Effect of saw palmetto extract on urodynamic parameters, bladder contractility and pharmacological receptors in female rats

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ABSTRACT

To clarify the efficacy of saw palmetto extract (SPE), a phytotherapeutic agent, in female rats, its pharmacological effects on urodynamic parameters, bladder contractility and autonomic receptors were investigated. The urodynamic parameters were monitored using a cystometric method, and specific binding of [N-methyl-3H]scopolamine chloride ([3H]NMS) (muscarinic receptors), (-)[125I]cyanopindolol ((-)[125I]CYP) (β-adrenoceptors) and contractile responses in the bladder were measured using radioligand binding assay and organ-bath methods. Orally administered SPE (160 mg/kg) in female rats significantly increased the micturition interval and decreased the frequency of micturition, with a slight increase in the mean micturition volume and decrease in the basal pressure. SPE (0.01-1.0 mg/ml) significantly attenuated the contraction of isolated rat bladder induced by electrical field stimulation and acetylcholine. SPE inhibited specific [3H]NMS binding in the rat bladder in a concentration dependent manner (IC50: 40.9 µg/ml), with little effect on specific ((-) [125I]CYP binding. SPE also inhibited specific [3H]NMS binding in CHO-K1 cell lines expressing human muscarinic M1 to M5 receptor subtypes. These results indicate that SPE improves the detrusor overactivity in female rats by antagonizing bladder muscarinic receptors. Therefore, SPE may be a potential therapeutic agent for improving lower urinary tract symptoms in female patients with overactive bladders.

Key words: Saw palmetto extract; female rats; urological parameters, bladder contractility, muscarinic receptors.

INTRODUCTION

Benign prostatic hyperplasia (BPH) and the associated lower urinary tract symptoms (LUTS) are common disorders in aging men, and LUTS due to overactive bladder (OAB) are also very common in aging women. The typical symptoms include increased frequency of urination, nocturia, urgency, hesitancy, and weak urine stream. Medical therapies to treat BPH and LUTS include α1-blockers, 5α-reductase inhibitors, antimuscarinic agents, phosphodiesterase 5 inhibitors and phyotherapy, several of which can be used in combination. Antimuscarinic agents are commonly used in men and women with LUTS, and the basic and clinical aspects of nine antimuscarinic agents in the treatment of LUTS have been extensively reviewed by Yamada et al. (2018). Medications with antimuscarinic effects are prescribed to elderly people who frequently experience anticholinergic adverse events in the peripheral
and central tissues such as dry mouth, constipation, dry eyes, cognitive dysfunction, confusion and falls (Yamada et al., 2018; Alagiakrishnan and Wiens, 2004; Mate et al., 2015). Currently, selective β-adrenoceptor agonists such as mirabegron and vibegron are clinically used for the treatment of LUTS with fewer adverse events than with antimuscarinic agents (Chapple et al., 2014; Yoshida et al., 2018).

Herbs are widely used in Europe for natural medicines, where half of the German urologists prefer prescribing plant-based extracts to synthetic drugs. Extracts from saw palmetto berries are widely used for the treatment of BPH in men, often as an alternative to pharmaceutical agents (Barnes et al., 2004; Avins et al., 2008; Suzuki et al., 2009; Pagano et al., 2014; Vela-Navarrete et al., 2018). The mechanism of the effect of this extract has been proposed, including inhibition of 5α-reductase (Iehlé et al., 1995), anti-androgenic effects (Sultan et al., 1984), and anti-proliferative effects (Paubert-Braquet et al., 1998). Furthermore, saw palmetto extract (SPE) has α1 adrenoceptor-inhibitory properties (Goepel et al., 1999), anti-inflammatory properties (Koch et al., 2001), and spasmytic activity (Gutierrez et al., 1996). We previously showed that intraduodenally-administered SPE significantly improves urodynamic symptoms in hyperactive bladders of male rats by increasing bladder capacity and prolonging the micturition interval (Suzuki et al., 2009; Oki et al., 2005; Suzuki et al., 2007; Nasrin et al., 2018). Such an improvement of urodynamic symptoms by SPE may arise partly from its binding to pharmacologically-relevant receptors in the urinary bladder (Suzuki et al., 2009; Oki et al., 2005; Suzuki et al., 2007). Notably, Debruyne et al. (2004) showed that SPE (Permixon) as compared with tamsulosin (α-blocker) improved significantly the irritative symptom in severe BPH patients. These results suggest that SPE may be pharmacologically effective in improving LUTS not only in males with BPH but also in females with OAB. Since the effect of SPE on urological function in females has not been clarified, the current study aimed to characterize, in female rats, the pharmacological effects of SPE on urodynamic parameters, bladder contractility, muscarinic receptors and β-adrenoceptors.

**MATERIALS AND METHODS**

**Materials:** [N-methyl-3H]scopolamine methyl chloride ([3H]NMS, 3.03 TBq/mmol) and (-)-[125I]cyanopindolol ([125I]CYP, 81.4 TBq/mmol), as selective radioligands of muscarinic and α-adrenergic receptors, respectively, were purchased from PerkinElmer Life Sciences, Inc. (Boston, MA, USA). Saw Palmetto Extract (SPE) was provided by EUROMED, S.A. (Barcelona, Spain). The most important constituents of saw palmetto fruits are free fatty acids, methyl and ethyl esters of fatty acids, sterols, fatty alcohols, carotenoids, essential oils and polysaccharides (http://www.euromed.es/saw-palmetto-extract/). SPE was suspended in 0.5% methyl cellulose. All other chemicals were obtained from commercial sources.

**Animals**

Female Sprague-Dawley rats at 8-10 weeks of age, used for measurement of urodynamic parameters and receptor binding and female Wistar rats at 12-14 weeks of age, used for measurement of contractile response in isolated bladders, were purchased from Japan SLC, Inc. (Shizuoka, Japan). Rats were housed in the laboratory with free access to food and water and maintained on a 12-h light-dark cycle in a room with controlled temperature (23 ± 1°C) and humidity (55 ± 5%). All experiments were approved by the Ethical Committee for Research at University of Shizuoka and Mukogawa Women's University, and performed in accordance with the guidelines for the Care and Use of Laboratory Animals of both universities. The membrane preparations of stable CHO-K1 cell lines expressing human muscarinic M1 to M5 subtype receptors were obtained from Euroscreen (Brussels, Belgium).

**Measurements of urodynamic parameters by cystometry**

Female rats were anesthetized by urethane (0.8 g/kg i.p., 0.4 g/kg s.c.). The bladder was then exposed through a short midline incision, and polyethylene tubing (SP-45, Natsume, Tokyo, Japan) was inserted into the dome of the bladder and ligated. The bladder catheter was connected via a T connector to a pressure transducer and to an infusion pump. Then, 0.9% saline maintained at 37°C was instilled into the bladder at a rate of 3.0 ml/h. Intravesical pressure was recorded continuously. After the stabilization for about 30 min, the following urodynamic parameters were recorded for 30 min in each animal: micturition interval, mean micturition volume, frequency of micturition, maximum micturition pressure, threshold pressure and basal bladder pressure. Voided urine was cumulatively collected into a urine cup placed on a microbalance. Furthermore, at the end of the cystometry recording, the saline infusion was stopped after confirming the first micturition, and the residual urine was collected by syringe. Absolutely, female rats intravesically pretreated with 0.5% acetic acid were orally administered SPE (160 mg/kg), which was suspended in 0.5% methyl cellulose.

**Measurement of contractile responses in the isolated bladder strips.**

The bladder was removed from each rat under sodium
pentobarbital anesthesia (120 mg/kg, i.p.) and immediately placed in an oxygenated Krebs solution (NaCl 118.4 mM, KCl 4.7 mM, CaCl₂ 2.5 mM, MgCl₂ 1.2 mM, NaHCO₃ 25 mM, Na₂HPO₄ 1.2 mM, glucose 11.1 mM) at 37°C. The upper part of the bladder was vertically divided into four parts. Each part of bladder strip sized approximately 2 mm wide × 5 mm length, was prepared from the middle third of the detrusor. The strips were then mounted in a 10-ml organ bath containing Krebs solution at pH 7.4 maintained at 37°C and constantly gassed with 95% O₂ and 5% CO₂. Isometric tension was recorded using a force-displacement transducer (Model t-7; NEC San-Ei, Tokyo, Japan) coupled to a dual-channel chart recorder (Model 8K21; NEC San-Ei). The strips were then subjected to a passive tension of 3 mg and allowed to equilibrate for 45 to 60 min before further experiments were conducted. Two platinum electrodes were placed on each side of the strips, and electrical field stimulation (EFS, 0.1 or 1.0Hz) was delivered by an Electronic Stimulator SEN-3301 (Nihon Kohden Co., Tokyo, Japan) in the form of single square-wave pulses of 10V, 0.5 ms duration and at 0.1 or 1.0 Hz. The EFS at 1.0 Hz was applied to the strip for 10 s and the stimulation interval was 20 min. Under the 0.1 Hz EFS condition, SPE (0.01-1.0 mg/ml) or vehicle was cumulatively added to the bath. SPE was dissolved in 100 mg/ml dimethyl sulfoxide. The EFS at 1.0 Hz was applied to the strips 10 min after adding SPE (1.0 mg/ml) or vehicle to the strip. The effect of SPE on the acetylcholine-induced contraction of the rat isolated bladder strip was also examined. SPE (0.01-1.0 mg/ml) or vehicle was applied to the organ bath 10 min before the stimulation with 10 μM acetylcholine. Data were calculated as a change in value with 100% as the contractile force at 0.1 Hz or 10 μM acetylcholine.

Measurements of binding activities of pharmacological receptors in the bladder

The autonomic (muscarinic and β-adrenergic) receptor binding activity of SPE in the rat bladder was examined by radioligand binding assay using [³H]N-methylscopolamine (NMS) and (-)[¹²⁵I]cyanopindolol (CYP) as selective radioligands of muscarinic and β-adrenergic receptors, respectively as previously described (Oki et al., 2005; Yamada et al., 1996).

The female rat bladders were dissected, washed with cold saline and minced with scissors. Each tissue was homogenized with a Kinematica Polytron homogenizer in 19 volumes of ice-cold 30 mM Na⁺/HEPES buffer (pH 7.5). The homogenate was centrifuged at 40,000 x g for 20 min at 4°C. The resulting pellet was finally resuspended in the same buffer for the binding assay. The radioligand binding assay for muscarinic receptors was performed using [³H]NMS. In the binding assay, the tissue homogenate was incubated with [³H]NMS (0.10 nM) in 30 mM Na⁺/HEPES buffer (pH 7.5). Incubation was performed for 60 min at 25°C. The reaction was terminated by rapid filtration (Cell harvester; Brandel Co. Ltd., Gaithersburg, MD, USA) through Whatman GF/B glass filters, and the filters were then rinsed 3 times with 3 ml of ice-cold buffer. Tissue-bound radioactivity was extracted from the filters overnight in scintillation fluid, and radioactivity was measured with a liquid scintillation counter. The specific binding of [³H]NMS was experimentally determined from the difference between counts in the absence and presence of 1 μM atropine. Also, the membrane preparations of stable CHO-K1 cell lines expressing human muscarinic M₁ to M₅ subtype receptors were diluted by ice-cold 30 mM Na⁺/HEPES buffer (pH 7.5) for the muscarinic receptor assays.

For the β-adrenoceptor binding assay, the tissue homogenate of the rat bladder was incubated with (-)[¹²⁵I]CYP (0.12 nM) for 60 min at 37°C in 10 mM Tris buffer (10 mM Tris, 154 mM NaCl, 0.55 mM ascorbic acid, pH 7.2). Incubation was terminated by rapid filtration over Whatman GF/C filters. Each filter was washed with an additional 10 ml of ice-cold buffer, and the radioactivity of the filters was determined by a gamma-counter (Beckman Gamma 4000) at an efficiency of 70%. The nonspecific binding of (-)[¹²⁵I]CYP was defined as radioactivity bound in the presence of 1 μM (-)-propranolol. Specific binding was defined as the difference between total binding and nonspecific binding.

Data analysis

Statistical analyses of the data were performed with Student’s t-tests. All data are expressed as the mean ± S.E. Significance was accepted at p<0.05.

RESULTS

Effects on urodynamic parameters

Following the intravesical infusion of saline containing 0.5% acetic acid in female rats as compared with saline infusion alone, the micturition interval was significantly shortened. Mean micturition volume and bladder capacity were also significantly decreased. The single oral administration of SPE at the dose of 160 mg/kg significantly increased the micturition interval (62.6%) and significantly decreased the number (frequency) of micturition (36.2%) in 0.5 % acetic acid-pretreated female rats, as compared with the vehicle administered rats (Figure 1, Table 1). SPE administration also slightly increased the mean micturition volume (26.1%) and decreased the threshold pressure (17.5%) and basal
Figure 1: Representative cystometric traces of bladder pressure and micturition interval of female rats after oral administration of saw palmetto extract (SPE). The typical tracings show cystometry of 0.5% acetic acid-pretreated female rats after oral administration of vehicle (Pre) and SPE (160 mg/kg) (Post).

Table 1: Effect of oral saw palmetto extract (SPE) administration on urodynamic parameters in female rats injected intravesically with 0.5% acetic acid. Urodynamic parameters (micturition interval, micturition volume, frequency of micturition, maximum micturition pressure, threshold pressure, and basal pressure) were measured by the cystometric method in 0.5% acetic acid-pretreated female rats administered orally vehicle or SPE (160 mg/kg). Each value represents the mean ± SE (n=7). Asterisks indicate a significant difference from the values for vehicle-treated rats,*p<0.05.

<table>
<thead>
<tr>
<th>Urodynamic parameters</th>
<th>Vehicle</th>
<th>SPE 160 mg/kg</th>
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<tbody>
<tr>
<td>Micturition interval (min)</td>
<td>5.69 ± 0.79</td>
<td>9.25 ± 1.23*</td>
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<tr>
<td>Micturition volume (mL)</td>
<td>0.46 ± 0.09</td>
<td>0.58 ± 0.07</td>
</tr>
<tr>
<td>Frequency of micturition (number/h)</td>
<td>13.0 ± 1.6</td>
<td>8.30 ± 0.86*</td>
</tr>
<tr>
<td>Maximum micturition pressure (mmHg)</td>
<td>28.3 ± 2.0</td>
<td>28.9 ± 2.8</td>
</tr>
<tr>
<td>Threshold pressure (mmHg)</td>
<td>7.56 ± 0.74</td>
<td>6.24 ± 0.73</td>
</tr>
<tr>
<td>Basal pressure (mmHg)</td>
<td>2.57 ± 0.31</td>
<td>1.84 ± 0.40</td>
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pressure (28.4%). In contrast, the maximum micturition pressure in these rats was unaffected by SPE administration.

Effects on bladder smooth muscles

SPE at concentrations of 0.1 and 1.0 mg/ml exerted a concentration-dependent inhibition (16.0 and 32.4%, respectively) of EFS (0.1 Hz)-induced contraction of isolated rat bladder (Figure 2A, B) and this inhibitory effect of SPE was significant. The lower concentration (0.01 mg/ml) of SPE had little effect on the EFS-induced contraction. SPE (1.0 mg/ml) significantly reduced (74.2%) the contraction of isolated bladder induced by FES at higher frequency (1.0 Hz). Also, SPE at 0.01, 0.1 and 1.0 mg/ml
exerted a concentration-dependent inhibition of acetylcholine (10 µM)-induced contraction of isolated rat bladder and the effect at 1.0 mg/ml was significant (Figure 2C). SPE itself at these concentrations did not alter the basic tone of bladder smooth muscles under the condition without stimulation at FES and 10 μM acetylcholine.

**Effects on pharmacological receptors in the bladder**

SPE at concentrations of 10 to 300 µg/ml inhibited specific [³H]NMS binding in the bladder from female rats in a concentration-dependent manner (Figure 3). The IC₅₀ values of SPE in inhibiting specific [³H]NMS binding was 40.9±5.6 µg/ml. The IC₅₀ value in the bladder of female rats was similar to that previously reported in male rat bladder (46.1±5.6 µg/ml) (Oki et al., 2005). These findings showed significant binding activities of bladder muscarinic receptors by SPE. In contrast, SPE (10-300 µg/ml) had little effect on specific (−)[¹²⁵I]CYP binding in the rat bladder (Figure 3). The [³H]NMS binding effect of SPE may be of a noncompetitive rather than competitive manner, as shown by the significant depression of maximum number of binding sites (Bₙₐₓ values) of the ligand (Suzuki et al.,...
Academia Journal of Medicinal Plants; Yamada et al. 176

Figure 3: Effect of saw palmetto extract (SPE) on specific binding of \[^{3}H\]NMS and (-\[^{125}\]I\]CYP in the bladder of female rats. Muscarinic receptors and β-adrenoceptors in the rat bladder in the absence and presence of SPE (10-300 µg/mL) was measured by radioreceptor binding assay using \[^{3}H\]NMS (0.10 nM) and (-\[^{125}\]I\]CYP (0.12 nM) as selective radioligands of muscarinic and β-adrenergic receptors, respectively. Each point represents the mean of 5 determinations of rat bladder.

Table 2: Inhibition (IC\textsubscript{50} values) by saw palmetto extract of specific \[^{3}H\]NMS binding in membranes of CHO-K1 cell lines expressing human muscarinic M\textsubscript{1} to M\textsubscript{5} receptor subtypes. Each value represents the mean ± SE (n=5).

<table>
<thead>
<tr>
<th>Subtype</th>
<th>IC\textsubscript{50} values (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M\textsubscript{1}</td>
<td>25.2 ± 4.8</td>
</tr>
<tr>
<td>M\textsubscript{2}</td>
<td>18.5 ± 6.1</td>
</tr>
<tr>
<td>M\textsubscript{3}</td>
<td>14.3 ± 8.4</td>
</tr>
<tr>
<td>M\textsubscript{4}</td>
<td>10.3 ± 5.6</td>
</tr>
<tr>
<td>M\textsubscript{5}</td>
<td>21.5 ± 3.6</td>
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Binding affinities in recombinant human muscarinic receptor subtypes

SPE at 3-100 µg/ml inhibited specific \[^{3}H\]NMS binding in the membranes of CHO-K1 cell lines expressing human muscarinic M\textsubscript{1} to M\textsubscript{5} receptor subtypes in a concentration dependent manner. The IC\textsubscript{50} values (µg/ml) of SPE was slightly lower in the subtypes of M\textsubscript{2} (18.5±6.1), M\textsubscript{3} (14.3±8.4) and M\textsubscript{4} (10.3±5.6) than in M\textsubscript{1} (25.2±4.8) and M\textsubscript{5} (21.5±3.6) (Table 2), while the differences in IC\textsubscript{50} values among these five muscarinic receptor subtypes were not significant.

DISCUSSION

Natural medicines have been extensively used for thousands of years in many regions of the world, including China, India, Egypt and Greece. These medicines have recently gained popularity in many western countries due to their reliable therapeutic effects and affordability. With increasingly extensive application of natural products and
their bioactive ingredients for disease treatment, therapeutic responses of such products need to be clearly identified to ensure their therapeutic efficacy and safety (Rao et al., 2019).

SPE is the phytotherapeutic agent most commonly used to treat BPH and LUTS, and it has been the most thoroughly studied (Suzuki et al., 2009; Pagano et al., 2014), although the exact mechanisms of action on bladder function are unclear. We previously showed that intraduodenally-administered SPE significantly improves urodynamic symptoms in the hyperactive bladder of male rats by increasing bladder capacity and prolonging the micturition interval (Suzuki et al., 2009; Oki et al., 2005; Suzuki et al., 2007; Nasrin et al., 2014). Such an improvement of urodynamic symptoms by SPE may arise from its binding to pharmacologically-relevant receptors in the urinary bladder.

In the present study, the infusion of diluted acetic acid caused a hyperactive bladder response in female rats, which was characterized by a decrease in bladder capacity and mean micturition volume with a concomitant shortening of the micturition interval. Such acetic acid-induced increase of micturition frequency and decrease of voided volume of urine was significantly attenuated by single oral administration of SPE (Figure 1 and Table 1). Therefore, in accordance with the previous findings in male rats (Oki et al., 2005), our study demonstrated that SPE also attenuated a hyperactive bladder response in female rats. The present study showed that SPE significantly increased bladder capacity in female rats, resulting in a prolonged micturition interval, decreased frequency of micturition and enhanced mean micturition volume. Similar effects were observed by antimuscarinic agents, which improve LUTS in patients with OAB mainly by antagonizing bladder muscarinic receptors, as recently reviewed by Yamada et al. (2018).

The spasmolytic effect of SPE on isolated smooth muscle preparations was previously reported by Gutierrez et al. (1996). They showed that SPE at 0.3-1.0 mg/ml relaxed agonist-induced contraction of smooth muscles of the thoracic aorta, uterus (female only) and urinary bladder isolated from male and female rats, with EC50 values of 0.35-0.56 mg/ml. In agreement with these findings, our study showed that similar concentrations (0.1-1.0 mg/ml) of SPE attenuated EFS- and acetylcholine-induced contraction of isolated female rat bladders (Figure 2). Furthermore, SPE competed with specific [3H]NMS binding sites in the bladder of female rats in a concentration dependent manner (Figure 3). In contrast, SPE showed little binding activity of bladder β-adrenoceptors, as shown by no inhibition of specific (−)[3H]ICYP binding. Such antimuscarinic activity of SPE may have an important role for the relaxant effect on the bladder smooth muscle. Therefore, the inhibitory effect of SPE on bladder hyperactivity may be partly attributable to the relaxation of bladder smooth muscle by antagonizing muscarinic receptors in the bladder. SPE was shown to inhibit specific [3H]NMS binding in the membranes of CHO-K1 cell lines expressing human muscarinic M1 to M5 receptor subtypes in a concentration-dependent manner, and there were few significant differences among the five (M1 - M5) subtypes of muscarinic receptors in the inhibitory potency (IC50 values) of SPE. These findings indicate that SPE binds to each muscarinic receptor subtype of M1 to M5 with similar affinity.

Conclusion
SPE significantly attenuated a hyperactive bladder response in female rats, with antagonistic effects on agonist-induced contraction and muscarinic receptors in the bladder. These results support the clinical efficacy of SPE for the treatment of LUTS in female patients with OAB.

REFERENCES


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