Specifying Human Platelet cAMP and cGMP Phosphodiesterase Inhibitory Activity of the Plants Used in Traditional Iranian Medicine for the Purpose of Erectile Dysfunction


Abstract: In the present study, cAMP and cGMP phosphodiesterase inhibitory (PDEI) activities of the ethanolic extracts of nineteen plants of Traditional Iranian Medicine (TIM) with aphrodisiac effects were investigated. The plants were extracted in a similar way and then three concentrations (0, 1, 5 mg mL⁻¹) from each were tested for PDEI activity against control and sildenafil. Among plants tested, 8 including Allium cepa, Trigonella foenum-graecum, Brassica rapa, Alpinia officinarum, Tribulus terrestris, Urtica pilulifera, Linum usitatissimum and Zingiber zerumbet exhibited a significant dose-dependent cAMP-PDEI activity and 6 including A. cepa, A. officinarum, T. terrestris, L. usitatissimum, Withania somnifera and Z. zerumbet gave a remarkable dose-dependent cGMP-PDEI effects in comparison to control. Among tested herbs, Zingiber officinalis and Peganum harmala at dose of 5 mg mL⁻¹ demonstrated better cGMP-PDEI in comparison to sildenafil. The results of this study give idea for discovery of safe and better drugs in management of erectile dysfunction.

Key words: Phosphodiesterase inhibitor, sildenafil, traditional Iranian medicine, cAMP, cGMP

INTRODUCTION

Phosphodiesterases (PDEs) are a class of enzymes that exist in nearly all tissues regulating the second messengers cAMP and cGMP involving in many diverse physiological functions. PDE inhibitors (PDEIs) are used for management of clinical disorders such as dementia, depression, cardiovascular diseases, diabetes, chronic obstructive pulmonary diseases, colitis, toxicities, pulmonary hypertension and Erectile Dysfunction (ED) (Abdollahi et al., 2003a, b, c; Icen et al., 2005; Khoshakhlagh et al., 2007; Milani et al., 2005). Inhibitors of PDE type 5 (PDE5) i.e., sildenafil, vardenafil and tadalafil are now used in treatment of ED but have some common side effects like headache, flushing and blurred vision. As reviewed by Rahimi et al. (2010), there are many single and combination herbal formulas in Traditional Iranian Medicine (TIM) for ED that needs experimental and clinical testing. The aim of the present study was to evaluate PDEI activity of the medicinal plants claimed to be beneficial for ED in TIM.

MATERIALS AND METHODS

Materials: Different parts of nineteen plants that were selected on the basis of TIM data were prepared from the clinic of Traditional Medicine, Tehran University of Medical Science (TUMS), Tehran, Iran, in May 2010. Voucher specimens have been deposited in the Central Herbarium of Medicinal Plants (ACECR, Karaj, Iran). All chemicals or reagents needed in this study were purchased from Sigma-Aldrich Company (Germany) unless otherwise stated.

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Extraction method: Dried and semi powdered plants (20 g) were extracted using ethanol 80% (3×60 mL) at room temperature for a week. After removal of the solvent in the vacuum at 30°C, the residues were used for further analysis.

Assay of platelet cAMP and cGMP PDEI activity: The human platelets as a rich source of PDE enzymes were purchased from Tehran blood transfusion organization and stored at 25°C in a shaker during the process of experiment. An aqueous stock solution of 40 mM NaCl, 0.1 mM MnCl₂, 125 µM phenol red were prepared and the pH of this solution adjusted at 8.0. The assay mixture (200 µL) for control contained 53 µL of stock solution and 49 µL of platelets. Then cAMP and cGMP (1.9 mM) were added separately to the wells. The method is based on the absorbance level of the incorporating phenol red with titratable proton liberated from hydrolysis of cyclic nucleotide to corresponding 5'-phosphate ester by PDE (Frielle et al., 1979). The 49 µL of plant extract that was dissolved in dimethyl sulfoxide (DMSO) 10% was added to 53 µL of stock solution and 49 µL of platelets and incubated for 20 min at 25°C. 49 µL of cAMP and cGMP (1.9 mM) were separately added to the wells and then after 5 min the absorbance of the test samples were read at 560 nm using a fluorescence microplate ELISA reader. The extracts with various concentrations ranging from 0.1 to 5 mg mL⁻¹ were tested. Each experiment was carried out in duplicate wells and the absorbance was read triplicate. Sildenafil was used as the standard solution and blank was 53 µL of stock solution and 49 µL of platelets with DMSO 10%. Three concentrations (0.1, 1, 5 mg mL⁻¹) of the extracts and sildenafil (40, 2, 20 µM) were tested.

Statistical analysis: Mean and standard error values were determined for all the parameters and the results were expressed as Mean±SEM. All data were analyzed using analysis of variance ANOVA followed by Newman Keuls. Differences between groups was considered significant when p = 0.05.

RESULTS AND DISCUSSION

Nineteen plants of TIM with aphrodisiac effects were selected and extracted with ethanol. The extraction ratio for each plant was shown in Table 1. As shown in Fig. 1, significant dose-dependent cAMP-PDEI activity were observed from eight plants including *Allium cepa*, *Trigonella foenum-graecum*, *Brassica rapa*, *Alpinia officinarum*, *Tribulus terrestris*, *Urtica pilulifera*, *Linum usitatissimum* and *Zingiber zerumbet*. They showed the most cAMP-PDEI activity at 0.1 mg mL⁻¹, whereas the extracts of *Zingiber officinalis* and *Peganum harmala* demonstrated the most cAMP-PDEI effect at 5 mg mL⁻¹. The rest of the extracts showed similar cAMP-PDEI effect in three tested concentrations in comparison to sildenafil. As shown in Fig. 2, six plants including *Allium cepa*, *Alpinia officinarum*, *Tribulus terrestris*, *Linum usitatissimum*, *Withania somnifera* and *Zingiber zerumbet* showed significant dose-dependent cGMP-PDEI activity. The most cGMP PDEI effect was seen with 0.1 mg mL⁻¹ of these plants, while *Zingiber officinalis* and *Peganum harmala* had the most effect at 5 mg mL⁻¹. The rest of the extracts showed the same cGMP-PDEI effect in the three concentrations used in comparison to sildenafil. In this study, a total of 19 plants were screened for assaying inhibitory potential on platelets cAMP and cGMP PDEIs. In comparison to sildenafil, 10 plants showed better cAMP-PDEI effect and 8 plants exhibited better cGMP-PDEI effect. These extracts showed the most PDEI activity at 0.1 mg mL⁻¹, whereas *Z. officinalis* and *P. harmala* showed a different pattern with the most PDEI effect at 5 mg mL⁻¹ that might be related to antiplatelet activity of both plants (Guh et al., 1995; Im et al., 2009; Thomson et al., 2002). *A. cepa* and *P. harmala* have been previously shown for antiasthmatic effect (Duke, 2002; Wagner et al., 1990). *A. cepa*, *B. rapa*, *L. usitatissimum*, *T. foenum-graecum*, *U. pilulifera*, *W. somnifera*, *Z. officinalis* and *Z. zerumbet* have already shown antioxidant activity (Hu et al., 2007; Singh et al., 2009; Kim et al., 2006). *A. cepa*, *A. officinarum*, *P. harmala*, *T. foenum-graecum*, *W. somnifera*, *Z. officinalis* and *Z. zerumbet* have already shown anticancer or antitumor effects (Brown et al., 2009; Huang et al., 2005; Kadle et al., 2007; Lamechour et al., 1999; Singh et al., 2009; Sux et al., 2001; Yasukawa et al., 2008). *A. cepa* and *Z. officinalis* are currently used as cardiotoxic and cardiovascular (Shoji et al., 1982; Park et al., 2009). *A. officinarum* and *P. harmala* are found as antispasm (Duke, 2002; Gruenwald et al., 2007). *A. officinarum*, *T. foenum-graecum*, *W. somnifera*, *U. pilulifera*, *Z. officinalis* and *Z. zerumbet* are used as anti-inflammatory agents (Aimbire et al., 2007; Kavalali and Tunel, 1997; Lee et al., 2009; Vyas et al., 2008; Zakaria et al., 2010). *P. harmala* and *T. terrestris* have shown vasodilator property (Berrougui et al., 2006; Phillips et al., 2006). *W. somnifera* and *Z. zerumbet* exhibited immunomodulatory action (Rasool and Varalakshmi, 2006; Yob et al., 2011). *P. harmala* and *Z. zerumbet* were identified as analgesic (Farouk et al., 2008; Zakaria et al., 2010b). *P. harmala*, *Z. officinalis* and
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<tr>
<th>Scientific name</th>
<th>Voucher number</th>
<th>Family name</th>
<th>Part used</th>
<th>Extraction ratio (%)</th>
<th>Persian name</th>
<th>Chemical constituents</th>
<th>Pharmacological effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allium cepa L.</td>
<td>1322</td>
<td>Liliaceae</td>
<td>Seed</td>
<td>10</td>
<td>Finiz</td>
<td>Alliins, fructosans, saccharose, othersugars, steroidsaponin, flavonoids (Gruenwald et al. 2007), essential oil (DerMarderosian, 2008)</td>
<td>Antiiasthmatic and antiallergic Wagner et al. (1990), antioxidant, anticancer Singh et al. (2009), cardiovascular support Park et al. (2009)</td>
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<td>Glandelia tournefortii L.</td>
<td>1329</td>
<td>Compositae</td>
<td>Seed</td>
<td>4.2</td>
<td>Kangar</td>
<td>Cal Erie acid derivatives, phenolic content Hagh et al. (2011)</td>
<td>Antioxidant Cherif et al. (2007)</td>
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<td>Scientific name</td>
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<td><em>Trigonella foenum-graecum</em> L.</td>
<td>1335</td>
<td>Papilionaceae</td>
<td>Seed</td>
<td>13.8</td>
<td>Shanbalie</td>
<td>Mucilages, protein, steroidal saponins, sterols, flavonoids, trigonelline, volatile oil Gruenwald et al. (2007)</td>
<td>-</td>
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<tr>
<td><em>Zingiber zerumbet</em> (L.) Sm</td>
<td>1339</td>
<td>Zingiberaceae</td>
<td>Rhizome</td>
<td>9.05</td>
<td>Zorombad</td>
<td>Zerumbone Huang et al. (2005), essential oil Zakaria et al. (2010a), harmalene, monoterpenes, sesquiterpenoids, flavonoids, aromatic compounds Yob et al. (2011)</td>
<td>-</td>
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Fig. 1: Inhibitory effects of 10 extracts on cAMP-PDE activity in vitro. Values are expressed as Mean±SE. **Significantly different from Sildenafil (Sild) groups at (p<0.01).

Fig. 2: Inhibitory effects of 8 extracts on cGMP-PDE activity in vitro. Values are expressed as Mean±SE. **Significantly different from Sildenafil (Sild) groups at (p<0.01).

Z. zerumbet have shown antithrombotic and antiplatelet aggregation effects (Im et al., 2009; Thomson et al., 2002; Guh et al., 1995; Yob et al., 2011). A. officinarum and U. pilulifera increase motility and count of sperm (DerMarderosian, 2008; Irshaid and Mansi, 2009). T. terrestris is currently used in management of ED (Gauthaman and Ganesan, 2008). All of these therapeutic effects could be relevant to PDEI properties (Rahimi et al., 2010). In our previous paper, it was indicated that main components of the plants with PDEI effects are flavonoids, alkaloids, saponins, lignans and coumarins (Rahimi et al., 2010) that are seen in all 19 plants tested here (Table 1). Among tested herbs, Z. officinalis and P. harmala at 5 mg mL⁻¹ showed better cGMP-PDEI in comparison to sildenafil. Z. officinalis includes volatile oils, alkaloid and oleoresins (DerMarderosian, 2008; Gruenwald et al., 2007; Syamkumar et al., 2003) while P. harmala contains alkaloid (Hemmateenejad et al., 2006; Pulpati et al., 2008).

CONCLUSION

The present study for the first time reports PDEI activities of TIM plants that is notable in the field of drug discovery for ED or other relevant diseases. It is required to complete animal and then clinical profile of these extracts. Also, combinative use of these plants may reveal more efficiency in ED with respect to synergetic effect of constituents.

ACKNOWLEDGMENT

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