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The hydro-alcoholic extracts of Sardinian wild thistles (*Onopordum* spp.) inhibit TNFα-induced IL-8 secretion and NF-κB pathway in human gastric epithelial AGS cells

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Chlorogenic acid (PubChem ID: 1794427)
1,3 Dicaffeoylquinic acid (PubChem ID: 6474640)
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ABSTRACT

Ethnopharmacological relevance: Thistles species (Family: Compositae) are traditionally used in the Mediterranean area, particularly in Sardinia. They are usually gathered from the wild and used for both food and therapeutic purposes, including gastrointestinal disorders.
Aim of the study: This work aims to evaluate the anti-inflammatory activity of eight wild thistles from Sardinia, in an in vitro model of gastric inflammation, and to identify the major active compounds in the extracts.
Materials and methods: The hydro-alcoholic extract of the aerial part of each species was prepared. After the induction of inflammation by the addition of tumor necrosis factor-α (TNFα) (10 ng/mL), AGS cells were treated with extracts/pure compounds under study. The inhibition of interleukin-8 (IL-8) release, IL-8 and NF-κB promoter activities and NF-κB nuclear translocation were evaluated. Extracts main components were identified by HPLC-PDA-MS/MS.
Results: Only *Onopordum horridum* Viv. and *Onopordum illyricum* L. hydro-alcoholic extracts reduced, in a concentration-dependent fashion, the IL-8 release and promoter activity in human gastric epithelial cells AGS. The effect was partially due to the NF-κB pathway impairment. *Onopordum* hydro-alcoholic extracts were also chemically profiled, and caffeoylquinic acid derivatives were the main compounds identified in the extract. Further investigations showed that 3,5 dicaffeoylquinic acid highly inhibited IL-8 secretion in AGS cells (IC_{50} 0.65 μM), thus suggesting that this compound contributed, at least in part, to the anti-inflammatory activity elicited by *O. illyricum* extracts.
Conclusions: Our results suggest that *Onopordum* species may exert beneficial effects against gastric inflammatory diseases. Thus, these wild plants deserve further investigations as preventive or co-adjuvant agents in gastric diseases.

1. Introduction

The aetiopathogenesis of gastritis, an inflammatory state of gastric mucosa, is mostly due to the presence of *Helicobacter pylori* (*H. pylori*), a Gram-negative pathogen affecting humans and classified as Type 1 carcinogen by WHO. (Brown, 2000; Israel and Peek, 2001).

Abbreviations: IL-8, Interleukin 8; NF-κB, Nuclear factor κB; WHO, World Health Organization; TNFα, Tumor necrosis factor alpha;; IL-1β, Interleukin 1β; VCAM-1, Vascular cell adhesion protein 1; ICAM-1, Intercellular Adhesion Molecule 1; TNF-R1, Tumor necrosis factor receptor 1;; IL-4, Interleukin 4; IFN-γ, Interferon γ; STAT3, Signal transducer and activator of transcription 3; Nrf2, Nuclear factor (erythroid-derived 2)-like 2; COX-1, Cyclooxygenase-1; COX-2, Cyclooxygenase-2; NO, Nitric oxide; 5-LOX, 5-lipoxygenase; AGS, Human gastric adenocarcinoma AGS cells; DMEM F12, Dulbecco's Modified Eagle Medium F12;; MTT, 3,4,5-dimethylthiazol-2-yl-2–5-diphenyltetrazolium bromide;; ELISA, Enzyme-linked immunosorbent assay; FBS, Foetal bovine serum; s.d., Standard deviation; EGCG, Epigallocatechin-3-gallate; DMSO, Dimethyl sulfoxide; LUC, Luciferase; HPLC, High-performance liquid chromatography; HRP, Horseradish peroxidase; TMB, 3,31,5,51 -tetramethylbenzidine; LDH, Lactate dehydrogenase; ANOVA, Analysis of Variance; IC_{50}, Half maximal inhibitory concentration; THP-1, Human monocytic leukaemia derived cells; PDA, Photodiode Array Detector;; MS/MS, Tandem mass spectrometry; UV, Ultraviolet; MRM, Multiple reaction monitoring

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Many pro-inflammatory molecules (e.g. TNFα, IL-8, NF-κB), released during gastritis, can be considered as potential therapeutic targets to prevent or treat *H. pylori*-induced gastric diseases (Bodger and Crabtree, 1998; Crabtree et al., 1993; Israel and Peek, 2001; Martin and Wallace, 2006; Zaidi et al., 2012). Emerging resistance to antibiotics and adverse effects of conventional drugs lead to search for new therapeutic strategies to counteract the inflammatory processes exerted by *H. pylori* infection (Zaidi et al., 2012).

Botanicals, from both wild or cultivated plants, are widely used all over the world, for nutritional and health purposes, as different types of products, including herbal medicinal products, food, food supplements, and functional foods.

Wild plants, traditionally used by the native populations, recently received attention for their therapeutic properties and the high content of fibres, vitamins, minerals, and polyphenols (Licata et al., 2016; Tuttolomondo et al., 2014). Some of them are traditionally used to treat gastrointestinal disorders such as dyspepsia, constipation, diarrhoea, gastritis, colitis (Atzei, 2003; Tuttolomondo et al., 2014) and have shown beneficial effects against gastritis (Colombo et al., 2013; Di Lorenzo et al., 2013; Sangiovanni et al., 2015).

Sardinia boasts a well-established culture on the traditional uses of wild plants (Atzei, 2003; Lancioni et al., 2007; Maxia et al., 2013). The so-called thistles mostly refer to Compositae species and are traditionally consumed and used for therapeutic purposes by Sardinian inhabitants (Atzei, 2003; Guarrera and Savo, 2016; Lancioni et al., 2007; Signorini et al., 2009). The aim of the present study was to investigate the anti-inflammatory activity of eight wild thistles species from Sardinia in a cell model of gastric inflammation. The species under study belong to the *Cardueae* Cass. Tribe (Family: Compositae) and to four genera: *Carduus* L. (*C. argyroa* Biv., *C. cephalanthus* Viv., *C. pycnocephalus* L., *C. nutans* subsp *macrocephalus* (Desf.) Nyman), *Onopordum* L. (*O. illyricum* L., *O. horridum* Viv.), *Silybum* L. (*S. marianum* (L.) Gaertn.), and *Ptilostemon* Cass. (*P. casabonae* (L.) Greuter). All these plants are traditionally used for food and medicinal purposes, also against gastrointestinal disorders (Atzei, 2003; Guarrera and Savo, 2016; Lancioni et al., 2007; Licata et al., 2016; Rinchen and Pant, 2014; Signorini et al., 2009).

The in vivo activity of *C. pycnocephalus* has been previously reported towards the rat paw oedema inflammation, while the in vitro inhibition of NF-κB pathway, IL-1β, TNFα, and the adhesion molecules VCAM-1, ICAM-1 and E-selectin release has been described for *S. marianum* extracts, demonstrating that the effects are mostly due to the presence of silymarin components (Al-Shammari et al., 2015; Giorgi et al., 2012; Kang et al., 2003; Manna et al., 1999). *In vivo* studies have shown the ability of *S. marianum* to inhibit TNF-R1, TNFα, IL-4 and IFN-γ expression (He et al., 2004; Schumann et al., 2003). Moreover, the in vitro NF-κB, STAT3 inhibitory activity and the Nrf2 activation were evaluated for six sesquiterpenes from *O. illyricum* (Formisano et al., 2017). *O. acanthium* inhibited COX-2 and NF-κB gene expression, NO production and 5-LOX, COX-1 and COX-2 enzymes activity in THP-1 cells (Lajter et al., 2015). However, no studies investigating the in vitro anti-inflammatory activity of the thistles species under study in human gastric epithelial cells have been reported so far.

A preliminary screening of the selected thistles hydro-alcoholic extracts was assessed to investigate their inhibitory effect on IL-8 released by human gastric epithelial cells (AGS). To elucidate the underlying molecular mechanisms, the extracts showing remarkable activity were tested on the NF-κB pathway. The extracts were also chemically profiled to identify the compounds responsible for the observed biological activity.

2. Materials and methods

2.1. Materials

Dulbecco's Modified Eagle's Medium/F12 (DMEM)/F12 (1:1), penicillin, streptomycin, L-glutamine, sodium pyruvate and trypsin-EDTA were from Gibco (Life Technologies Italia, Monza, Italy). DMEM, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were from Sigma Aldrich (Milan, Italy). All reagents used for analytical determinations and biological assays were HPLC grade. Human TNFα and Human IL-8 Elisa Development Kit were from Peprotech Inc. (London, UK). Foetal bovine serum (FBS), and disposable material for cell culture were purchased by Euroclone (Euroclone S.p.A., Pero-Milan, Italy). Human adenocarcinoma cells (AGS, CRL-1739) were purchased from LGC Standard S.r.l., Milano, Italy. 1,5 dicaffeoylquinic acid (purity > 99.4%), 3,5 dicaffeoylquinic acid (purity > 98.2%), 1,3 dicaffeoylquinic acid (purity > 99.36%), were purchased from Phytolab (Vestenbergsgreuth, Germany), chlorogenic acid (purity > 99.6%) was from Sequoia Research Products (Pangbourne, UK), epigallocatechin-3-*O*-gallate (purity > 99%, EGCG), and DMSO were from Sigma-Aldrich (St Louis, USA). The plasmid NF-κB-LUC containing the luciferase gene under the control of three κB sites was a gift of Dr N. Marx (Department of Internal Medicine-Cardiology, University of Ulm, Germany). Native IL-8-LUC promoter was kindly provided by Dr T. Shimohata (Department of Preventive Environment and Nutrition, University of Tokushima Graduate School, Japan). Britelite™ plus was from Perkin Elmer (Monza, Italy). HPLC-grade acetonitrile and methanol were purchased from Sigma (Bellefonte, USA). De-ionized water (18.2 MΩ cm) was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). Formic acid (purity > 98%) was obtained from Sigma (Bellefonte, USA).

Table 1
Localities and dates of collection, local name (Atzei, 2003; Congia, 1998), voucher numbers, and No. of individuals of the eight *Cardueae* species.

Species	Local name	Localities and dates of collection	Coordinates	Voucher specimen	No. of individuals
Carduus argyroa	Càdru, Cardu	Decimomannu, 27 May 2015	39°17'47.96"N – 8°58'14.95"E	CAG-803	10
Carduus cephalanthus	Cardu	Capo Testa, 12 June 2015	41°14'33.80"N –9°8′49.25"E	CAG-807	6
Carduus nutans* subsp. *macrocephalus	Gàrdu pissiaiòlu	Gennargentu, 18 June 2015	39°57'35.77"N – 9°19'12.46"E	CAG-802	13
Carduus pycnocephalus	Ardu pissiarolu, baldu aininu, cardu pisciau	Monte dei Sette Fratelli, 21 May 2015	39°20'43.60"N – 9°17'43.74"E	CAG-805	10
***Onopordum illyricum* L.**	Ardu nieddu, cardu santu, cardu molentinu	Monte dei Sette Fratelli, 21 May 2015	39°20'43.60"N – 9°17'43.74"E	CAG-798	10
***Onopordum horridum* Viv.**	Aldu nieddu	Gennargentu, 18 June 2015	39°53'54.9"N –9°26'27.9"E	CAG-186/14	10
***Ptilostemon casabonae* (L.) Greuter**	Caldu drummitu, cardu de Casteddu	Gennargentu, 18 June 2015	39°53'54.9"N –9°26'27.9"E	CAG-796	10
***Silybum marianum* (L.) Gaertn**	Ardu biancu, cardu tufu, cima de cardu	Uta, 27 May 2015	39°17'48.0"N –8°58'14.9" E	CAG-801	10