Angiotensin I Converting Enzyme Inhibitory Activities of Hydroalcoholic Extracts of Nardostachys jatamansi, Prangos ferulacea and Marrubium vulgare

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1. Background

Hypertension is one of the major risk factors for cardiovascular diseases. Lifestyle changes, physical exercise and intake of healthy diet are some common issues associated with reducing the risk of hypertension. There are several classes of pharmacological agents used in the treatment of hypertension. Angiotensin I converting enzyme (ACE) cleaves angiotensin I to angiotensin II and inactivates bradykinin. The synthetic ACE inhibitors are used widely to treat cardiovascular disorders. They may cause many adverse effects, including dry cough, allergic reactions and skin rashes, so investigation for new natural sources could be helpful.

Objectives: The aim of the present study was to evaluate angiotensin I converting enzyme inhibitory activities of hydroalcoholic extracts of Nardostachys jatamansi, Prangos ferulacea and Marrubium vulgare.

Materials and Methods: ACE inhibitory activity was measured according to the methods of Cushman & Cheung with some modifications. Captopril was used as positive control.

Results: The ACE inhibitory activities of hydroalcoholic extracts were as follows; M. vulgare > N. jatamansi > P. ferulacea. The least IC50 value was related to the hydroalcoholic extract of M. vulgare (0.791 mg/ml). The IC50 values of N. jatamansi and P. ferulacea were 2.147 and 4.057 mg/ml, respectively.

Conclusions: The results supported the traditional antihypertensive use of these plants, especially M. vulgare, by inhibitory effects on ACE enzyme.

Keywords: Angiotensin-Converting Enzyme Inhibitors; Hypertension; Nardostachys jatamansi; Marrubium vulgare
tion estimates that approximately 80% of population in developing countries use the traditional medicine for primary health care. The plant products also play an important role in health care for the remaining 20% (10).

There are many medicinally antihypertensive herbs such as Lime blossom (Tilia europea), Kudzu (Pueraria lobata), Garlic (Allium sativum), Saffron (Crocus sativus), Valerian (Valeriana officinalis), Mistletoe (Viscum album) and Rauwolfia serpentine. They are devoid of side effects like weakness, tiredness, drowsiness, impotence, cold hands and feet, depression, insomnia, abnormal heartbeats, skin rash, dry mouth, dry cough, headache, dizziness, constipation or diarrhea and do not have any interaction with other antihypertensive drugs (11).

The plants in this study were Nardostachys jatamansi (Indian Valerian), Prangos ferulacea and Marrubium vulgare (Horehound). They have been used for hypertension treatment in the traditional medicine (12).

2. Objectives

The aim of the present study was to evaluate angiotensin I converting enzyme inhibitory activities of three plants mentioned above.

3. Materials and Methods

3.1. Chemicals

All the chemicals were purchased from sigma-Aldrich Chemie Gmbh (USA) and Merck (Germany) companies. The chemicals were of analytical grade. ACE (angiotensin-converting enzyme, EC 3.4.15.1) from rabbit lung, substrate (HHL, hippuryl-histidyl-leucine) of ACE (St. Louis, MO) and all other chemicals were used reagent grade chemicals.

3.2. Plant Materials

The rhizome of N. jatamansi and the flowering branches of P. ferulacea were collected in Khuzestan and aerial parts of M. vulgar were collected in Mazandaran province during the flowering period in summer 2010. The plants were identified at the Herbarium of Department of Pharmacognosy, school of pharmacy, Ahvaz, Iran, where voucher specimens were preserved.

3.3. Extraction

The plant material (200 g) was powdered and extracted with EtOH-water (80 - 20) using maceration method. Then the extract was filtered and concentrated under reduced pressure with rotary evaporator (Heidolf, Germany). Next, lyophilized by freeze dryer (Operon, Korea). Finally, the absorbance was measured at 228 nm using a UV-spectrophotometer (Jasco, Japan) (13). The produced hippuric acid was extracted with 500 μL of ethylacetate. After centrifugation (800 g, 15 minutes) 200 μL of the upper layer was transferred into a test tube and evaporated at room temperature for two hours in a vacuum. Hippuric acid was dissolved in 1.0 mL of distilled water. Finally, the absorbance was measured at 228 nm using a UV-spectrophotometer (Jasco, Japan) (13). The produced hippuric acid was extracted with 500 μL of ethylacetate. After centrifugation (800 g, 15 minutes) 200 μL of the upper layer was transferred into a test tube and evaporated at room temperature for two hours in a vacuum. Hippuric acid was dissolved in 1.0 mL of distilled water. Finally, the absorbance was measured at 228 nm using a UV-spectrophotometer (Jasco, Japan) (13).

ACE inhibition test was performed on three samples: positive control, negative control and fractions prepared from three mentioned plants. Captopril was used as positive control.

The following concentrations of 0.0078, 0.0156, 0.0312, 0.0625, 0.125, 0.25 and 0.5 mg/mL of captopril were used. Following extract concentrations were prepared 0.625, 1.25, 2.5, 3.75 and 5 mg/mL by serial dilution method. For blank preparation, borate buffer was used instead of enzyme solution. The negative control had no ACE inhibitory activity and maximum absorbance was related to negative control.

3.5. Statistical Analysis

For all tests, the inhibition assay was performed in triplicate and ACE inhibition percentage calculated as follows:

\[
\%\text{inhibition} = 100 \times \left(1 - \frac{A}{C}\right)
\]

\(A = \text{Absorbance test - Absorbance blank}\)

\(C = \text{Absorbance negative control}\)

Linear chart was plotted for ACE inhibition percentage versus the logarithm of concentration for each sample. The \(IC_{50}\) values were determined by constructing a dose-inhibition curve. The \(IC_{50}\) value was defined as the concentration of inhibitor required to inhibit 50% of the ACE. \(IC_{50}\) values can be used to compare the potency of plants extract.

4. Results

Plants extracted with Ethanol-water (80 - 20) using maceration method and the percentage yield of plant extract entered in Table 1. We evaluated the inhibitory effects of hydroalcoholic extracts of N. jatamansi, P. ferulacea and
M. vulgare on ACE enzyme. The ACE inhibitory activities of plants were represented as percentage ACE inhibition by the extracts. Percentage of ACE inhibition by different concentrations of captopril and studied plants was calculated. The plants demonstrated ACE inhibitory activities in a concentration dependent manner (Figure 1). The IC₅₀ values of hydroalcoholic extract of M. vulgare, N. jatamansi, and P. ferulacea were 0.791, 2.147 and 4.057 mg/mL, respectively and that of standard, captopril, was 0.0087 mg/mL (Figure 2).

Table 1. The Percentage Extract Yield of Studied Plants

<table>
<thead>
<tr>
<th>Plants</th>
<th>Yield</th>
</tr>
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<tbody>
<tr>
<td>Marrubium vulgare</td>
<td>8.63</td>
</tr>
<tr>
<td>Nardostachys jatamansi</td>
<td>6.74</td>
</tr>
<tr>
<td>Prangos ferulacea</td>
<td>11.32</td>
</tr>
</tbody>
</table>

*Data are presented as %.

5. Discussion

This study showed that M. vulgare has the least IC₅₀ (0.791 mg/mL) and the most inhibitory effect among the studied plants. The IC₅₀ of Indian valerian was 2.147 mg/mL, which shows that the inhibitory effect is less than M. vulgare and more than P. ferulacea. P. ferulacea had the minimal inhibitory effect among the other plants with IC₅₀ = 4.057 mg/mL. Previous studies showed antioxidant (14), antibacterial (15) and abortifacient effects of P. ferulacea (16, 17).

In a study, nine herbs used in the traditional medicine of Lebanon were tested about their inhibitory effect on ACE enzyme. N- hexane extract of Hyssopus officinalis had the least IC₅₀ and so the best inhibitory effect among them with IC₅₀ = 52 μg/mL. According to our results, its inhibitory effect is more than our studied plants. Chloroform extract of M. radiatum had IC₅₀ of 110 μg/mL, but the IC₅₀ of hydroalcoholic and n-hexane extract was between 60 - 65 μg/mL. Hydroalcoholic extract of M. vulgare in our study had a less inhibitory effect than hydroalcoholic, n-hexane and chloroform extract of M. radiatum (12).

Previous studies showed analgesic (18), antispasmodic (19), anti-diabetic (20), hepatoprotective (21, 22), antioxidant effects and (23, 24) prevention of hyper-cholesterolemia (25) of M. vulgare.

In a study, 20 traditional herbs of Zulu were selected for screening of ACE inhibitory effect and the most inhibitory effect related to Adenopodia spicata (26).

Antioxidant effect of M. vulgare has been proven due to having flavonoid and phenolic acid. This plant contains tannin and the inhibitory effect of tannins and flavonoid on ACE has been demonstrated (27). In our study it had the least IC₅₀.

Another checking plant is N. jatamansi, which its effects on improving memory and learning (28), treatment of nervous headache (29) and hypertension (30) have been proven.

In a study on aqueous extract of Salviae miltiorrhiza root in Korea for assessing ACE inhibitory effect, obtained IC₅₀ was 170 μg/mL. This plant is a rich source of pigments and some phenolic compounds like lithospermic acid B (31).

In another study on leaves of Vaccinium ashei reade, obtained IC₅₀ was 46 μg/mL. Inhibitory effect is more than the three plants in our study. This plant contains 18.7% tannin. Perhaps, this proper inhibitory effect is related to tannin percentage in this plant. Tannins are polyphenolic compounds that their inhibitory effect on ACE has been proven (32).

Muthuswamy Umamaheswari in India performed a study on seed extract of Apium graveolens Linn. (celery) and observed potential antioxidant and ACE inhibitory effects (IC₅₀ = 666.26 μg/mL). Phytochemical screening of the extract showed that it contains tannins, phenolics, flavonoids glycosides and steroids (33).

Phoenix sylvestris is used as a diuretic in India. A study
found that this plant has 48% inhibitory effect in 0.33 mg/ml concentration. Moreover, if we remove tannins of this plant, its inhibitory effect reduces to 8%, indicating the inhibitory role of tannins (34).

Due to non-specific ACE inhibitors and adverse effects of chemical inhibitors of ACE, it is necessary to investigate for new natural compounds.

The present study was conducted to assess anti-hypertensive effects according to ACE inhibition activity of three different spices used as traditional medicinal herbs in Iran. It is suggested to perform further clinical studies to confirm the effects and safety of the studied plants.

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References


