Inhibition of Xanthine Oxidase by Some Iranian Plant Remedies Used for Gout

Ali Roohbakhsh[1], Jamal Shamsara[1], Mohammad Hasanzadeh Khayyat[2], Gholamreza Karimi[3,4]

1. School of Medicine, Rafsanjan University of Medical Sciences, Rafsanajan, Iran.
2. Department of Medicinal Chemistry, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Department of Pharmacodynamics & Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
4. Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Summary

Gout is a metabolic disease with a distinct increase in the uric concentration in body fluids. Xanthine oxidase is an enzyme that converts hypoxanthine and xanthine to uric acid and has an important role in pathogenesis of some diseases. Flavonoids are a big group of natural compounds and have a distinct inhibitory activity against xanthine oxidase. In vitro assay of xanthine oxidase inhibitory activity was determined by measurement of decreased uric acid absorbance by UV spectroscopy at 295 nm. Aqueous extracts of *Mentha pulegium*, *Hypericum perforatum* and *Matricaria chamomilla* had dominant inhibitory activity on xanthine oxidase. *Cynara scolymus* and *Phasaeolus vulgaris* also showed some xanthine oxidase inhibitory activity. *Hedera hlix*, *Trachyspermum copticum, Zea mays, Cinnamomum zeylanicum, Fraxinus excelsior, Cichorium intybus, Capparis spinosa* and *Trigonella foenum-graceum* had no significant effect on xanthine oxidase activity. As *Matricaria chamomilla* also has anti-inflammatory effects, it can be useful for treatment of gout in which inflammation is an important problem.

Keywords: Gout, Matricaria *chamomilla*, Xanthine oxidase

Corresponding Author:

Gholamreza Karimi
Address: Department of Pharmacodynamics & Toxicology, School of Pharmacy, Mashhad, Iran.
P.O.Box 91775-1365
Tel: +98-511-8823255
Fax: +98-511-8823251
Email: Karimig@mums.ac.ir

Introduction

Gout has been typically associated with excess consumption of rich food and wine. The incidence of gout is increasing and because of limited therapeutic options, treatment of gout remains challenging. Hyperuricaemia is defined as serum uric acid>0.42 mmol/L (1). Overproduction or underexcrition of uric acid causes huperuricaemia or gout. Purines (ATP, GTP, and nucleic acids) metabolize to uric acid and excretion of uric acids removes nitrogenous wastes from body. In supersaturated concentration it forms monosodium urate crystals and deposits in joints and induce inflammatory response and mechanical pressure (2, 3).

Urate-lowering therapy is the main approach in treatment of gout. The target level of serum uric acid usually is <0.36 mmol/L to dissolve the urate crystals and inhibit gout attack (1, 3). Urate-lowering therapy could be done by decreasing the uric acid production or increasing its excretion. Allopurinol and its metabolite, oxypurinol inhibit xanthine oxidase resulted in decrease of uric acid production regardless of cause of hyperuricaemia. However, approximately 20% of patients experience side-effects (including gastrointestinal intolerance and skin rash) with allopurinol, with up to 5% discontinuing therapy. Probenecid and benzbromarone increase uric acid excretion. The main problems with these drugs are urate nephropathy or the formation of uric acid stones (1, 4, 5). Combination therapy is a next choice for resistant cases. Febuxostat is a new agent which is a non-purine-selective inhibitor of xanthine oxidase. Febuxostat inhibits both the oxidized and reduced forms of xanthine oxidase. It is more selective than allopurinol and has a longer half live. Treatment of gout by febuxostat is effective, safe and promising but more long-term data are required on clinical efficacy and safety (6). Uricase or uric oxidase is an enzyme which catalyses the conversion of uric acid to alantoin. It has an advantage in comparison to allopurinol and can break down existing urate. The most common adverse effect with uricase is gout flare and another one is the formation of hydrogen peroxide as a byproduct (6, 7). Despite of increasing incidence of gout the current therapeutic options for it were limited and many patients remain untreated.

Wide variety of plants used by Iranian people for the treatment of gout or diseases with similar symptoms such as rheumatism or arthritis, but no systematic investigations on xanthine oxidase inhibitory activity of these plants have been reported until now and we postulate that these may contain xanthine oxidase inhibitors. The putative therapeutic effects of these traditional medicines may be attributed due to the presence of flavonoids and certain other phenols. Thus, screening of the various plant extracts for the xanthine oxidase inhibitor activity may play a crucial role in identifying a potent chemical entity for treating gout and related inflammatory disorders.

In the present study, 13 medicinal plants were selected to screen their xanthine oxidase inhibitor activity based on their ethnomedical use in the treatment of gout and rheumatic swellings of the joints.

Material and methods

General

Xanthine and xanthine oxidase were purchased from sigma (Germany). Allopurinol was purchased from EGIS (Hungury). Other chemical and reagents were reagent grade and were purchased from sigma (Germany).

Plant collection

The plant materials collected through 2007-2008 from different parts of Mashhad. They were authenticated by department of pharmacognosy of our institute and preserved in Department of pharmacognosy, School of pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

Preparation of crude extracts

The air-dried plant materials were cut into small pieces. 10-15 grams of them were extracted with 200 ml of water at 85º C for 5 min. Then it was filtered and concentrated. We used different pats of plants which noted in table 1.

Assay of xanthine oxidase activity

The inhibitory effects of extracts on xanthine oxidase activity were determined spectrophotometrically at 295 nm under aerobic condition (8). The reaction mixture consisted of 80 mM sodium pyrophosphate buffer (pH 7.5), 120 mM xanthine and 0.1 U enzyme. The increase in absorption at 295 nm indicating the formation of uric acid at 25°C were followed and the initial velocity was calculated. Water extracts dissolved directly in the buffer. All determinations were performed in duplicate. All samples were assayed for xanthine oxidase inhibitory activity at concentrations of 10, 50, 100, 150, 200 and 300 µg/ml (dried extract:ml), respectively. Those showing greater than 50% inhibition at 300 µg/ml were tested further to ascertain the determined values and calculate IC_{50}.

Statistical analysis

Values were represented as mean. Data were analyzed using ANOVA followed by Tukey-Kramer test by SigmaPlot 6.0 (Systat Software Inc.). $P < 0.05$ was considered significant. The IC_{50} was calculated by CalcuSyn 2.0 (Biosoft).

Results

Xanthine oxidase inhibitory activity

As the results were shown in the table 1, among the plant extracts were tested, the *Mentha pulegium*, *Matricaria chamomilla* and *Hypericum perforatum* showed the highest xanthine oxidase inhibitory activity. They inhibited xanthine oxidase 61.4%, 55.9%and 35% at 300 µg/ml respectively. *Cynara scolymus* and *Phasaeolus vulgaris* also inhibited xanthine oxidase significantly (25% and 26% at 300 µg/ml, respectively). *Hedera hlix*, *Trachyspermum copticum*, *Zea mays*, *Cinnamomum zeylanicum*, *Fraxinus excelsior*, *Cichorium intybus*, *Capparis spinosa* and *Trigonella foenum-graceum* had no significant effect on xanthine oxidase activity. The IC_{50} value of allopurinol,

being clinically used as a xanthine oxidase inhibitory drug, was 7.6 μM under the assay condition.

Table 1. Xanthine oxidase inhibitory activity: inhibition of xanthine oxidase by aqueous extracts from different plants at a final concentration of 10-300μg/ml. Each value is represented from duplicate measurements. The IC_{50} was also reported. (*: P<0.05, **:P<0.01,***:P<0.001)

Species/family	Parts	%Xanthine oxidase inhibition						IC_{50} (μg/ml)
		10 μg/ml	50 μg/ml	100 μg/ml	150 μg/ml	200 μg/ml	300 μg/ml	
Hedera hlix Araliaceae	Leaf	1.9	1.9	2.9	7.5	3.1	5.8	-
Phaseolus vulgaris Papilinaceae	Fruit	3.3	4.8*	9.3***	16***	19.2***	26***	>300
Trachyspermum copticum Umbelliferae	Fruit	0	0	0	0.9	0.9	0.9	-
Zea mays Graminaceae	Aerial parts	0.5	1.8	1.8	0	0	0	-
Cinnamomum zeylanicum Lauraceae	Aerial parts	0	0	0	0	0	4.9	-
Mentha pulegium Labiatae	Aerial parts	0	10***	27***	41.2***	48***	61.4***	207.1
Fraxinus excelsior Oleaceae	Leaf	1.1	2.7	4.7	1.5	6.2	0	-
Cichorium intybus Compositae	Aerial parts	0	0	0	0	0	0	-
Matricaria chamomilla Compositae	Aerial parts	0	9.6***	25***	35.2***	40***	55.9***	249.4
Cynara scolymus Compositae	Aerial parts	3.2	4.6*	7.6**	12***	20***	25***	>300
Hypericum perforatum Hupericaceae	Aerial parts	0	0	5.4	20***	34.5***	35***	336.1
Capparis spinosa Capparidaceae	Aerial parts	1.7	0.3	6.9	2.3	0	1.1	-
Trigonella foenum-graceum Papilionaceae	Aerial parts	5.2	4.9	3	0	0	0	-

Discussion

Our results indicate that *Mentha pulegium*, *Hypericum perforatum* and *Matricaria chamomilla* inhibited xanthine oxidase enzyme significantly. *Cynara scolymus* and *Phaseolus vulgaris* also inhibited the xanthine oxidase but to lower extents. As in traditional medicine they were used for management of gout it is thought that their anti-gout activity, at least by part, was related to this xanthine oxidase inhibitory activity. These plants have high level of flavonoids (luteolin, apigenin, and hesperidin), xanthone and coumarin (9) which the xanthine oxidase inhibitory activity of some plants containing these components (especially flavonoids) has been proven previously (10-14). Therefore, it seems that the inhibitory effects of the water extract of these plants are mainly dependent to flavonoids.

Previous phytochemical attention to the *Mentha pulegium* and *Cynara scolymus* had led to characterization of hesperidin and rutin respectively (9), which was found to be a xanthine oxidase inhibitor (15, 16). The flavonoids luteolin and apigenin, and quercetin isolated previously from the flower of *Matricaria chamomilla* (9), were shown to be xanthine oxidase inhibitory (10, 12, 17). *Hypericum perforatum* was indicated to also contain quercitrin and rutin (9). From these published results, it could be predicted that most of the natural xanthine oxidase inhibitors present in the active extracts might be polyphenols. *Phaseolus vulgaris* also contains flavonoids (9).

We postulated that the inhibition of xanthine oxidase enzyme by these plants was mainly dependent on their flavonoids components whereby decreased xanthine oxidase activity. As inhibitory mechanism of quercetin (11) was mixed and results of other studies demonstrated the mixed type inhibitory effects of some anti-gout plants (18), the inhibitory effects of these plant extracts also might be mixed. According to previous studies and our results it seems that *Matricaria chamomilla* is a good choice for treatment of gout due to its anti-inflammatory (19) and xanthine oxidase inhibitory activity. We suggest the further investigation on serum uric acid lowering effect of these tested plants especially *Matricaria chamomilla in vivo*.

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