidant properties as well as its opposite effect on some intracellular mediators.

1. Introduction

Gastro-Esophageal Reflux (GER) designates the permanent or intermittent reflux of gastric contents to the esophagus through the esophageal hiatus [1,2]. The physiological GER that exists in all subjects, essentially after meals, is not accompanied by symptoms or esophageal mucosal lesions [3], whereas pathological GER is characterized by the presence of those symptoms and/or lesions known as esophagitis. The reflux of the gastric contents is then frequent and/or prolonged [4,5]. The most aggressive elements are the concentrations of H^+ ions and pepsin. However, the harmful effects depend not only on the concentration, but also on the exposure duration of esophageal mucosa [3,4]. The reflux of pancreatic enzyme probably has no relative importance, except after total gastrectomy or in case of achlorhydria [2]. Several biochemical mechanisms have been proposed to be involved in the pathophysiology of esophagitis, the most important of which is oxidative stress which is a cellular imbalance between the antioxidant and the prooxidant systems in favor of high production of reactive oxygen species (ROS) [6,7]. The oxidative stress may later have different cellular sources and the most important one is mitochondria [8]. The superoxide radicals produced by the NADPH oxidase route can then give rise, by successive reductions to other species, such as hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^•$) which are characterized by high reactivity [7,9]. Several investigations have reported that oxidative lesions are involved in numerous digestive diseases such as ulcerative colitis [10], gastric ulcer [11] and esophagitis [12,13].

Special importance is given to medicinal plants which act on the esophageal and gastric mucosa to treat esophagitis [14]. *Artemisia campestris* is a perennial herb belonging to Compositae genus and Asteraceae family, from 30 to 80 cm in height. This plant has very small flowerheads, narrow (1–1.5 mm), ovoid or conical, with scarous involucre and it contains only 3–8 yellow flowers bordered of red, and with peduncle provided with whitish to brownish hairs. It is distributed in Europe, Siberia, Asia Minor and Africa, and it grows particularly in the steppe and desert. Flowering takes place from August to October [15]. From the clinical and experimental

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Table 1
Characterisation of phenolic compounds of ACAE by HPLC-DAD-ESI–MS/MS.

compound	t_R (min)	UV λ_{max} (nm)	$[M-H]^-$ (m/z)	Fragment ion (m/z)	composition (%)	Tentative identification
1	16.5	242, 298 (sh), 325	353	191; 179	4.3	chlorogenic acid
2	17.2	242, 296 (sh), 323	179		2.15	caffeic acid
3	21.4	259, 357	477	315	5.37	isorhamnetin hexoside
4	23.9	241, 298 (sh), 327	515	353, 191, 179	0.24	3,4-dicaffeoylquinic acid
5	24.1	241, 298 (sh), 329	515	353, 191, 179	0.78	3,5-dicaffeoylquinic acid
6	24.6	241, 298 (sh), 329	515	353, 191, 179	0.93	4,5-dicaffeoylquinic acid
7	25.1	265, 349	477	301	0.05	quercetin-3-*O*-glucuronide
8	25.7	267, 337	431	269	6.42	apigenin-7-*O*-*hexo*side
9	27.7	267, 339	577	517, 401, 269	15.52	apigenin-6-*C*-glucuronide-8-*C*-pentoside
10	30.03	253, 270 (sh), 347	285		2.74	kaempferol
11	32.88	267, 337	269		62.42	apigenin

studies performed on *Artemisia campestris*, it seems that most of its beneficial effects are related to its antioxidant properties which are mainly due to its ability to scavenge reactive oxygen species and/or inhibit lipid peroxidation [16,17]. For this reason, the Atemisia extracts are known to exhibit many beneficial health effects such as renoprotective [18], antidiabetic [19] and gastroprotective [11] effects.

All these pharmacological effects are related to the wide range of biologically active compounds identified in the artemisia, particularly phenolic compounds such as isochlorogenic acid and 3,5-dicaffeoylquinic acid [20], rhamnetin, quercetin and Petunidin-3-*O*-acetyl glucoside [21], as well as kaempferol and apigenin [14]. The literature shows that most of these compounds had potent pharmacological properties. In fact, the isochlorogenic acid is known for its hepatoprotective and antiinflammatory effects [22]. 3,5-dicaffeoylquinic acid has been shown to mitigate inflammatory mediator production [23]. Moreover, Zhong et al. [24] reported that kaempferol and apigenin exhibited a strong ROS scavenging activity. In addition, they have been shown to alleviate intestinal inflammation [25] as well as gastric ulcer [26]. Accordingly, this leads us to think of the effect of ACAE on esophageal injuries. In view of these biological activities, we sought to investigate on how artemisia extract could prevent the development of esophageal injuries in rat models. Currently, some of the experimental animal models are used to study the pathogenesis and pathophysiology of the esophagitis and gastroesophageal reflux. A model of GER in rats is one of the common models in the esophagus diseases research and it resembles human esophagitis in histology, such as the infiltration of inflammatory cells and the appearance of edematous zones in the esophageal mucosa [3].

To test our hypothesis, the present study was undertaken to determine the protective effect of an aqueous extract of *A. campestris* against gastric acid reflux-induced esophageal mucosal injury. We also studied the implication of oxidative stress and some intracellular mediators in such esophageal protection.

2. Materials and methods

2.1. Chemicals

Butylated hydroxytoluene (BHT), bovine catalase, Epinephrine, trichloroacetic acid and 2-Thio-barbituric acid (TBA) were from Sigma chemicals Co (Germany). All other chemicals used were of analytical grade.

2.2. Sampling and Artemisia campestris aqueous extract (ACAE) preparation

Artemisia campestris L. was collected during March 2014 from Béja governorate (Tunisia). The artemisia leaves (10%, weight/volume) were dried in an incubator at 40 °C during 72 h and then ground in an electric mixer. The plant powder was subsequently dissolved in distilled water and incubated at room temperature for 24 h under magnetic stirring. The sample was then centrifuged at 10.000*g* for 10 min and the supernatant was lyophilized, aliquoted and stored at −80 °C until use. The chemical composition of ACAE (Table 1 and Fig. 1) was determined according to Sebai et al. [14].

2.3. Animals

Adult male *Wistar* rats (200–220 g, 15 weeks old) were provided by Pasteur Institute of Tunis and used in accordance with the Tunis University ethics committee for the use and care of Laboratory animals and in accordance with the NIH recommendations [27]. They were provided with food and water *ad libitum* and maintained at room temperature of 22–25 °C.

2.4. Experimental model

The rats were divided into six groups of 10 animals each. GER was induced to all used animals except control group. Following light ether anesthesia, rats were laparotomised to ligate the junction between the forestomach and the corpus as well as the pylorus [28] and they were then deprived of food and water. However, Groups I and II served respectively as normal and GER controls and had per orally (*p.o.*) a physiological solution. Groups III, IV, and V were treated with different doses of ACAE (100, 200 and 400 mg/kg, *b.w., p.o.*), while Group VI received famotidine (20 mg/kg, *b.w., p.o.*). Six hours later, animals were autopsied; their esophageal portion of the digestive tract was rapidly excised, cleaned, macroscopically examined and homogenized in phosphate buffer saline to measure the biochemical parameters such as MDA levels, H_2O_2, calcium, free iron, protein and −SH groups, as well as antioxidant enzyme activities.

2.5. Esophagitis severity evaluation

The severity of esophagitis was macroscopically scored, using an ulcer index. The following scale was used: 0, no injury; 1, erosion of mucosal epithelium; 2, the length of hemorrhagic ulcer area520 mm; 3, the length of hemorrhagic ulcer area 20–30 mm; 4, the length of hemorrhagic ulcer area 30–40 mm; 5, the length of hemorrhagic ulcer area440 mm or perforation [12,13].

2.6. Histopathological analysis

Immediately after sacrifice, the esophageal segments were harvested and washed with ice-cold saline. Tissue fragments were then fixed in a 10% neutral buffered formalin solution, embedded in paraffin, and used for histopathological examination. From this, 5 μm thick sections were cut, deparaffinized, hydrated, and stained with hematoxylin plus eosin (H + E). Tissue preparations

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