Editura AcademicPres

Available online: www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Horti Agrobo, 2017, 45(1):59-64. DOI:10.15835/nbha45110549

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Original Article

Polyphenolic Profile, Anti-Inflammatory and Antinociceptive Activity of an Extract from *Arctium lappa* L. Roots

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Abstract

The aim of the study was to assess the polyphenolic profile, anti-inflammatory and antinociceptive activity of *Arctium lappa*, a medicinal plant traditionally used in the treatment of gout, hepatitis and other inflammatory disorders. Polyphenolic profile of a hydro-glycero-ethanolic extract from *Arctium lappa* roots (ALE) was evaluated by HPLC-MS method. Anti-inflammatory effect of the *Arctium lappa* extract (ALE) was determined by carrageenan-induced rat paw oedema test, while antinociceptive effect was determined by acetic acid induced writhing test in mice and Randall Selitto test in rats. HPLC-MS analysis of the extract showed the presence of chlorogenic acid (158.9 µg/mL) and quercitrin (14.4 µg/mL). The administration of ALE reduced the oedema formation in the carrageenan-induced rat paw oedema test, especially at dose of 500 mg/kg, the results being statistically significant and dose-dependent. Also, ALE showed statistically significant and dose-dependent antinociceptive effects in the acetic acid induced writhing test in mice and Randall Selitto test in rats. The results of the pharmacological experiments suggest that the anti-inflammatory and antinociceptive effects of the *Arctium lappa* extract (ALE) may be related to the ability of polyphenols such as chlorogenic acid to inhibit the synthesis and release of some pro-inflammatory mediators. Our experimental data justify the traditional use of this plant in the management of some inflammatory diseases.

Keywords: burdock, chlorogenic acid, pain threshold, rat paw oedema test, writhing test

Introduction

Arctium lappa L. (Asteraceae), commonly known as burdock, has been widely used therapeutically in Europe, North America and Asia for hundreds of years (Chan *et al.*, 2011). In folk medicine, burdock roots were popular remedies for hypertension, gout, hepatitis and other inflammatory disorders (Predes *et al.*, 2011).

Arctium lappa L. is an important natural source for several classes of bioactive compounds such as flavonoids and lignans (Ferracane *et al.*, 2010). *A. lappa* has demonstrated potent antioxidant effects *in vitro* and *in vivo* (Duh, 1998; Liu *et al.*, 2014) and protective effects in an acute experimental colitis model (De Almeida *et al.*, 2013).

Several studies reported that burdock roots had hepatoprotective (Lin *et al.*, 2002), antimicrobial (Pereira *et al.*, 2005), anti-inflammatory and radical scavenge effects (Lin *et al.*, 1996). Antiproliferative and apoptotic effects of lignans from *A. lappa* were demonstrated on leukemic cells (Matsumoto *et al.*, 2006), the antitumor effects of arctigenin being also revealed on pancreatic cancer cell lines (Awale *et al.*, 2006).

Received: 29 Aug 2016. Received in revised form: 09 Feb 2017. Accepted: 18 Mar 2017. Published online: 23 Mar 2017.

In addition, arctigenin, a phenylpropanoid dibenzylbutyrolactone lignan, suppressed lipopolysaccharide (LPS)-stimulated NO production in a dose-dependent manner (Zhao *et al*., 2009) and also inhibited some pro-inflammatory cytokines, such as TNF-α (Cho *et al*., 2004).

Although a variety of studies investigated mainly *in vitro* the properties of lignans from *A. lappa*, the polyphenols from this species were less studied. Thus, the aim of our study was to evaluate the polyphenolic profile of *A. lappa* by HPLC-MS method and to assess the anti-inflammatory and antinociceptive activity of this plant, using several *in vivo* rodent experimental models.

Materials and Methods

Plant material

The roots of *Arctium lappa* L. were collected from Cavnic, Maramures County, Romania, in October 2015. After identification and authentication, a voucher specimen was deposited at the Herbarium of the Department of Pharmacognosy and Phytotherapy, Vasile Goldis Western University of Arad, Arad, Romania (item no. 327/2015).

Preparation of the extract

Dried roots of *Arctium lappa* were reduced to a fine powder with a mechanical grinder. The powdered plant material (40 g) was extracted by maceration for 10 days at room temperature with a solvent mixture of water: glycerol: ethanol 1:1:1 in a 1:5 ratio (plant material/solvent). After filtration, 100 mL *Arctium lappa* extract were obtained. For the pharmacological studies, the extract was concentrated at reduced pressure on a rotary evaporator and suspended in a mixture of Tween 80 and normal saline solution (1:100 v/v).

Polyphenolic profile

Initially, the hydro-glycero-ethanolic extract from *Arctium lappa* roots was analyzed by a HPLC-MS method, in order to determine the polyphenolic profile (Conea *et al*., 2014).

The experiment was carried out using an Agilent 1100 HPLC Series system (Agilent, USA) equipped with degasser, binary gradient pump, column thermostat, autosampler and UV detector. The HPLC system was coupled with an Agilent 1100 mass spectrometer (LC/MSD Ion Trap VL). For the separation, a reverse-phase analytical column was employed (Zorbax SB-C18 100 x 3.0 mm i.d., 3.5 μm particle); the temperature was 48°C. The detection of the compounds was performed on both UV and MS mode. The UV detector was set at 330 nm until 17.5 min, then at 370 nm. The MS was equipped with a Turbo-Ion spray (ESI) interface, negative ion mode. ESI settings were: negative ionization, ion source temperature 360°C, gas: nitrogen, flow rate 12 L/min, nebulizer: nitrogen at 70 psi pressure, capillary voltage 3000 V. The analysis mode was multiple reaction monitoring (MRM) and single ion monitoring (SIM). The chromatographic data were processed using ChemStation and DataAnalysis software from Agilent, USA. The mobile phase was a binary gradient prepared from methanol and solution of acetic acid 0.1% (v/v) in distilled water. The elution started with a linear gradient, beginning with 5% methanol and ending at 42% methanol, for 35 minutes; isocratic elution followed for the next 3 minutes with 42% methanol. The flow rate was 1 mL min^{-1} and the injection volume was 5 μL.

The MS signal was used only for qualitative analysis based on specific mass spectra of each polyphenol. The MS spectra obtained from standard solutions of 18 polyphenols were integrated in a mass spectra library. Later, the MS traces/spectra of the analyzed samples were compared to spectra from library, which allows positive identification of compounds, based on spectral mach. The UV trace was used for quantification of identified compounds from MS detection. The detection limits were calculated as minimal concentration producing a reproductive peak with a signal-to-noise ratio greater than three. Quantitative determinations were performed using an external standard method. Calibration curves in the 0.5-50 μg mL^{-1} range with good linearity ($R^2 > 0.999$) for a five point plot were used to determine the concentration of polyphenols in plant samples.

18 polyphenolic standards were used. The standards were: caffeic acid, chlorogenic acid, p-coumaric acid, kaempferol, apigenin, rutoside, quercetin, quercitrin, isoquercitrin, fisetin, hyperoside, myricetin (Sigma, Germany), ferulic acid, gentisic acid, sinapic acid, patuletin, luteolin (Roth, Germany), caftaric acid (Dalton, USA).

Animals

For the pharmacological experiments, ten groups of male Charles River Wistar (Crl:WI) rats (n=6) with a mean weight of 195 g and five groups of male Swiss albino mice (n=6) with a mean weight of 32 g were obtained from the Practical Skills and Experimental Medicine Centre of the Iuliu Haţieganu University of Medicine and Pharmacy Cluj-Napoca (Romania). The animals were housed in polycarbonate type IV-S open-top cages (Tecniplast, Italy) and maintained under standard conditions (22 ± 2 °C, a relative humidity of 45 ± 10%, 12:12-h light: dark cycle). The animals had access to a standard pelleted food (Cantacuzino Institute, Bucharest, Romania) and filtered water *ad libitum* throughout the experiment, except for the day when the test substances were administered. All experimental protocols were approved by the Ethics Committee of the Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania and were conducted in accordance with the EEC Directive 63/2010, which regulates the use of laboratory animals for scientific purposes.

Carrageenan-induced rat paw oedema test

The acute anti-inflammatory activity of the *Arctium lappa* root extract (ALE) was assessed by the carrageenan-induced rat paw oedema method (Winter *et al*., 1962), modified by the introduction of a commercially available plethysmometer (Griesbacher *et al*., 1994; Adeyemi *et al*., 2002). Initially, the *A. lappa* extract was orally administered to three groups of Crl:WI rats (n = 6), by gastric intubation, in graded doses (125 mg/kg, 250 mg/kg and 500 mg/kg b.w.), 1 hour before the induction of the inflammation. The rats in the negative control group (n = 6) were orally treated with normal saline solution. The animals from the positive control group (n = 6) were orally treated with a reference anti-inflammatory drug, diclofenac 20 mg/kg b.w. Oedema was induced by a subplantar injection of 0.1 mL 1 % (w/v) λ-carrageenan into the left hind paw of each rat. The paw volume of each animal was determined before carrageenan injection and then at 1, 2, 3 and 4h after the induction of inflammation, with a digital plethysmometer (model 7140, Ugo Basile, Varese, Italy).

The anti-inflammatory effect of the standardized extract from *Arctium lappa* root was determined at each time interval with the

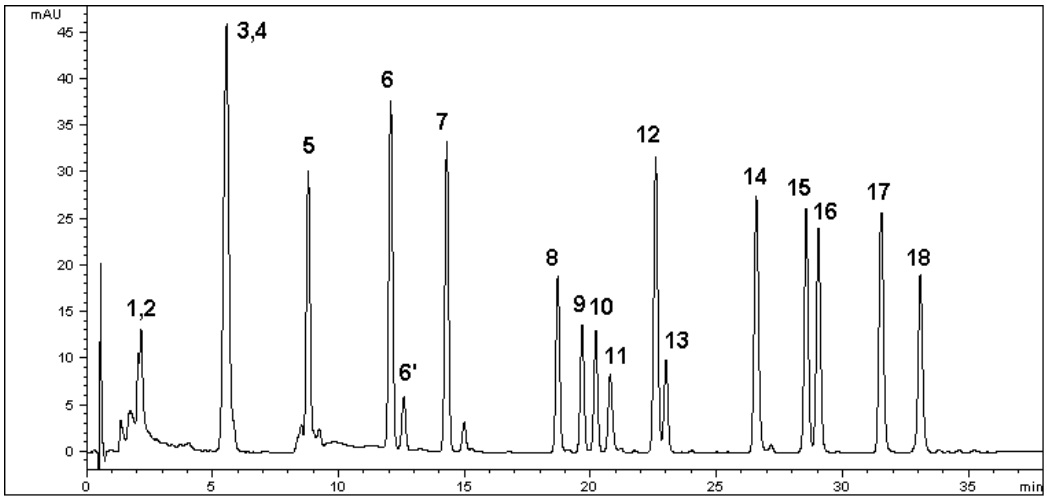

Fig. 1. HPLC chromatogram of 18 polyphenolic standards (UV detection at 330 nm and 370 nm)

formula: Inhibition of oedema (%) = [1 – (Ot/Oc)] × 100, where Ot is the oedema in the treated group and Oc is the oedema in the negative control group.

Acetic acid induced writhing test in mice

The antinociceptive effect of the extract from *Arctium lappa* roots (ALE) was evaluated by the writhing test in mice, using 1% (v/v) acetic acid solution administered intraperitonealy to induce abdominal constrictions (Koster *et al.*, 1959). Initially, the extract from *Arctium lappa* root was orally administered to three groups of Swiss albino mice (n = 6) by gastric intubation, in graded doses (125 mg/kg, 250 mg/kg and 500 mg/kg b.w.). The mice in the negative control group (n = 6) were treated orally with normal saline solution (10 mL/kg). The animals from the positive control group (n = 6) were orally treated with a reference anti-inflammatory drug, diclofenac 20 mg/kg b.w. After 30 minutes, all the mice were injected intraperitoneally with 0.1 mL of 1% acetic acid solution, in order to induce abdominal constrictions (writhes). The animals were placed in an observation box, the writhes being counted over a period of 20 minutes. For scoring purposes, a writhe was indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.

The antinociceptive activity was evaluated by calculating the percentage of inhibition of the writhes with the formula:

% inhibition = (Mean no. of writhes for control group – Mean no. of writhes for treated group) × 100 / Mean no. of writhes for control group.

Randall-Selitto test in rats

The antinociceptive activity of the extract from *Arctium lappa* roots (ALE) was also assessed by the Randall Selitto test. Thus, the pain threshold of the inflamed, oedematous hind paw of the rats subjected to constant force was determined with an analgesimeter (Randall and Sellito, 1957). For this experiment, five groups of male Crl:WI rats (n = 6) were used. Initially, the animals received an intraplantar injection of λ-carrageenan (1% w/v, 0.1 mL) in sterile saline solution. Thirty minutes after the induction of inflammation, 125, 250 and 500 mg/kg b.w. of ALE were orally administered to three test groups, while the normal saline solution (10 mL/kg) was given to the negative control group and a reference analgesic, diclofenac (20 mg/kg b.w.) was orally administered to the positive control group. After 1, 2, 3, and 4 h, pressure was applied to the inflamed paw using an analgesimeter (model 37215, Ugo Basile, Varese, Italy), generating linearly increasing force, until the animal produced a response characterized by removal of the paw, interpreted as mechanical hypernociception. The instrument recorded the maximal amount of pressure (expressed in grams) withstood by the rats, at each time interval.

Statistical analysis

Data were expressed as mean values ± S.E. and were statistically analyzed by one-way ANOVA method. The differences between the treated groups and the control group were evaluated by Dunnett's 't' test, p values < 0.05 being considered statistically significant.

Table 1. Retention time values and parameters of the calibration line equation for the standards with UV detection (A = peak area (mAUxs), x = concentration (μg mL^{-1})

No.	Phenolic compound	Retention time (min.)	Calibration line equation
1	Caftaric acid	2.10	qualitatively
2	Gentisic acid	2.15	qualitatively
3	Caffeic acid	5.6	qualitatively
4	Chlorogenic acid	5.6	qualitatively
5	p-Coumaric acid	8.7	A = - 0.325 + 33.23 x
6	Ferulic acid	12.2	A = - 1.016 + 39.55 x
7	Sinapic acid	14.3	A = - 0.236 + 37.10 x
8	Hyperoside	18.6	A = 0.107 + 19.29 x
9	Isoquercitrin	19.6	A = - 0.273 + 12.97 x
10	Rutoside	20.2	A = 0.226 + 13.47 x
11	Myricetin	20.7	A = -0.544 + 26.45 x
12	Fisetin	22.6	A = 0.241 + 19.19 x
13	Quercitrin	23.0	A = 0.047 + 10.69 x
14	Quercetin	26.8	A = -1.152 + 36.32 x
15	Patuletin	28.7	A= - 0.429 + 31.44 x
16	Luteolin	29.1	A = - 0.760 + 28.97 x
17	Kaempferol	31.6	A = - 1.270 + 30.15 x
18	Apigenin	33.1	A = - 0.908 + 20.40 x

Table 2. Compounds identified in *Arctium lappa* root extract

No.	Compound	Retention time (min.)	Specific ions for identification Ion [M-H] > Ions from spectrum	MS analysis mode	Content in *Arctium lappa* extract (µg/mL)
1	Chlorogenic acid	5.6	353.5>178.7, 190.7	MRM	158.9
2	Quercitrin	23.0	447.1	SIM	14.4

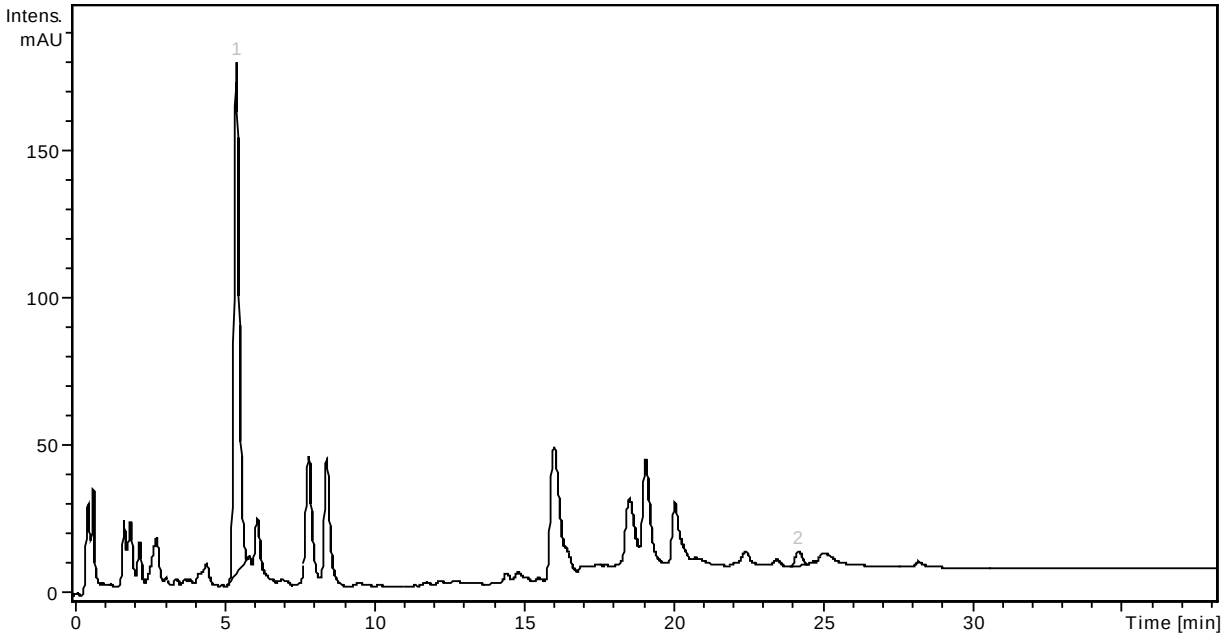

Fig. 2. HPLC chromatogram of the polyphenols from *Arctium lappa* root extract

Results and Discussion

Polyphenolic profile

Under the previously described chromatographic conditions, the polyphenols eluted in less than 35 minutes (Fig. 1).

The UV lambda used in the analytical method is corresponding to the maximum absorption spectra for phenolic acids (330 nm, all eluting before 17 min.) and for flavonoids (370 nm, all eluting after 18 min). Therefore, phenolic acids were detected at 330 nm, while flavonoids (glycosides and aglycons) were detected at 370 nm. The retention time values and the parameters of the calibration line equation for the standards are presented in Table 1. UV trace was used only for quantification, after positive identification by means of mass spectrometry, much more sensitive and specific than UV detector.

HPLC chromatogram of the polyphenols from *Arctium lappa* root extract (ALE) is presented in Fig. 2. The HPLC-MS analysis showed the presence of chlorogenic acid and quercitrin in the hydro-glycero-ethanolic extract from *Arctium lappa* roots (Table 2) confirming the previous findings of a HPTLC study (Toniolo *et. al.*, 2014). The unassigned peaks from the chromatogram did not correspond to any of the standards used in the analytical method, therefore we could assume the presence of other polyphenols, not yet identified.

Thus, our study identified chlorogenic acid as the major polyphenolic compound of the studied extract. Previous research demonstrated the role of chlorogenic acid as an

Table 3. Effect of the extract from *Arctium lappa* (ALE) on carrageenan-induced rat paw oedema

Group	Dose (mg/kg)	Oedema at 1h ± S.E. (mL) (% inhibition)	Oedema at 2h ± S.E. (mL) (% inhibition)	Oedema at 3h ± S.E. (mL) (% inhibition)	Oedema at 4h ± S.E. (mL) (% inhibition)
Control (normal saline)	-	2.23 ± 0.21 (-)	2.68 ± 0.20 (-)	3.01 ±0.14 (-)	3.47± 0.23 (-)
ALE	125	1.59 ± 0.12* (28.69)	2.22 ± 0.08* (14.92)	2.62 ± 0.11* (12.95)	2.45 ± 0.05* (29.39)
ALE	250	1.29 ± 0.18** (42.15)	2.15 ± 0.13* (19.77)	2.36 ± 0.13** (21.59)	2.18 ± 0.10** (37.17)
ALE	500	1.15 ± 0.17** (48.43)	1.83 ± 0.32** (31.71)	2.16 ± 0.26* (28.23)	2.04 ± 0.27** (41.21)
Diclofenac (reference)	20	1.63 ± 0.10* (26.90)	1.08 ± 0.09** (59.70)	1.13 ± 0.09** (62.45)	1.53± 0.10** (55.90)

(*p ≤ 0.05 vs.control, **p ≤ 0.01 vs.control, S.E. - standard error)

Table 4. Effect of the extract from *Arctium lappa* (ALE) in the acetic acid induced writhing test in mice

Group	Dose (mg/kg)	No. of writhes (X±S.E.)	% of inhibition
Control (normal saline)	10 mL/kg	56±2.84	-
ALE	125 mg/kg	43.5±2.48**	22.32
ALE	250 mg/kg	35.16±1.73**	37.21
ALE	500mg/kg	28.5±1.74**	49.10
Diclofenac	20 mg/kg	21.33±1.64**	61.91

(*p ≤ 0.05 vs. control, **p ≤ 0.01 vs. control, S.E. - standard error)

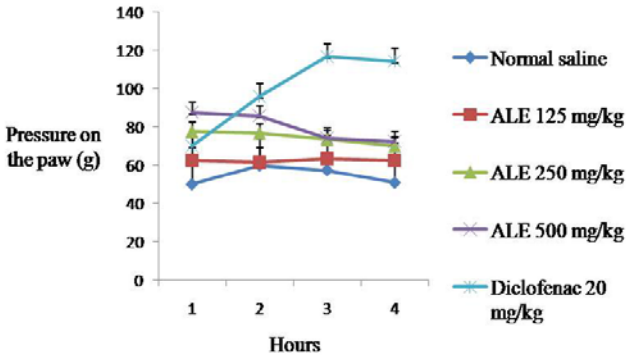

Fig. 3. Effects of the extract from *Arctium lappa* (ALE) in the Randall-Sellito test in rats

antioxidant, anti-inflammatory and antinociceptive agent. Chlorogenic acid significantly inhibited NO production and also the expression of COX-2 and iNOS, without any cytotoxicity in lipopolysaccharide (LPS)-stimulated murine RAW 264.7 macrophages and BV2 microglial cells (Hwang *et al.*, 2014) and also presented anti-inflammatory and antinociceptive effects in several *in vivo* experimental models (Dos Santos *et al.*, 2006).

Carrageenan-induced rat paw oedema test

The results of the carrageenan-induced rat paw oedema test are shown in Table 3. The inflammatory oedema developed soon after the subplantar injection of carrageenan, reaching its peak at 4 h (oedema of 3.47 ± 0.23 mL).

The administration of a reference anti-inflammatory drug, diclofenac 20 mg/kg b.w., inhibited the oedema formation in the treated rats, the effect being maximal at 2-3 h and slightly decreasing afterwards. The administration of the extract from *Arctium lappa* roots (ALE) reduced the oedema formation, especially at dose of 500 mg/kg, the results being statistically significant and dose-dependent. Additionally, in the first phase of oedema formation, ALE at the dose of 500 mg/kg was superior to diclofenac, with an inhibition rate of 48.43%, 1 h after the induction of the inflammation.

In carrageenan-induced rat paw oedema test, the initial inflammatory reaction (0–1 h), has been attributed to the release of histamine, serotonin, bradykinin and also complement and reactive oxygen species. In contrast, the second accelerating phase of swelling (2-4 h) has been correlated with the elevated production of prostaglandins in the inflammatory area (Vineagar *et al.*, 1987). Our experimental data suggest that several mechanisms may be responsible for the anti-inflammatory effect of the extract from *Arctium lappa* root. Aside from a possible reduction of prostaglandin concentration in the affected tissue, it appears that the extract was able to influence also the first phase of carrageenan-induced oedema formation, probably by inhibiting the release of other pro-inflammatory mediators.

Acetic acid induced writhing test in mice

The results of the acetic acid induced writhing test in mice are presented in Table 4. The intraperitoneal injection of a 1% acetic acid solution induced 56 writhes in the control group. The oral administration of the extract from *Arctium lappa* roots (ALE) produced a significant inhibition of the writhes. The results showed a 49.10% inhibition for the *A. lappa* extract at dose of 500 mg/kg. Although the results are inferior to the reference drug, diclofenac, which produced a 61.91% inhibition of the writhes, they are statistically significant and dose-dependent.

Acetic acid is known to trigger an irritative reaction in the peritoneum, which induces the writhing response due to the sensitization of nociceptors by prostaglandins (Yan *et al.*, 2015).

Our results indicate that the antinociceptive effect of ALE might be mediated by the peripheral inhibition of prostaglandin synthesis or actions, thereby causing a reduction in the number of writhes.

Randall-Selitto test in rats

The results of the Randall-Selitto test in rats are presented in Fig. 3. The oral administration of the extract from *Arctium lappa* roots (ALE) produced a significant peripheral antinociceptive effect at the dose of 500 mg/kg, with a pain threshold of 87.5 g.

The antinociceptive effect was visible especially in the first two hours after the substance administration, which may suggest a dual mechanism: inhibition of prostaglandins but also a diminution of the release of other pro-inflammatory mediators. Our results demonstrated a significant and dose-dependent antinociceptive effect for ALE, confirming data from previous studies, which investigated the peripheral antinociceptive effects of polyphenolic compounds (Küpeli and Yesilada, 2007; Toker *et al.*, 2004).

Conclusions

Our study evaluated the polyphenolic profile, anti-inflammatory and antinociceptive activity of *Arctium lappa* L. root extract (ALE). The HPLC-MS analysis showed the presence of chlorogenic acid (158.9 µg/mL) and quercitrin (14.4 µg/mL). The administration of *Arctium lappa* root extract reduced the oedema formation in the carrageenan-induced rat paw oedema test, especially at dose of 500 mg/kg, the results being statistically significant and dose-dependent. Also, ALE showed statistically significant and dose-dependent

antinociceptive effects in the acetic acid induced writhing test in mice and Randall Selitto test in rats.Both anti-inflammatory and antinociceptive effects of *Arctium lappa* root extract may be related to the ability of polyphenols to inhibit the synthesis and release of some pro-inflammatory mediators.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

Adeyemi OO, Okpo SO, Ogunti OO (2002). Analgesic and antiinflammatory effect of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). Fitoterapia 73:375-380.

Awale S, Lu J, Kalauni S, Kurashima Y, Tezuka Y, Kadota S, Esumi H (2006). Identification of arctigenin as an antitumor agent having the ability to eliminate the tolerance of cancer cells to nutrient starvation. Cancer Research 66(3):1751-757.

Chan YS, Cheng LN, Wu JH, Chan E, Kwan YW, Lee SM, Leung GP, Yu PH, Chan SW (2011). A review of the pharmacological effects of *Arctium lappa* (burdock). Inflammopharmacology 19(5):245-254.

Cho MK, Jang YP, Kim YC, Kim SG (2004). Arctigenin, a phenylpropanoid dibenzylbutyrolactone inhibits MAP kinases and AP-1 activation via potent MKK inhibition: the role in TNF-α inhibition. International Immunopharmacology 4(10-11):1419-1429.

Conea S, Vostinaru O, Vlase L (2014). LC-MS Analysis and anti-inflammatory activity of a tincture from *Eryngium planum* in a rat model of acute inflammation. Digest Journal of Nanomaterials and Biostructures 9(3):1039-1045.

De Almeida AB, Sánchez-Hidalgo M, Martin AR, Luiz-Ferreira A, Trigo JR, Vilegas W, Dos Santos LC, Souza-Brito AR, De La Lastra CA (2013). Anti-inflammatory intestinal activity of *Arctium lappa* L. (Asteraceae) in TNBS colitis model. Journal of Ethnopharmacology 146(1):300-310.

Dos Santos MD, Almeida MC, Lopes NP, de Souza GE (2006). Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. Biological and Pharmaceutical Bulletin 29(11):2236-2240.

Duh PD (1998). Antioxidant activity of burdock (*Arctium lappa* Linne): its scavenging effect on free-radical and active oxygen. Journal of the American Oil Chemists Society 75(4):455-461.

Ferracane R, Graziani G, Gallo M, Fogliano V, Ritieni A (2010). Metabolic profile of the bioactive compounds of burdock (*Arctium lappa*) seeds, roots and leaves. Journal of Pharmaceutical and Biomedical Analysis 51(2):399-404.

Griesbacher T, Sutliff RL, Lembeck F (1994). Anti-inflammatory and analgesic activity of the bradykinin antagonist, icatibant (HOE 140), against an extract from *Porphyromonas gingivalis*. British Journal of Pharmacology 112:1004-1006.

Hwang SJ, Kim YW, Park Y, Lee HJ, Kim KW (2014). Anti-inflammatory effects of chlorogenic acid in lipopolysaccharide-stimulated RAW 264.7 cells. Inflammation Research 63(1):81-90.

Koster R, Anderson M, De Beer EJ (1959). Acetic acid for analgesic screening. Federation Proceedings 18:412.

Küpeli E, Yesilada E (2007). Flavonoids with anti-inflammatory and antinociceptive activity from *Cistus laurifolius* L. leaves through bioassay-guided procedures. Journal of Ethnopharmacology 112(3):524-530.

Lin CC, Lu JM, Yang JJ, Chuang SC, Ujiie T (1996). Anti-inflammatory and radical scavenge effects of *Arctium lappa*. American Journal of Chinese Medicine 24(2):127-137.

Lin SC, Lin CH, Lin CC, Lin YH, Chen CF, Chen IC, Wang LY (2002). Hepatoprotective effects of *Arctium lappa* L. on liver injuries induced by chronic ethanol consumption and potentiated by carbon tetrachloride. Journal of Biomedical Science 9(5):401-409.

Liu W, Wang J, Zhang Z, Xu J, Xie Z, Slavin M, Gao X (2014). In vitro and in vivo antioxidant activity of a fructan from the roots of *Arctium lappa* L. International Journal of Biological Macromolecules 65:446-453.

Matsumoto T, Hosono-Nishiyama K, Yamada H (2006). Antiproliferative and apoptotic effects of butyrolactone lignans from *Arctium lappa* on leukemic cells. Planta Medica 72(3):276-278.

Pereira JV, Bergamo DC, Pereira JO, De Castro Franca S, Rodrigues Pietro RCL, Silva-Sousa YC (2005). Antimicrobial activity of *Arctium lappa* constituents against microorganisms commonly found in endodontic infections. Brazilian Dental Journal 16(3):192-196.

Predes FS, Ruiz ALTG, Carvalho JE, Foglio MA, Dolder H (2011). Antioxidative and *in vitro* antiproliferative activity of *Arctium lappa* root extracts. BMC Complementary and Alternative Medicine 11:25.

Randall LO, Selitto JJ (1957). A method for measurement of analgesic activity on inflammed tissue. Archives Internationales de Pharmacodynamie et de Therapie 111:409-419.

Toker G, Küpeli E, Memisoglu M, Yesilada E (2004). Flavonoids with antinociceptive and anti-inflammatory activities from the leaves of *Tilia argentea* (silver linden). Journal of Ethnopharmacology 95:393-397.

Toniolo C, Nicoletti M, Maggi F, Venditti A (2014). HPTLC determination of chemical composition variability in raw materials used in botanicals. Natural Product Research 28(2):119-126.

Vineagar R, Traux JF, Selph JH, Johnston PR, Vinable AH, McKenzie RK (1987). Pathway to carrageenan-induced inflammation of the hind limb of the rat. Federation Proceedings 6:118-126.

Winter CA, Risley EA, Nuss GW (1962). Carrageenan-induced oedema in the hind paw of rat as an assay for anti-inflammatory activity. Proceedings of the Society for Experimental Biology and Medicine 111:544-547.

Yan WX, Zhang JH, Zhang Y, Meng D-L, Yan D (2015). Anti-inflammatory activity studies on the stems and roots of *Jasminum lanceolarium* Roxb. Journal of Ethnopharmacology 171:335-341.

Zhao F, Wang L, Liu K (2009). *In vitro* anti-inflammatory effects of arctigenin, a lignan from *Arctium lappa* L., through inhibition on iNOS pathway. Journal of Ethnopharmacology 122(3):457-462.