

Nettle Extract (*Urtica dioica*) Affects Key Receptors and Enzymes Associated with Allergic Rhinitis

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A nettle (*Urtica dioica*) extract shows *in vitro* inhibition of several key inflammatory events that cause the symptoms of seasonal allergies. These include the antagonist and negative agonist activity against the Histamine-1 (H₁) receptor and the inhibition of mast cell tryptase preventing degranulation and release of a host of pro-inflammatory mediators that cause the symptoms of hay fevers. The nettle extract also inhibits prostaglandin formation through inhibition of Cyclooxygenase-1 (COX-1), Cyclooxygenase-2 (COX-2), and Hematopoietic Prostaglandin D₂ synthase (HPGDS), central enzymes in pro-inflammatory pathways. The IC₅₀ value for histamine receptor antagonist activity was 251 (±13) µg mL⁻¹ and for the histamine receptor negative agonist activity was 193 (±71) µg mL⁻¹. The IC₅₀ values for inhibition of mast cell tryptase was 172 (±28) µg mL⁻¹, for COX-1 was 160 (±47) µg mL⁻¹, for COX-2 was 275 (±9) µg mL⁻¹, and for HPGDS was 295 (±51) µg mL⁻¹. Through the use of DART TOF-MS, which yields exact masses and relative abundances of compounds present in complex mixtures, bioactives have been identified in nettle that contribute to the inhibition of pro-inflammatory pathways related to allergic rhinitis. These results provide for the first time, a mechanistic understanding of the role of nettle extracts in reducing allergic and other inflammatory responses *in vitro*. Copyright © 2009 John Wiley & Sons, Ltd.

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INTRODUCTION

More than 20 million people, over 40% of whom are children, suffer from seasonal allergies in the United States (Holgate *et al.*, 2003; Law *et al.*, 2003). In the last few years, several investigators have shown that there is a strong genetic and environmental component to the allergic inflammatory response (Holgate *et al.*, 2003; Bousquet *et al.*, 2004; Schatz, 2007; Schoefer *et al.*, 2008). Although there are numerous over-the-counter (OTC), prescription, and herbal-based medications on the market for allergies, many of these products suffer from undesirable side-effects like headache, dry mouth and/or drowsiness. Despite this, allergy and sinus treatment drugs for airborne pollen and/or particulate allergens are among the safest drugs in the world, with an extremely low number of adverse effects from use (Scadding, 1999; Lipworth, 2001; Hore *et al.*, 2005; Kawai *et al.*, 2007; Bousquet *et al.*, 2008).

Nettle (*Urtica dioica* L.) has been used for hundreds of years to treat a variety of disorders ranging from allergic rhinitis to hypertension (Thornhill *et al.*, 2000; Legssyer *et al.*, 2002). Nettle is a temperate herbaceous species that is a common and aggressive weed found in moist soils throughout the USA and Europe but is also cultivated commercially (Whitney *et al.*, 2006). *Urtica dioica* belongs to the family *Urticaceae*. The Latin root

of *Urtica* is *uro*, meaning 'I burn', indicative of the stings caused by glandular hairs on the leaves that contain formic acid and histamine, two agents known to cause the 'stinging' and skin irritation after contact. Dermatological reactions from exposure to the formic acid released with gentle mechanical stress to the leaves can range from mild irritation to severe dermatitis (Edwards Jr. *et al.*, 1992; Morgan *et al.*, 2003; Bossuyt *et al.*, 1994; Caliskaner *et al.*, 2004).

Clinical evidence shows that freeze-dried extracts of nettle reduce allergy symptoms (Mittman, 1990). However, the precise nature of the effectiveness of nettle extracts for allergies is unclear, but clinical data and recent findings suggest the benefits likely arise from its anti-inflammatory activities (Mittman, 1990; Tunon *et al.*, 1995; Konrad *et al.*, 2005). Here we report that a proprietary nettle extract has *in vitro* anti-inflammatory activities that target key processes that generate the symptoms of allergic rhinitis and we identify key functional bioactives that contribute to these activities. The activities include (1) H₁ receptor inactivation and inhibition, which blocks histamine production and release; (2) Tryptase inhibition, which blocks mast cell degranulation and the subsequent release of cytokines and chemokines that cause allergy symptoms; (3) COX-1 and COX-2 inhibition, which blocks prostaglandin formation; and (4) Hematopoietic Prostaglandin D₂ Synthase (HPGDS) inhibition, which specifically blocks Prostaglandin D₂ production, a primary pro-inflammatory mediator in allergic rhinitis. Key functional bioactives include 4-shogaol, piperine, 8-dehydrogingerdione, deoxyharringtonine and carnosol.

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MATERIALS AND METHODS

Botanical extraction. Cut leaves of *Urtica dioica* were obtained from Blessed Herbs (Oakham, MA, USA). Nettle leaves were ground to a powder with particle size ca. 20–40 mesh and the ground nettle leaf was extracted with HPLC grade water (Sigma-Aldrich, St Louis, MO, USA) and combinations of water and ethanol (20, 40, 60 and 80% ethanol) at 20, 40 and 60 °C. After 2 h, the slurries were filtered through Fisher P4 filter paper with pore size of 4–8 µm and centrifuged at 2000 rpm for 20 min. The supernatants were collected and evaporated to dryness at 50 °C in a vacuum oven overnight yielding a dark green powder. The extracts were then screened in a set of bioassays and the extract with the greatest inhibitory activities against the inflammatory targets was more fully characterized. All other reagents and chemicals used were supplied with the assays or purchased individually from the assay suppliers.

DART TOF-MS chemical characterization of *Urtica dioica*. A Jeol DART AccuTOF-MS (Model JMS-T100LC; Jeol USA, Peabody, MA, USA) was used for chemical characterization of the nettle extract. The DART settings were loaded as follows: DART Needle voltage = 3000 V; Electrode 1 voltage = 150 V; Electrode 2 voltage = 250 V; Temperature = 250 °C; He Flow Rate = 2.52 LPM. The following AccuTOF mass spectrometer settings were loaded: Ring Lens voltage = 5 V; Orifice 1 voltage = 10 V; Orifice 2 voltage = 5 V; Peaks voltage = 1000 V; Orifice 1 temperature was turned off. The samples were introduced by placing the closed end of a borosilicate glass capillary tube into the nettle extract, and the coated capillary tube was placed in the He plasma. The sample remained in the He plasma stream until signal was observed in the total-ion-chromatogram (TIC). The sample was removed and the TIC was brought down to baseline levels before the next sample was introduced. A Polyethylene glycol 600 (Ultra Chemicals, Kingston, RI, USA) was used as an internal calibration standard giving mass peaks throughout the desired range of 100–1000 amu.

The key bioactives in the nettle extract were identified using Jeol USA software (Peabody, MA, USA) and proprietary software programs that incorporate the relative abundances, exact masses, mass spectral differences of the chemistries found in the extract and *in vitro* activity profiles of the extract (Roschek *et al.*, 2009). The theoretical IC₅₀ values for the identified bioactives were calculated assuming that all of the inhibitory activity in the extract was attributed to individual bioactives.

Histamine receptor (H₁) inhibition assays. Histamine receptor (H₁) activity was evaluated with the nettle extract using Geneblazer H1 HEK 293T Division Arrested Cells (Invitrogen, Carlsbad, CA, USA). Cells were seeded onto a tissue culture treated 384-well plate according to manufacturer's specifications using DMEM with FBS 10% penicillin (100 U mL⁻¹), streptomycin (100 µg mL⁻¹), non-essential amino acids (0.1 mM), and HEPES buffer. Cells were incubated overnight for 16–20 h in a CO₂ incubator (5% CO₂) at 37 °C allowing them to adhere to the plate. To determine if the nettle

extract was an agonist for the H₁-receptor, cells were exposed to serial dilutions of the extract for 5 h. For antagonist screening, cells were exposed to serial dilutions of the extract for 30 min and then exposed to histamine (0.5 µM) at 37 °C for 4.5 h in a CO₂ incubator (5% CO₂). After the 5-h incubation period, CCF4-AM substrate (Invitrogen, Carlsbad, CA, USA) was loaded in each well and incubated for 1 h at room temperature, according to the manufacturer's protocols. Plates were then excited at 409 nm and the emission read at 460 nm and 530 nm. The background subtracted fluorescence emission ratio (Em 460/530 nm) was obtained on a Biotek Synergy 4 plate reader and percent inhibition of H₁-receptor activity in the presence of the extract as an agonist and antagonist was determined relative to histamine and triprolidine activity. A known H₁-antagonist, triprolidine was used as a control and achieved an IC₅₀ of 19 nM in the H₁-receptor assay.

Mast cell tryptase inhibition assay. Tryptase activity was determined in a cell-free assay by monitoring the production of the chromophore *p*-nitroaniline (pNA) generated by the cleavage of tosyl-gly-pro-lys-pNA by the tryptase enzyme according to the manufacturer's protocol (Millipore Inc., Westbury, MA, USA). In a 96-well format, 10 µL of tryptase was added to 10 µL of serial dilutions of the extract, followed by 20 µL of tosyl-gly-pro-lys-pNA and 160 µL of 1X reaction buffer. Absorbance at 405 nm was monitored for 2 h at 37 °C using a Biotek Synergy 4 microplate reader and percent inhibition of tryptase was determined by comparing the extract results to 100% enzyme activity (positive control) and no enzyme (negative control). In addition, a commercially available tryptase inhibitor, protamine was used for comparison, and achieved an IC₅₀ of 103 µM.

Cyclooxygenase-1 and -2 (COX-1/COX-2) inhibition assays. All reagents and solutions were prepared according to the protocols established by Cayman Chemicals (Ann Arbor, MI, USA) for the COX-1 and COX-2 inhibition assays. Briefly, extracts were dissolved in neat DMSO and diluted in reaction buffer to a final DMSO concentration of 1% (v/v). Reactions were conducted with COX-1 or COX-2 enzymes in the presence of Heme as a co-factor. The enzymes were incubated at 37 °C for 15 min with serial dilutions of the nettle extract or reaction buffer to determine 100% enzyme activity (positive control), and heat inactivated enzymes were used to as negative controls (0% enzyme activity). Arachidonic acid (100 µM) was added to each well and the reaction was stopped after 2 min by addition of 1 M HCl. The product of the reaction, Prostaglandin H₂, was reduced to Prostaglandin F_{2α} using SnCl₂ and quantified using Enzyme Immunoassay (EIA) provided by the supplier. Percent inhibition of the COX enzymes was determined by comparing the extract loaded wells with the positive and negative controls. Commercially available COX-1 and COX-2 inhibitors (SC-560 and Dup-697, respectively) were used for comparison. The COX-1 inhibitor achieved an IC₅₀ value of 48 nM, while the COX-2 inhibitor achieved an IC₅₀ of 1.2 µM in our assays.

Hematopoietic Prostaglandin D₂ Synthase (HPGDS) inhibition assay. The HPGDS inhibitory activity of the nettle extract was determined following the protocol

provided by Cayman Chemical Co. (Ann Arbor, MI, USA). Briefly, the extract was dissolved in neat DMSO and diluted in reaction buffer to a final concentration between 1 and 2% DMSO (v/v). The HPGDS was incubated in the presence of serial dilutions of the extract for 2 min and the reactions were stopped by the addition of 1M HCl. The samples were diluted 1:5000 times before being added to the EIA plate coated with goat antimouse IgG. Prostaglandin D₂ EIA monoclonal antibody and AChE tracer were added and the reaction incubated for 2 h at room temperature. The plate was washed, Ellman's reagent was added and the plate was incubated at room temperature in the dark for 1 h. A Biotek Synergy 4 microplate reader was used to measure absorbance at 410 nm to determine the percent inhibition of HPGDS activity by comparing extract loaded wells with 100% HPGDS activity (positive control) and no enzyme activity (negative control). The commercially available HPGDS inhibitor hgl-79 reached an IC₅₀ value of 53 μ M.

RESULTS AND DISCUSSION

DART TOF-MS analysis of the nettle extract. A newly developed ionization source for mass spectrometry, termed DART (Direct Analysis in Real Time), is coupled to a time-of-flight (TOF) mass spectrometer, making it possible to identify, rapidly and accurately, the chemical components in botanicals and extracts at atmospheric pressure, with no sample preparation or processing. The DART ion source utilizes electronic excited-state helium and nitrogen atoms as plasmas. These excited atoms ionize samples directly for mass spectrometric analysis. DART is capable of analyzing surface materials without direct exposure of the samples to elevated temperatures and/or electrical potentials thereby minimizing sample decomposition (Cody *et al.*, 2005).

The DART mass spectrum of the most active nettle extract is shown in Fig. 1. The X-axis shows the mass distribution of the chemical entities present in the mass range of 100 to 650 amu ($m/z = [M + H]^+$). The nettle extract has a broad diversity of chemical species and Table 1 provides the 100 compounds identified in the optimized nettle extract by exact mass measurements

from DART TOF-MS analysis. Many polyphenols (eugenol, flavones), saccharides (glycogen), amino acids (proline, leucine), fatty acids (linolenic acid), and phyto-sterols (cholecalciferol, carnosol) have been identified in nettle by determining the exact mass and isotopic ratios of each compound using DART TOF-MS.

Effect of the nettle extract on H₁ histamine receptor activity. The nettle extract shows both H₁-receptor antagonism (competition of normal ligand binding) and negative agonism (binding to the receptor thereby preventing the triggering of downstream events) activity, mechanisms-of-action similar to traditional OTC antihistamines. The histamine receptor antagonist activity was 251 (\pm 13) μ g mL⁻¹ while the histamine receptor negative agonist activity was 193 (\pm 71) μ g mL⁻¹ (Table 2). Because the nettle extract contains bioactives that are effective H₁-receptor antagonists, it will prevent histamine from binding to the receptor, thereby blocking the normal cascade of histamine triggered allergic responses. Commercial antihistamines such as diphenhydramine are H₁-receptor antagonists (Simmons, 2004). However, diphenhydramine readily crosses the blood-brain barrier (BBB) and binds to central nervous system (CNS) H₁-receptors causing drowsiness. Many compounds identified in nettle that contribute to the antihistamine activity do not have chemical and physical properties that would allow penetration of the BBB (Table 3) (Pajouhesh *et al.*, 2005). Therefore, the majority of the nettle antihistamine bioactives (e.g., DL-methyl-m-tyrosine, isopropyl- β -D-thiogalactopyranoside, and deoxyharringtonine have ClogP-[N+O] values <0) would most likely act only on peripheral H₁-receptors minimizing the likelihood of drowsiness. The nettle bioactives that function as H₁-receptor negative agonists will shut down normal cell signaling events that occur when histamine binds to the H₁-receptor and, as a consequence, limit the inflammation response associated with histamine binding.

Among the bioactives identified in the nettle extract using proprietary data analysis methods that incorporate the relative abundances, exact masses, mass spectral differences and *in vitro* bioactivity (Roschek *et al.*, 2009), several are inhibitors of H₁-receptor function with theoretical IC₅₀ values that are less than 250 μ M (Table 3). Key bioactives such as DL-methyl-m-tyrosine, isopropyl- β -D-thiogalactopyranoside, phosphatidylcholine,

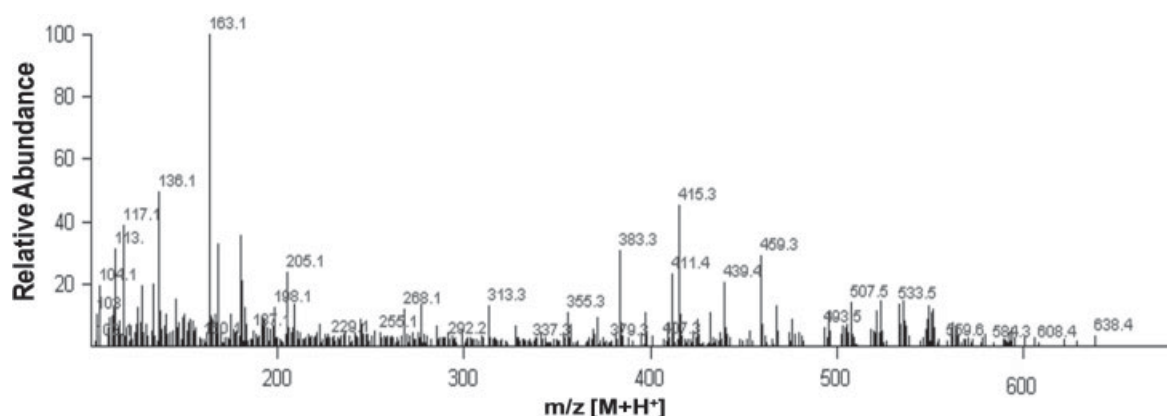


Figure 1. DART TOF-MS fingerprint of the nettle extract showing the mass distribution (X-axis) and the relative abundances of each of the chemical species (Y-axis).

Table 1. Compounds present in the nettle extract, as determined by DART TOF-MS analysis

Compound Name	Measured Mass	Relative Abundance (%)	Compound Name	Measured Mass	Relative Abundance (%)
aminobutyric acid	104.0712	19.4027	1,6-dioxaspiro[4.4]non-3-ene	201.0927	1.7105
1,4-benzoquinone	109.0304	5.6214	4-aminoantipyrine	204.1046	3.3994
2-acetylpyrrole	110.0661	9.1518	4-methyl-7-ethoxycoumarin	205.0775	23.7605
resorcinol	111.0536	9.5431	Carvylacetate	209.1562	13.1682
6-azacytosine	113.0467	31.2587	Flavan	211.1169	2.1584
creatinine	114.062	6.2286	Harmine	213.1023	1.9492
proline	116.0706	3.4373	vitamin B5	220.1107	3.1573
levulinic acid	117.0551	38.5861	pantothenic acid	220.1107	3.1573
Indole	118.0597	3.0894	1,2-O-isopropylidene-D-glucoside	221.107	4.382
L-threonine	120.0583	1.3511	Hydrocotarnine	222.1122	6.8038
acetophenone	121.0661	6.5386	Flavones	223.0708	0.9831
vitamin B3 (niacinamide)	123.0582	4.2889	6-benzylaminopurine	226.1141	2.7388
pyrogallol	127.047	19.4107	Carnosine	227.1129	3.3847
leucine	132.1008	4.7604	Kavain	231.1032	1.6417
glutaric acid	133.0504	19.6285	Dihydrokavain	233.1186	1.9648
2-deoxy-D-ribose	135.0566	1.3173	Harmol	235.1107	3.455
adenine	136.0633	49.4889	isopropyl-β-D-thio-galacto-pyranoside	239.0939	0.9677
anisaldehyde	137.0664	10.9016	Phosphatidylcholine	243.1153	2.7201
tyrosol	139.0812	6.4353	6-aminochrysene	244.1076	8.621
histidinol	142.0903	4.2666	Osthole	245.0978	6.9901
3-hydroxy-2,3 dihydromaltol	145.0536	14.7854	4-shogaol	249.126	1.5636
nonanol	145.1654	0.634	Pterostilbene	257.1251	2.8696
galactal	147.0665	7.4541	1,2,5,6-di-O-iso propylidene	261.1266	2.8952
cinnamide	148.082	2.0246	Lotaustralin	262.1355	2.575
hydroxycinnamaldehyde	149.0549	9.1354	abscisic acid	265.137	1.6699
chitosan	150.0821	9.813	Adenosine	268.107	11.7278
ribose	151.0693	4.5401	3,4-dimethoxychalcone	269.124	3.4104
anthranilic acid methyl ester	152.0755	7.5959	podocarpic acid	275.172	3.8764
adonitol/arabitol	153.079	8.4557	Homatropine	276.1514	2.762
4-deoxyribose	154.0905	7.7181	linolenic acid	279.2302	0.974
diphenyl	155.0777	4.8341	Piperine	286.139	2.132
methylcoumarin	161.0618	1.0294	Isopilosine	287.1357	2.4398
L-2-amino adipic acid	162.0799	4.2859	7-shogaol	291.1887	4.1135
levoglucosan	163.0628	100	Pukateine	296.1367	0.7118
eugenol	165.0855	8.4339	Convolvamine	306.1629	1.7648
phenylalanine	166.0922	10.241	Bioallethrin	313.2726	12.8033
synephrine	168.0994	32.6556	8-dehydrogingerdione	319.1892	1.5626
2,3-dimethoxyhydroquinone	171.066	0.8038	incensole oxide	323.2634	0.949
shikimic acid	175.062	10.1962	deoxyharringtonine	330.1772	1.1885
DL-a aminopimelic acid	176.0905	5.3511	Carnosol	331.1828	1.885
cinnamyl acetate	177.0838	4.0369	integerrimin	336.1791	0.7261
dimethyl-L-tartrate	179.0593	3.0288	10-gingerdione	349.2333	2.1386
glucosamine	180.0902	35.5968	incensole oxide acetate	365.2665	0.9937
coniferyl alcohol	181.0892	21.0621	9-amino-campthothecin	368.156	0.5496
mannitol	183.0873	6.8052	eleutheroside B	373.1404	0.1661
n-acetyl-L-glutamine	189.0821	2.7638	cholecalciferol	385.3388	3.0758
4-methylmethoxycoumarin	191.0687	0.7431	Solanidine	398.3456	4.7657
carvacryl acetate	193.1216	8.4651	mogroside backbone (-3H ₂ O)	423.3612	4.7064
ferulic acid	195.0668	1.1773	alpha-tocotrienol	425.3518	8.6566
DL-a-methyl-m-tyrosine	196.0951	4.9624	hecogenin	431.317	0.9077

Table 2. Summary of *in vitro* inhibition activity (IC₅₀ values) of the nettle extract against COX-1, COX-2, tryptase, HPGDS, and H₁ receptor endpoints associated with allergic rhinitis. Values for one standard deviation are provided and n = 3 in all cases

Target	IC ₅₀ value (µg mL ⁻¹)
H ₁ negative agonist	193 (±71)
H ₁ antagonist	251 (±71)
Tryptase	172 (±28)
COX-1	160 (±47)
COX-2	275 (±9)
HPGDS	295 (±51)

4-shogaol, piperine, 8-dehydrogingerdione, deoxyharringtonine and carnosol have theoretical IC₅₀ values for inhibition of the H₁-receptor between 35 and 243 µM (Table 3).

Inhibition of mast cell tryptase. The nettle extract also effectively inhibits mast cell tryptase activity with an IC₅₀ of 172 (±28) µg mL⁻¹. Tryptase is a mast cell secretory protease, and functions in mast-cell-related allergic and inflammation responses (Rice *et al.*, 2000b). Tryptase controls mast-cell degranulation which releases prostaglandins, particularly Prostaglandin D₂, as well as cytokines, leukotrienes, and other factors that initiate

Table 3. List of bioactives in the nettle extract determined to contribute to the *in vitro* anti-allergy and anti-inflammatory properties assessed. The chemical class, ClogP-(N+O) values, and theoretical IC₅₀ values are provided. The theoretical IC₅₀ values for inhibition of H₁ receptor function, tryptase, COX-1, COX-2 and HPGDS were determined based on the relative abundance measurements from the DART-MS analysis

Compound Name	Molecular Mass (M)	Chemical Class	ClogP – (N+O)†	Theoretical IC ₅₀ (µM)					
				H ₁ Agonist	H ₁ Antagonist	Tryptase	COX-1	COX-2	HPGDS
Resorcinol	110.037	phenol	-1.8	828	1077	618	1261	978	2248
6-azacytosine	112.039	alkaloid	-5.1	2665	3466	1988	4059	3148	7235
Proline	115.063	amino acid	-3.4	285	371	213	434	337	774
Levulinic acid	116.047	fatty acid	-3.1	3175	4129	2369	4836	3751	8620
Threonine	119.058	alkaloid	-5.4	109	141	81	165	128	295
Vitamin B3 (niacinamide)	122.048	vitamin	-0.3	335	436	250	511	396	910
Leucine	131.095	amino acid	-4.6	347	452	259	529	410	943
Adenine	135.055	alkaloid	-5	3500	4551	2611	5331	4134	9502
Levoglucoson	162.053	sacharide	<-5.0	5894	7665	4398	8979	6963	16 003
Synephrine	167.095	alkaloid	-3.1	1867	2427	1393	2843	2205	5068
Shikimic acid	174.053	phenolic acid	-6.2	560	728	418	853	662	1520
DL-methyl-m-tyrosine	195.09	amino acid	-6.2	243	317	182	371	287	661
4-methyl-7-ethoxycoumarin	204.079	coumarin	-0.2	1112	1446	830	1694	1314	3020
Vitamin B5	219.111	vitamin	-7.5	137	179	103	209	162	373
Isopropyl-b-D-thio-galactopyranoside	238.088	sacharide	-5.6	39	51	29	59	46	106
osthole	244.11	coumarin	0.6	274	356	204	417	323	743
Phosphatidylcholine	242.115	phospholipid	ND*	108	140	80	164	127	292
4-shogaol	248.121	phenol	-0.2	60	78	45	91	71	163
Piperine	285.137	alkaloid	-1.3	72	93	54	109	85	195
8-dehydrogingerdione	318.183	phenol	0.1	47	61	35	71	55	127
deoxyharringtonine	329.163	alkaloid	-6.3	35	45	26	53	41	94
carnosol	330.183	phenol	0.6	54	71	41	83	64	148

† ClogP – (N + O) < 0 = chemical not likely to cross BBB.

* ND = could not be determined.

the allergic response (Rice *et al.*, 2000a; Skoner, 2001). Inhibition of tryptase will therefore block mast-cell degranulation preventing the release of a host of pro-inflammatory mediators into the bloodstream that generate the symptoms of allergic rhinitis. Among the bioactives in nettle, several have theoretical IC₅₀ values <80 µM (Table 3), and these include many of the same bioactives that are effective against the H₁-receptor.

Inhibition of prostaglandin synthesis. Cyclooxygenases (COX-1 and COX-2) are key enzymes that trigger many inflammation events associated with allergic rhinitis (FitzGerald, 2003). By inhibiting COX-1 and COX-2 enzymes, the conversion of arachidonic acid to prostaglandins is prevented. Prostaglandins are known inflammatory mediators that trigger many downstream responses associated with allergies and arthritis (Trivedi *et al.*, 2006). Hematopoietic Prostaglandin D₂ synthase (HPGDS) is located in mast cells and basophils (FitzGerald, 2003; Prussin *et al.*, 2003; Nantel *et al.*, 2004), and converts COX-1 and COX-2 prostaglandin products into Prostaglandin D₂. Prostaglandin D₂ is a specific pro-inflammatory allergic mediator released from mast cells after degranulation and from basophils. The IC₅₀ values for the nettle extract COX-1 and COX-2 inhibition were 160 (±47) µg mL⁻¹ and 275 (±9) µg mL⁻¹, respectively (Table 2). The IC₅₀ value for the inhibition of HPGDS was 295 (±51) µg mL⁻¹ (Table 2). The bioactives in nettle block the upstream processes, COX-1 and COX-2, in the allergic pro-inflammatory pathways, and inhibit HPGDS activity which blocks the formation of specific allergy-related prostaglandins.

Key bioactives in the nettle extract include 4-shogaol, deoxyharringtonine and carnosol that are effective inhibitors of COX-1 and COX-2, and possess theoretical IC₅₀ values between 40 and 90 µM (Table 3). These same compounds show theoretical IC₅₀ values between ca. 90 and 170 µM for inhibition of HPGDS (Table 3). Key bioactives found in here in the nettle extract (Table 3) and reported to have COX-1 or COX-2 inhibitory activity include resorcinol (Mutoh *et al.*, 2000) and Vitamin B3 (Jonas *et al.*, 1996). Both of these compounds show COX-1 and COX-2 inhibition activities, though not as great as other bioactives identified in the nettle extract (Table 3). Gingerols, particularly 4-shogaol and 8-dehydrogingerdione, are likely inhibitors of inflammation (Funk *et al.*, 2005; Grzanna *et al.*, 2005), as well as polyphenolics like shikimic acid (Pajonk *et al.*, 2006). The amino acid tyrosine has been shown to absorb specific allergens released by mast cells (Palmer *et al.*, 1977) and the presence of DL-methyl-m-tyrosine in the nettle extract likely reduces the load of endogenous allergens such as histamine and Prostaglandin D₂ in the body. Coumarin derivatives such as 4-methyl-7-ethoxycoumarin and osthole are also likely contributors to the anti-inflammatory and anti-allergy components of nettle (Liu *et al.*, 1998; Matsuda *et al.*, 2002).

The identified bioactive phenols and alkaloids including shogaol, dehydrogingerdione, piperine, deoxyharringtonine and carnosol have theoretical IC₅₀ values below 100 µM (Table 3), indicating that these compounds are the major contributors to the *in vitro* activity of the nettle extract. Although phenols have been reported to have anti-inflammatory properties (Grzanna *et al.*, 2005; Jia *et al.*, 2007), this is the first report that these specific phenols, as well as the alkaloids present in nettle, contribute to the *in vitro* anti-allergenic activity of nettle.

CONCLUSIONS

Although the historic use of nettle for inflammatory diseases such as allergies is well documented, the precise role of nettle in the pro-inflammatory pathways that characterize hay fevers has been obscure. The nettle extract examined here shows strong *in vitro* inhibition activity against several endpoints associated with allergic rhinitis. These inhibitory activities are the result of direct effects of the functional bioactives in the extract on the receptors and enzymes evaluated and not due to cellular toxicity of the extract (data not shown). In addition to the inhibition of H₁-receptors, through antagonistic and negative agonistic activity, the bioactive constituents in the nettle extract inhibit the HPGDS enzyme, reducing the production of Prostaglandin D₂, an allergy specific pro-inflammatory prostaglandin, *in vitro*. Additionally, the nettle extract inhibits mast cell tryptase *in vitro*. Tryptase is a central regulator of mast cell degranulation and the subsequent release of a range of pro-inflammatory cytokines and chemokines that cause allergy symptoms such as sneezing, nasal congestion, itchy and watery eyes and related discomfort. Lastly, bioactives in nettle are *in vitro* inhibitors of the COX-1 and COX-2 enzymes that are upstream in the inflammatory pathway and block the formation of a range of pro-inflammatory prostaglandins. The combination of these inhibitory activities will likely contribute to the known benefits of nettle for inflammatory disorders (Tunon *et al.*, 1995; Konrad *et al.*, 2005). The nettle extract described here has broad *in vitro* anti-inflammatory activities and the synergistic interactions of the many functional bioactives present in this nettle extract address multiple steps in the pro-inflammatory cascade associated with inflammatory disorders including allergic rhinitis.

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