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ORIGINAL ARTICLE

Kynurenic acid content in anti-rheumatic herbs

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Abstract

Introduction: The use of herbal medicines is common among people living in rural areas and increasingly popular in urbanized countries. Kynurenic acid (KYNA) is a metabolite of kynurenine possessing anti-inflammatory, anti-oxidative and pain reliving properties. Previous data indicated that the content of KYNA in the synovial fluid of patients with rheumatoid arthritis is lower than in patients with osteoarthritis. Rheumatoid arthritis is a chronic, systemic inflammatory disorder affecting about 1% of the world's population.

Aim: The aim of the presented study was to investigate the content of KYNA in 11 herbal preparations used in rheumatic diseases.

Materials and methods: The following herbs were studied: bean pericarp, birch leaf, dandelion root, elder flower, horsetail herb, nettle leaf, peppermint leaf and willow bark. An anti-rheumatic mixture of the herbs Reumatefix and Reumaflos tea were also investigated. The herbs were prepared according to producers' directions. In addition, the herbal supplement Devil's Claw containing root of *Harpagophytum* was used. KYNA content was measured using the high-performance liquid chromatography method, and KYNA was detected fluorometrically.

Results: KYNA was found in all studied herbal preparations. The highest content of KYNA was found in peppermint, nettle, birch leaf and the horsetail herb. The lowest content of KYNA was found in willow bark, dandelion root and in the extract from the root of *Harpagophytum*.

Conclusion: These findings indicate that the use of herbal preparations containing a high level of KYNA can be considered as a supplementary measure in rheumatoid arthritis therapy, as well as in rheumatic diseases prevention.

Key words

arthritis, herb, kynurenic acid

INTRODUCTION

Kynyrenic acid (KYNA) is a metabolite of tryptophan formed along kynurenine pathway. It is an endogenous antagonist of ionotropic glutamate receptors and α7 nicotinic acetylcholine receptor [1, 2]. Moreover, it is an agonist of the G-protein-coupled receptor (GPR35), which is predominantly expressed in immune and gastrointestinal tissues [3]. It was stated that KYNA has anti-inflammatory [4, 5], anti-oxidative [6] and analgesic properties [7]. A previous study indicated that the content of kynurenic acid (KYNA) in synovial fluid of patients with rheumatoid arthritis is lower than in patients with osteoarthritis [8, 9]. Rheumatism is a term used to describe numerous medical problems affecting the joints and connective tissue. The symptoms include pain, weakness, inflammation and swelling. There are more than 100 rheumatic diseases. One of them is rheumatoid arthritis. This is a chronic, systemic inflammatory disorder. About 1% of the world's population is afflicted by rheumatoid arthritis.

Herbal medicines and traditional treatments are common among people living in rural areas [10]. The World Health Organization (WHO) estimates that in some Asian and African countries, even 80% of the population depend on traditional medicine for primary health care [11].

In recent years, the developed regions of Europe, North America, Australia and New Zealand have increasingly using a variety of herbal medicines and commercial mixtures of herbs [10, 11]. Therefore, the aim of the presented study was to investigate the content of KYNA in chosen herbal preparations used in rheumatic diseases.

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MATERIALS AND METHOD

Herbal preparations. The following herbs were studied: bean pericarp (*Phaseoli pericarpium*), birch leaf (*Betulae folium*), dandelion root (*Taraxaci radix*), elder flower (*Sambuci flos*), horsetail herb (*Equiseti herba*), nettle leaf (*Urtice folium*), peppermint leaf (*Menthae piperitae folium)*(all obtained from Kawon, Gostyn, Poland) and willow bark (*Salicis cortex* (Flos, Mokrsko, Poland). Moreover, an anti-rheumatic mixture of the herbs: Reumatefix (*Salicis cortex, Urtice folium, Equiseti herba, Betulae folium, Menthae piperitae folium, Taraxaci folium and radix* (Herbapol, Kraków, Poland) and Reumaflos tea (*Phaseoli pericarpium, Equiseti herba, Betulae folium, Urtice folium* (HB Flos, Płońsk, Poland) were investigated. The herbs were prepared according to producers' directions. The herbal supplement Devil's Claw (NOW Foods, Bloomingdale, IL 60108, USA) in the form of capsules containing root of Harpagophytum (*Radix Harpagophyti*) was also used.

KYNA determination. The content of KYNA in all chosen herbs was investigated according to the method described previously [12]. In brief, herbs were prepared according to producers 'guidelines. Obtained extracts were centrifuged

(4,000 rpm, 5 min) and 1 ml of supernatant was collected for further experiments. Capsules were weighed and distilled water added to them (1:5 w/v). They were then homogenized and centrifuged (4,000 rpm, 5 min) and 1 ml of supernatant was collected for further experiments. Subsequently, proteins were denaturated with 50% trichloroacetic acid and removed by centrifugation (4,000 rpm, 5 min). The supernatant was acidified with 0.1 N HCl, and applied to columns containing cation exchange resin (Dowex 50 W+: 200–400 mesh) prewashed with 0.1 N HCl. Subsequently, the columns were washed with 1 ml of 0.1 N HCl and 1 ml of water. Fraction containing KYNA was eluted with 4 ml of water. Eluate was subjected to high performance liquid chromatography (HPLC) and KYNA was detected fluorometrically (Hewlett Packard 1050 HPLC system: ESA catecholamine HR-80, 3 µm, C18 reverse-phase column, mobile phase: 250 mM zinc acetate, 25 mM sodium acetate, 5% acetonitrile, pH 6.2, flow rate of 1.0 ml/min; fluorescence detector: excitation 244 nm, emission 398 nm).

Statistics. Data are expressed in microgram of KYNA/g dry weight and presented as a mean value ± standard error of the mean (SEM).

RESULTS

The highest concentration of KYNA was detected in peppermint leaves, nettle leaves and birch leaves. An intermediate level of KYNA was found in horsetail herb, Reumaflos tea, elder flower and Reumatefix. Low content of KYNA was assessed in bean pericarp and willow bark. Dandelion root and Devil's Claw capsules composed of root of Harpagophytum contained a very low amount of KYNA (Tab. 1).

Table 1. Content of kynurenic acid in herbal preparations.

Herbal preparation	Form	KYNA [µg/g dry weight]
Peppermint leaf *(Menthae piperitae folium)*	loose herbs	3.82 ± 0.46
Nettle leaf (*Urtice folium*)	loose herbs	2.71 ± 0.38
Birch leaf (*Betulae folium*)	loose herbs	2.68 ± 0.40
Horsetail herb (*Equiseti herba*)	loose herbs	2.27 ± 0.36
Reumaflos tea	sachets	2.08 ± 0.35
Elder flower (*Sambuci flos*)	loose herbs	1.73 ± 0.19
Reumatefix	sachets	1.66 ± 0.23
Bean pericarp *(Phaseoli pericarpium)*	loose herbs	0.57 ± 0.07
Willow bark (*Salicis cortex*)	loose herbs	0.26 ± 0.05
Dandelion root (*Taraxaci radix*)	loose herbs	0.05 ± 0.01
Root of Harpagophytum (*Radix Harpagophyti*, Devil's Claw)	capsules	0.03 ± 0.01

Data are means ± standard error of the mean (SEM).

The highest content of KYNA in a maximum daily dose of herbal medicines appeared in elder flower, peppermint, nettle and birch leaves. An intermediate level was estimated in horsetail herb, Reumatefix, bean pericarp and Reumaflos tea. A low level was assessed in willow bark and dandelion root, and the lowest in the root of *Harpagophytum* (Tab. 2).

Table 2. Content of KYNA in a maximum daily dose of herbal medicines.

Herbal preparation	Maximum daily dose*	KYNA [µg/day]
Elder flower (*Sambuci flos*)	6 x 3 g	31.11
Peppermint leaf *(Menthae piperitae folium)*	3 x 2 g	22.91
Nettle leaf (*Urtice folium*)	3 x 3 g	24.37
Birch leaf (*Betulae folium*)	3 x 3 g	24.15
Horsetail herb (*Equiseti herba*)	3 x 2 g	13.61
Reumatefix	3 x 2 g	9.98
Bean pericarp *(Phaseoli pericarpium)*	3 x 5 g	8.51
Reumaflos tea	2 x 2 g	8.32
Willow bark (*Salicis cortex*)	1 x 3 g	0.78
Dandelion root (*Taraxaci radix*)	3 x 3 g	0.46
Root of harpagophytum (*RadixHarpagophyti*, Devil's claw)	3 x 2 capsules	0.12

* Maximum daily dose specified by the supplier. Data were calculated according to the content of KYNA in herbs depicted in Table 1, and maximum daily dose specified by the supplier.

DISCUSSION

KYNA is a constituent of human synovial fluid [8, 9, 13]. Its level was found to be lower in patients with rheumatoid arthritis in comparison with patients with osteoarthritis [8, 9]. In *in vitro* studies, KYNA inhibited the proliferation and viability of fibroblasts and enhanced the anti-proliferative action of antirheumatic drugs, celecoxib and nimesulide [13]. Moreover, KYNA's anti-inflammatory [4, 5] and anti-oxidative [6] action was documented. These findings suggest that KYNA may be implicated in the pathogenesis of arthritis. Therefore, we investigated the presence and measured the content of KYNA in antirheumatic herbal preparations. A high content of KYNA was found in peppermint, nettle and birch leaves. An intermediate level was detected in herbal mixtures like Reumaflos and Reumatefix. It should be noted that they are composed of herbs containing a high amount of KYNA, e.g. nettle leaf and birch leaf. Surprisingly, a very low content of KYNA was found in the popular anti-rheumatic dietary supplement Devil's claw. For preparation of this supplement, the roots of the plant *Harpagophytum procumbens* are used**.** Similarly, low level of KYNA was found in dandelion root. This is in agreement with our previous study which showed that in fresh plant material of dandelion the content of KYNA the in roots is over 40 times lower than in the leaves [14]. Likewise, the content of KYNA in the herbal preparation of nettle roots was 30 times lower than in the leaves [14]. These findings point to the conclusion that KYNA concentration is higher in leaves in comparison to the roots of plants.

The calculation of KYNA content in maximal daily dose of the investigated herbal preparations showed that the highest amount of KYNA can be delivered with elder flower, peppermint, nettle and birch leaves. Since KYNA is absorbed from the digestive system [12], it seems that the use of the tested herbal preparations can be considered as a supplementary measures in rheumatoid arthritis therapy, as well as in rheumatic diseases prevention. Therefore, the pharmacological properties of KYNA and its presence in high concentration in some medicinal herbs may suggest that it should be considered a new valuable dietary supplement in rheumatology.

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