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Pharmacological characterization and chemical fractionation of a liposterolic extract of saw palmetto (*Serenoa repens*): Effects on rat prostate contractility

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ABSTRACT

Ethnopharmacological relevance: Saw palmetto (*Serenoa repens*) was first used medicinally by native American Indians to treat urological disorders. Nowadays, saw palmetto extracts are widely used in Europe and North America to treat the urinary symptoms associated with benign prostatic hyperplasia even though its mechanisms of action are poorly understood. This study aimed to characterize the bioactive constituents of a lipid extract of saw palmetto that are able to affect contractility of the rat prostate gland. The mechanism of action will also be investigated.
Materials and methods: A commercially available lipid extract of saw palmetto was subjected to fractionation using normal phase column chromatography. Composition of fractions was assessed by proton nuclear magnetic resonance spectroscopy (^{1}H NMR) and mass spectrometry (MS). Contractile activities of these fractions were evaluated pharmacologically using isolated preparations of rat prostate gland and compared to the activity of the crude extract.
Results: Saw palmetto extract inhibited contractions of the rat prostate gland which were consistent with smooth muscle relaxant activity. Only the ethyl acetate fraction resulting from chromatography inhibited contractions of isolated rat prostates similarly to the inhibition produced by the crude lipid extract. Comparison with authentic samples and analysis of NMR data revealed that this bioactivity was due to the fatty acid components present in the ethyl acetate fraction. Bioassay using various pharmacological tools identified multiple contractile mechanisms which were affected by the bioactive constituents.
Conclusion: A fatty acid component of saw palmetto extract causes inhibition of prostatic smooth muscle contractions via a non-specific mechanism.

1. Introduction

The pathogenesis of benign prostatic hyperplasia (BPH) involves a combination of two elements: the static and dynamic components. The static component involves prostatic growth, caused by testosterone diffusing into the prostate cells and being converted to dihydrotestosterone by the enzyme 5α-reductase. The dynamic component relates to prostatic smooth muscle tone, which is under the influence of the sympathetic nervous system and mediated by α_1-adrenoceptors. Conventional pharmacotherapies for BPH consist of 5α-reductase inhibitors (dutasteride and finasteride) and α_1-adrenoceptor antagonists (tamsulosin, alfuzosin, prazosin, terazosin and doxazosin). Smooth muscle contraction in the urethra, prostate and bladder neck is considered to be the main contributor to the voiding symptoms associated with BPH. Hence, α_1-adrenoceptor antagonists are regarded as the preferred and more effective drug class (Miano et al., 2008).

There are a number of phytotherapeutic agents currently used for the treatment of BPH (Lowe and Ku, 1996). Most of these extracts are derived from the roots, seeds, bark or fruits of plants such as the African plum, purple cone flower, pumpkin seeds, rye, stinging nettle, red clover and saw palmetto. Of these, extracts of saw palmetto berries which were first used for the medicinal treatment of urological disorders by the native American Seminole Indians, are by far the most popular treatment for urinary symptoms caused by BPH (Gerber, 2000; Wilt et al., 2000). There are several different saw palmetto preparations on the market but

Abbreviations: ATP, adenosine-5′-triphosphate disodium salt; 2-APB, 2-aminoethoxydiphenyl borate; BPH, benign prostatic hyperplasia; ESI/APCI, electrospray ionization/atmospheric pressure chemical ionization; IP_3, inositol triphosphate; MS, mass spectrometry; PDA, phorbol-12,13-diacetate; PKC, protein kinase C; ^{1}H NMR, proton nuclear magnetic resonance spectroscopy; TLC, thin layer chromatography

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the most widely used are the liposterolic extracts of saw palmetto. Liposterolic oily extracts are prepared with either *n*-hexane, 90% ethanol (w/w) or by supercritical fluid extraction with CO_2 and are normally standardised to contain 70 to 95% of free fatty acids and sterols. Despite the presence of free fatty acids, the liposterolic extracts also contain small amounts of phytosterols, fatty alcohols, flavonoids and polyprenoids (Plosker and Brogden, 1996).

Over the years, various mechanisms of action have been proposed for the beneficial effects of extracts of saw palmetto. It is believed that saw palmetto works by a variety of mechanisms, befitting its polypharmaceutic nature. The pharmacological activity of saw palmetto includes; inhibition of 5α-reductase activity (Di Silverio et al., 1992; Iehle et al., 1995), anti-inflammatory activity (Paubert-Braquet et al., 1997), anti-androgen properties (Ravenna et al., 1996) and anti-edema effects (Tarayre et al., 1983). However, saw palmetto extract also appears to have spasmolytic and smooth muscle relaxant activity as demonstrated in the urinary bladder of guinea pigs and rats (Gutierrez et al., 1996). This indicates that saw palmetto extracts may alleviate symptoms by inhibiting both the dynamic and static components of BPH.

Previously, we have demonstrated that commercially available ethanolic extracts of saw palmetto contain the indirectly acting sympathomimetic tyramine (Cao et al., 2006; Chua et al., 2011). This effect causes an indirect α_1-adrenoceptor mediated contraction via the release of noradrenaline from sympathetic neurons, which would cause urethral obstruction and be detrimental to BPH sufferers. The present study was undertaken to examine the acute pharmacological properties of a widely used liposterolic extract of saw palmetto on the isolated rat prostate gland *in vitro*. In addition, chemical fractionation was performed to elucidate the bioactive compounds contained in saw palmetto that mediate its pharmacological activity.

2. Materials and methods

2.1. Animals and tissues

Male Sprague-Dawley rats (200–300 g) were housed at the Monash Clayton and Parkville animal houses in a controlled environment of 22 °C and exposed to a photoperiod of 12 h light/12 h dark. Animals were provided with food and water *ad libitum*. The animals were killed by cervical dislocation. An abdominal incision was made, exposing the male urogenital tract and the left and right lobes of the prostate were removed providing two prostate preparations from each rat. The excess fat and connective tissues were carefully removed and prostates were then placed in a Petri dish containing Krebs–Henseleit solution (mM: NaCl 118.1, KCl 4.69, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.7, $MgSO_4$ 1.1 and $CaCl_2$ 2.5, carbogenated to pH 7.4). Prior approval for animal experimentation was obtained from the Monash University Standing Committee on Animal Ethics in Animal Experimentation (Ethics number VCPA.2009.15).

2.2. Isolated organ bath studies

Isolated prostates were mounted in separate 10 ml glass, water-jacketed organ baths, containing Krebs–Henseleit solution bubbled with 5% CO_2 in O_2, and maintained at 37 °C. One end of the prostate was attached to a perspex tissue holder and the other to an isometric Grass FT03 force-displacement transducer (Telefactor, West Warwick, RI, USA), which measured the force produced by the tissue. A PowerLab data acquisition system (Chart v5) (ADInstruments, Bella Vista, NSW, Australia) received information from the force transducer for recording on a personal computer. Preparations were equilibrated for 60 min, under a resting force of 0.5–0.8 g. To ensure the viability of the tissue, nerve terminals within the prostatic smooth muscle were electrically stimulated via two vertical parallel platinum electrodes incorporated in the tissue holder, which was connected to a Grass S88 stimulator delivering pulses of 0.5 ms, 60 V, at 0.01 Hz.

2.2.1. Effects of saw palmetto on nerve-mediated contraction

To assess whether the liposterolic extract of saw palmetto affected contractile responses produced by nerve-stimulation, frequency response curves (0.5 ms, 60 V, 0.1–20 Hz) to electrical field stimulation were conducted using a frequency progression ratio of approximately one third of a log unit. The pulses were delivered for 10 pulses at low frequencies ($\leq$ 1 Hz) and for 10 s at higher frequencies ($\geq$ 1 Hz). Trains of electrical field stimulation were delivered at intervals of 10 min. An initial frequency–response curve was constructed to determine the contractile response of each tissue at each frequency. A subsequent frequency–response curve was constructed 1 h later in the presence of liposterolic extract of saw palmetto or vehicle after the tissue had been exposed to the extract or vehicle for 15 min. One prostate from each rat was used to construct frequency–response curves to saw palmetto extract while the contralateral prostate was used as a vehicle control.

In a subset of experiments, fractions produced by chromatographic fractionation and commercially available fatty acids were used instead of the liposterolic extract of saw palmetto, using the same discrete frequency–response curve protocol as above. The effect of triglycerides was also examined using the commercially available glyceryl trioleate. The above protocol was adapted using a single frequency of 10 Hz.

2.2.2. Effects of saw palmetto extract on contractile responses induced by noradrenaline, ATP, acetylcholine and KCl

Following the initial 60 min equilibration period discrete concentration–response curves to saw palmetto were constructed against various agonists. The agonists used were noradrenaline (1 μM), acetylcholine (10 μM), ATP (10 mM) and high potassium Krebs–Henseleit solution (mM: NaCl 38.0, KCl 80.0, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.7, $MgSO_4$ 1.1 and $CaCl_2$ 2.5, carbogenated to pH 7.4) (KCl (80 mM)). In these experiments, one prostate from each rat was used to construct concentration–response curves to saw palmetto extract while the contralateral prostate was used as a vehicle control. Agonists were added to the organ bath 15 min after addition of each concentration of saw palmetto extract. Once the contractile responses of the agonist had reached a plateau, the tissue was washed out with four to five times the bath volume, followed by the addition of the next saw palmetto extract concentration.

2.2.3. Effects of various pharmacological tools on actions of saw palmetto extract

Following the 60 min equilibration period, frequency–response curves to electrical field stimulation were constructed to determine the contractile response of the tissue at each frequency. A subsequent frequency–response curve was constructed in the presence of a test drug after the tissue had been exposed to the drug for 15 min. The test drugs used were the β-adrenoceptor antagonist, propranolol (1 μM); the Rho-kinase inhibitor, Y27632 (10 μM); the protein kinase C (PKC) activator, phorbol-12,13-diacetate (PDA) (1.0 μM); the calmodulin antagonist, w-7 (100 μM); the L-type Ca^{2+} channel blocker, nifedipine (1.0 μM); the inositol triphosphate (IP_3) receptor antagonist, 2-aminoethoxydiphenyl borate (2-APB) (60 μM) and ryanodine (10 μM);. A final frequency–response curve was established in the presence