



Pharmacological characterization of *Cirsium ligulare* Boiss. (Asteraceae) herb decoction

Farmakološka karakterizacija dekokta herbe *Cirsium ligulare* Boiss. (Asteraceae)

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Abstract

Background/Aim. Data on phytochemical and pharmacological investigations of genus *Cirsium* Mill. (Asteraceae) are scarce. Some data suggest that decoctions or infusions prepared from these plants are used in folk medicine as tonics, particularly in inflammatory, liver and stomach diseases. So far there have been no pharmacological investigations related to *Cirsium ligulare* (*C. ligulare*) Boiss. Accordingly, the aim of this study was to estimate antioxidative, anti-inflammatory and gastroprotective activities of aqueous extracts of *C. ligulare* herb prepared as 5% and 10% decoctions. **Methods.** Antioxidative activity was determined using the method of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. Investigations of anti-inflammatory (a model of systemic inflammatory response induced by endotoxin of *Escherichia coli* and carrageenan-induced rat paw oedema model for local inflammatory response), as well as gastroprotective effects (a model of stress-ulcer induced by absolute ethanol), were conducted in adult female Wistar rats that were given the aqueous extracts of *C. ligulare* herb *per os*. Indomethacin and ranitidine were used as reference drugs for evaluation of local anti-inflammatory and gastroprotective effects, respectively. **Results.** The results demonstrated that aqueous extracts of *C. ligulare* herb produced strong antioxidative activity, diminished body weight loss induced by endotoxin, significantly reduced carrageenan-induced paw oedema, and prevented the ulcerogenic action of absolute ethanol. Both anti-inflammatory and gastroprotective activities of the extract tested were comparable to those of the reference drugs. **Conclusion.** Presented results justify the traditional use of *C. ligulare* herb decoctions and further phytochemical and pharmacological investigations are warranted.

Key words: cirsium; asteraceae; pharmacologic actions; herbal medicine; rats, wistar.

Apstrakt

Uvod/Cilj. Biljke roda *Cirsium* su hemijski i farmakološki nedovoljno ispitane. Tradicionalno se dekokti ili infuzi ovih biljaka koriste kao tonici, naročito kod zapaljenjskih procesa, kao i kod bolesti jetre i želuca. Vrsta *Cirsium ligulare* (*C. ligulare*) Boiss. do sada nije farmakološki ispitivana. Stoga je cilj ovog istraživanja bio da se proceni antioksidantna, antiinflamatorna i gastroprotektivna aktivnost vodenog ekstrakta herbe *C. ligulare* izrađenog kao 5% i 10% dekokt. **Metode.** Ispitivanje antioksidantne aktivnosti vršeno je testom neutralizacije 2,2-diphenyl-1-picrylhydrazyl (DPPH) radikala. Ispitivanje antiinflamatornog (model sistemskog inflamatornog odgovora indukovani endotoksinom bakterije *Escherichia coli* i karageninom-indukovani edem šape pacova kao model lokalne inflamcije), kao i gastroprotektivnog efekta (model ulkusa indukovani apsolutim etanolom), sprovedeno je na odraslim ženka pacova Wistar soja, kojima su *per os* dati vodeni ekstrakti herbe *C. ligulare*. Kao referentni lekovi prilikom ispitivanja lokalnog antiinflamatornog i gastroprotektivnog delovanja, primenjeni su indometacin i ranitidin, redom. **Rezultati.** Rezultati pokazuju da su ispitivani vodeni ekstrakti herbe *C. ligulare* ispoljili jaku antioksidantnu aktivnost, smanjili gubitak telesne mase indukovani endotoksinom, značajno redukovali karageninom indukovani edem šape pacova i spečili ulcerogeni efekat apsolutnog etanola. Antiinflamatorna i gastroprotektivna aktivnost ispitivanih ekstrakata bila je uporediva sa aktivnošću referentnih lekova. **Zaključak.** Dobijeni rezultati u značajnoj meri opravdavaju tradicionalnu primenu dekokta herbe *C. ligulare*, a planiraju se i dodatna hemijska i farmakološka ispitivanja ove droge.

Ključne reči: cirsium; asteraceae; farmakološka dejstva; medicina, biljna; pacovi, wistar.

Introduction

Genus *Cirsium* Mill. (Asteraceae) includes about 120 species widely distributed in Europe, North Africa, West and North Asia, as well as North and Central America¹. According to available data, these species contain flavonoids²⁻⁴, phenolic acids, essential oils, sterols, triterpenes, as well as alkaloids, polyacetylenes, hydrocarbons, aliphatic aldehydes and guaianolides–sesquiterpene lactones^{5,6}. Traditionally, they are used as tonics, antiphlogistics, diuretics, diaphoretics, adstringents and venoactive remedies^{6,7}, as well as anti-hemorrhagic, diuretic and anticancer agents⁴, against a cough and peptic ulcers⁸.

Cirsium ligulare (*C. ligulare*) Boiss. is one of 22 *Cirsium* species present in the Serbian flora⁹. In folk medicine of Serbia and Montenegro, according to our ethnomedicinal experience, decoctions or infusions prepared from its herb are used as tonics, particularly in inflammatory, liver and stomach complaints. However, data on *C. ligulare* research are scarce. Chlorogenic acid and flavonoids (apigenin, apigenin 7-glucoside, kaempferol 3-glucoside and kaempferol 3-rhamnoglucoside) were identified in methanolic extracts of *C. ligulare* leaves and inflorescences². In the essential oil of its inflorescence, beside predominant aliphatic hydrocarbons, small amounts of thymol (2.4%), eugenol (0.6%) and carvacrol (0.3%) were present⁵. To the best of our knowledge, no pharmacological investigations of this species have been conducted.

Thus, the aim of this study was to estimate antioxidative, anti-inflammatory and gastroprotective activities of aqueous extracts of *C. ligulare* herb, prepared in the traditional manner as decoctions.

Methods

Chemicals

Dimethylsulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl, lipopolysaccharides (LPS) from *Escherichia coli* 0128:B8 (Cat. No L3880) and indomethacin were obtained from Sigma Chemical Co. (St. Louis, USA); absolute ethanol (96%, v/v) from Merck (Darmstadt, Germany); L-ascorbic acid from Lachema (Neratovice, Czech Republic); rutin from Roth (Karlsruhe, Germany), and ranitidine from Zdravlje-Actavis Company (Leskovac, Serbia).

Plant material

The herb of *C. ligulare* was collected in summer 2004 from natural habitat in the river Lim valley, close to its spring from Plavsko lake (North-Eastern region of Montenegro). Once harvested, the plant material was dried at room temperature. It was identified by Prof. P. Marin, PhD (Institute of Botany, Faculty of Biology, University of Belgrade, Serbia).

Preparation of decoctions

Prior to extraction, the plant material was reduced to a coarse powder. Decoctions were prepared by boiling 5.0 and 10.0 g of powdered material in 100 mL of distilled water for

5 min to prepare 5 and 10% (w/v) decoctions. After 10 min, the extracts were allowed to cool at room temperature and filtered. Extracted plant material was washed with distilled water and the volume of the prepared decoctions was adjusted to 100 mL using these washings. Decoctions were each time prepared *ex tempore*.

Animals

Adult female Wistar rats, weighing 200–250 g, were used in the experiments in which anti-inflammatory and anti-ulcer activities of decoctions of *C. ligulare* were tested. Before the experiments, the animals were housed in groups of 5 to a plastic cage in standard laboratory condition (room temperature of 22 ± 1 °C, 30% humidity, 12/12 h light/dark cycle) with free access to food and tap water. Animal studies were conducted in accordance with the internationally accepted principles for laboratory animal use and care in the European Community (EEC Directive of 1986; 86/609/EEC) adopted by Ethical Committee of Military Medical Academy, Belgrade.

Antioxidant activity (DPPH radical assay)

Four milliliters of 5% decoction of the drug, prepared as described above, was mixed with 1 mL of 0.5 mM DPPH in methanol, and final volume was adjusted up to 5 mL. The mixture was vigorously shaken and left 30 min in dark. Absorbance was measured at 517 using methanol as blank. One milliliter of 0.5 mM DPPH diluted with 4 mL of methanol was used as a control. Scavenging (SC) of DPPH radical was calculated using the equation:

$$SC(\%) = 100 \times (A_0 - A_s) / A_0 \quad (1)$$

A_0 - the absorbance of the control (containing all reagents except the test compound); A_s - the absorbance of the tested sample.

The SC_{50} value represents the concentration of the sample that caused 50% of DPPH radical scavenging. The relative activity of the samples was compared to those of L-ascorbic acid and rutin¹⁰.

Anti-inflammatory activity

Lipopolysaccharide (LPS)-induced systemic inflammatory response

A systemic inflammatory reaction was induced by s.c. injection of LPS given in a dose of 5 mg/kg. This dose was chosen as a non-lethal one, but sufficient for producing a systemic inflammatory response, on the basis of previously performed experiments in which the LD50 of LPS for this species and this route of administration had been established (approximately 20 mg/kg).

Before the experiments, the animals were divided into six groups of 15 rats in each, and their body mass, as well as the average consumption of food and water were determined. After that, they were given following treatments:

Group 1 – intact controls drinking tap water; Group 2 – animals drinking 5% decoction of *C. ligulare* herb Group 3 –

animals drinking 10% decoction of *C. ligulare* herb; Group 4 – animals injected by 5 mg/kg s.c. of LPS and drinking tap water; Group 5 – animals injected by 5 mg/kg s.c. of LPS and drinking 5% decoction of *C. ligulare* herb; Group 6 – animals injected by 5 mg/kg s.c. of LPS and drinking 10% decoction of *C. ligulare* herb.

The experiment lasted 30 days after injecting LPS and, during this period, tap water or extracts (5 and 10% decoctions of *C. ligulare* herb) were given to animals *ad libitum*. The body mass and consumption of food and water or the extract were measured each day, always at the same time (9 a.m.).

Carrageenan-induced rat paw oedema test

The carrageenan-induced rat paw oedema was used as an experimental model of local inflammatory reaction as reported earlier¹¹.

The experimental groups consisted of 6–8 animals each. In order to estimate an anti-inflammatory activity of an aqueous extract of *C. ligulare* herb, the animals were given 10% decoction of *C. ligulare* herb instead tapping water for seven days. On the day 8 of the experiment, a local inflammatory reaction was induced by the injection of 0.1 mL of carrageenan-saline solution (0.5%, w/v) into the plantar surface of the right hind paw of the rat. A saline was injected in a volume of 0.1 mL into the plantar surface of the left hind paw that served as the control (non-inflamed) paw. The animals were sacrificed 3 h after the carrageenan and saline injections and paws were cut off for weighing. The difference in weight between right and left paw (in mg) served as an indicator of the inflammatory response intensity (i.e. extent of the oedema). The control animals, instead of decoction, were drinking tap water for seven days before inducing paw oedema. The positive control group consisted of the animals treated with the well-known non-steroidal anti-inflammatory drug (NSAID) indomethacin, dissolved in DMSO and given in a dose of 2 mg/kg p.o. 60 min prior to injection of carrageenan solution. This dose of indomethacin reduced the carrageenan-induced rat paw oedema by approximately 50%, as had shown in previously performed pilot-experiment.

Effects of the treatments applied were estimated regarding the differences in rat paw oedema extent, i.e. intensity of inflammatory response, between the experimental groups. The difference in weight between right and left paw, active drug (decoct or indomethacin)-treated versus tap water (vehicle)-treated rats (the control group), served as an indicator of the anti-inflammatory activity of drugs tested (the extract and indomethacin). The inflammatory response was calculated using the equation:

$$\text{Inflammatory response (\%)} = \frac{e}{c} \times 100 \quad (2)$$

e – the difference in the paw weight in the active drug/treatment groups; c – difference in the paw weight in the control groups.

Gastroprotective activity

In order to study the anti-ulcer activity of decoction of *C. ligulare* herb an experimental model of acute gastric mucosal damage induced by absolute ethanol (1 mL/rat p.o.) was used.

The experimental groups consisted of 6–8 animals each. For seven days the animals were given 10% decoction of *C. ligulare* herb instead of tap water, while the control animals were given tap water all the time. On the day 8 after the beginning of the experiment the animals were given 1 mL of absolute ethanol through the gastric tube (the animals were starved at least 18 h before giving ethanol). Animals in the positive control group were treated by a single dose of ranitidine (20 mg/kg p.o.), the well-known anti-ulcer drug, given 60 min prior the absolute ethanol. This dose of ranitidine was chosen as a proven anti-ulcer dose on the basis of previously performed pilot-experiment.

The animals were sacrificed 1 h after ethanol administration, their stomachs removed and opened along the great curvature. Lesions were examined under an illuminated magnifier (3×). The intensity of gastric lesions was assessed in accordance with a modified scoring system of Adami et al.¹².

Results

Antioxidative activity

Antioxidative activity of 5% decoction of *C. ligulare* herb was determined using the method of DPPH radical scavenging, and the results are presented in Table 1. The anti-DPPH activity of investigated decoction was comparable with the activity of vitamin C and rutin.

Table 1
Antioxidative activity of *Cirsium ligulare* herb decoction

Sample tested	SC ₅₀
5% decoction of the <i>C. ligulare</i> herb	8.44 ± 1.17
L-ascorbic acid	4.09 ± 0.08
Rutin	5.75 ± 0.18

SC₅₀ – concentration of the sample that caused 50% of 2,2-difenil-1-picrylhydrazyl (DPHH) radical scavenging.

Anti-inflammatory activity

Results of LPS-induced systemic inflammatory response experiment are presented in Table 2. In our experimental conditions, main changes were caused by LPS dose of 5 mg/kg s.c. related to the changes in body mass of treated animals. Namely, LPS led to the significant decrease of body mass gain in rats compared to the intact controls, particularly in the first two weeks after its injection. Reduced body mass in these animals was maintained during the whole period of observation. The usage of decoctions prepared from *C. ligulare* herb in both concentrations alleviated body mass decrease just within the first two weeks of the experiment when the body mass decrease induced by LPS was the most pronounced.

In the model of carrageenan-induced rat paw oedema, the 10% decoction of *C. ligulare* herb also produced a significant anti-inflammatory effect (Table 3). Results of this

Table 2
Influence of *Cirsium ligulare* herb decoctions on body mass changes (% of basal values) in rats with lipopolysaccharide induced systemic inflammatory response

Treatment	Days			
	7	14	21	28
Group 1	16.81 ± 1.08	32.74 ± 1.95	40.7 ± 2.07	53.1 ± 2.21
Group 2	14.83 ± 1.42	23.81 ± 1.89	36.74 ± 1.85	49.1 ± 1.97
Group 3	12.56 ± 1.15	22.76 ± 1.72	32.56 ± 2.31	47.51 ± 2.18
Group 4	-0.62 ± 0.9 ^{a,b}	6.26 ± 0.58 ^{a,b}	21.52 ± 1.46 ^a	27.8 ± 1.79 ^{a,b}
Group 5	2.12 ± 0.24 ^{a,b}	13.34 ± 1.4 ^{a,c}	25.56 ± 1.67 ^a	31.8 ± 1.23 ^{a,b}
Group 6	2.23 ± 0.21 ^{a,b}	14.65 ± 1.42 ^{a,c}	27.25 ± 2.1 ^a	31.5 ± 1.17 ^{a,b}

Group 1 – intact controls given tap water; Group 2 – animals given 5% decoction of *C. ligulare* herb; Group 3 – animals given 10% decoction of *C. ligulare* herb; Group 4 – animals injected by 5 mg/kg *sc* of lipopolysaccharide (LPS) and given tap water; Group 5 – animals injected by 5 mg/kg *sc* of LPS and given 5% decoction of *C. ligulare* herb; Group 6 – animals injected by 5 mg/kg *sc* of LPS and given 10% decoction of *C. ligulare* herb

^a*p* < 0.05 vs Group 1; ^b*p* < 0.05 vs Groups 2 and 3; ^c*p* < 0.05 vs Group 4.

Table 3
Influence of *Cirsium ligulare* herb decoction on the carrageenan-induced paw oedema (local inflammatory response) in rats

Treatment	Inflammatory response (%)
None (the control group)	100.00 ± 25.98
10% decoction of <i>C. ligulare</i> herb	70.05 ± 9.02 ^a
Indomethacin* (2 mg/kg <i>p.o.</i>)	59.15 ± 7.09 ^b

*Reference drug; ^a*p* < 0.05; ^b*p* < 0.01 vs the control group.

experiment clearly demonstrate that 7-day drinking of the 10% decoction of *C. ligulare* herb produced significant anti-inflammatory effect comparable to that of indomethacin, a strong NSAID, given in a dose of 2 mg/kg which corresponded to its ED₅₀ (the dose that reduced the carrageenan-induced rat paw oedema by approximately 50%). However, *post-mortem* examination of the stomach of treated animals, revealed no changes in gastric mucosa in animals drinking the 10% decoction of *C. ligulare* herb, while those given indomethacin had some gastric lesions, mostly hyperaemia and petechiae.

Gastroprotective activity

The results of the present study demonstrated that 7-day use of the 10% decoction of *C. ligulare* herb significantly re-

duced the ulcerogenic effect of absolute ethanol in rats. Moreover, this effect was comparable to that achieved by a single dose of ranitidine, a well-known anti-ulcer drug (Table 4).

Discussion

Antioxidative activity

The method of DPPH radical neutralization is often used for preliminary estimation of the antioxidative potential of various compounds because it is simple and provides a screening of antioxidative activities of a number of samples in a very short period of time. Each substance with SC50 value ≤ 50 µg/mL is considered as a very strong antioxidant. The values of SC50 between 50–100 µg/mL, also imply satisfactory antioxidative activity^{13,14}.

Table 4
Influence of *Cirsium ligulare* herb decoction on the ethanol-induced acute stress-ulcer in rats

Treatment	Intensity of gastric lesions**
None (the control group)	5.90 ± 1.14
10% decoction of <i>C. ligulare</i> herb	2.26 ± 1.10 ^a
Ranitidine* (20 mg/kg <i>p.o.</i>)	1.77 ± 1.08 ^a

*Reference drug; ^a*p* < 0.01 vs the control group

**0 = no lesions; 0.5 = slight hyperaemia or ≤ 5 petechiae; 1 = ≤ 5 erosions ≤ 5 mm in length; 1.5 = ≤ 5 erosions ≤ 5 mm in length and many petechiae; 2 = 6–10 erosions ≤ 5 mm in length; 2.5 = 1–5 erosions > 5 mm in length; 3 = 5–10 erosions > 5 mm in length; 3.5 = >10 erosions > 5 mm in length; 4 = 1–3 erosions ≤ 5 mm in length and 0.5–1 mm in width; 4.5 = 4–5 erosions ≤ 5 mm in length and 0.5–1 mm in width; 5 = 1–3 erosions > 5 mm in length and 0.5–1 mm in width; 6 = 4 or 5 grade 5 lesions; 7 = ≥ 6 grade 5 lesions; 8 = complete lesion of the mucosa with haemorrhage (according to Adami et al.¹²).

Our preliminary chemical investigations on *C. ligulare* herb decoction have shown the presence of flavonoids (e.g. rutin and isoquercitrin) and phenolic acids (e.g. chlorogenic and rosmarinic acid) (unpublished data). This is partially in line with results presented earlier by Kozyra et al.². Since many flavonoids and phenolic acids are proven to be strong anti-DPPH scavengers^{15,16}, presence of the above mentioned compounds could partly explain the obtained strong DPPH scavenging activity (Table 1).

Anti-inflammatory activity

For studying an anti-inflammatory activity of aqueous extracts of *C. ligulare* herb, two experimental models of inflammatory reaction were used. The first one was based on producing a systemic inflammatory response by the use of LPS, a major component of the outer membrane of Gram-negative bacteria, which acts as the typical endotoxin. LPS binds the CD14/TLR4/MD2 receptor complex which acts as a promoter of the proinflammatory cytokines secretion in many cells, particularly in macrophages. In animals non-lethal doses of LPS induce multisystemic responses that are manifested as changes in secretion of pituitary-hypophyseal-adrenal axis hormones, changes in body temperature and body mass, changes in normal sleeping cycle, etc.¹⁷⁻²⁰. As expected, LPS led to the significant decrease of body mass gain in rats during the whole period of the experiment. Though both decoctions alleviated body mass decrease within the first two weeks of the experiment, in the last two weeks everyday-drinking of the aqueous extract of the *C. ligulare* herb failed to influence the body mass gain in animals with LPS-induced inflammatory reaction. On the other hand, the extracts themselves did not significantly influence the body mass gain in intact animals. Contrary to the changes in body mass, there were no changes in consumption of food and drink in any of the experimental groups during the experiment. This finding implies an absence of anorectic effect of LPS and suggests that decrease of body mass gain in animals given LPS might be the consequence of its harmful effect on utilization of nutritive substances. It has been shown that LPS causes gut mucosal injury¹⁹ that might be the reason of poor intestinal absorption of nutritive substances. If this assumption is correct it could mean that the aqueous extracts of *C. ligulare* herb tested may at least partly reduce this injury and, consequently, improve absorption of nutritive components from food.

The model of carrageenan-induced rat paw oedema is often used for estimating the anti-inflammatory activity of various substances. It is known that injection of carrageenan in rat paw produces an acute local inflammatory response consisting of two phases. During the first hour after injection of carrageenan (the early phase) many vasoactive substances including bradykinins, prostaglandins, histamine, and 5-hydroxytryptamin, are released. The second phase is characterized by neutrophil infiltration, as well as by the additional production of prostaglandins²¹⁻²³.

Since the production of prostaglandins is the key factor involved in both phases of carrageenan-induced inflammati-

on it might be proposed that the anti-inflammatory effect of the decoction of *C. ligulare* herb may be the consequence of the inhibition of synthesis and/or release of these arachidonic acid metabolites. Currently, no data about such an effect of the extract exist. However, during the second phase of carrageenan-induced acute inflammation induced, a great amount of free radical species are produced by activated polymorphonuclear cells additionally damaging the inflamed tissue²⁴. Several investigations have shown that many active constituents from medicinal plants with strong antioxidative activity, such as flavonoids and phenolic acids, act as anti-inflammatory agents due to the capability to prevent neutrophil infiltration and neutralize free radical species in an inflamed area^{24, 25}. Regarding the high DPPH-scavenging capacity of the *C. ligulare* herb decoct, it could be hypothesized that its strong anti-inflammatory effect in the model of carrageenan-induced acute inflammation is a consequence of its antiradical activity.

Moreover, observed anti-inflammatory effect could be substantiated by results of previous *in vivo* investigations which showed that orally administered rosmarinic and chlorogenic acids, phenolics preliminary identified in investigated *C. ligulare* decoctions, reduce oedema in the same animal model of inflammation^{26,27}.

Gastroprotective activity

Absolute ethanol is noxious for the stomach and is often used as a model substance for induction of gastric lesions. It affects the gastric mucosa topically and disrupts its barrier causing significant microvascular changes including rapid and strong vasoconstriction accompanied by rapid and vigorous arterial dilation. As a consequence, ischemia followed by reperfusion occurs with formation of oxygen free radicals which additionally disturb gastric mucosa barrier²⁸⁻³⁰. It has been demonstrated that oxygen-derived free radicals are directly involved in this process because their removing stimulates healing of gastric lesions induced by ethanol³¹.

As in the case of acute inflammatory response, substances with antioxidant properties (e.g. phenolic compounds like flavonoids and phenolic acids), may produce protective effects against the ulcerogenic action of absolute ethanol³²⁻³⁶. Since the decoction of *C. ligulare* herb, tested in this study, showed high free-radical scavenging activity it could be suggested that not only its anti-inflammatory effect but also the gastroprotective one, might just in part be the consequence of this anti-radical activity of phenolic compounds, the main active substances of the extract, as responsible for producing such effects³⁷.

Conclusion

The results of the present study clearly suggested that the aqueous extracts of *C. ligulare* herb produced very strong DPPH-radical scavenging activity, as well as significant anti-inflammatory and gastroprotective effects in experimental models of acute inflammation and acute stress-ulcer, respectively, in the rat. Both anti-inflammatory and anti-ulcer effects of the extract tested were close to those of the

reference drugs, indomethacin and ranitidine, respectively, and might be a consequence of its high antioxidant activity.

These results justify the traditional use of *C. ligulare* herb decoctions, but further phytochemical and pharmacological investigations are warranted.

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