Pharmacological basis for the medicinal use of Michelia champaca in gut, airways and cardiovascular disorders

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ABSTRACT

Objective: To discover the mechanism behind ameliorative effects of Michelia champaca (M. champaca) in gastrointestinal, respiratory and cardiovascular disorders. Methods: Anti-spasmodic potential was evaluated by trying the M. champaca extract (aqueous:ethanolic) on rabbit aorta, trachea and jejunum in vitro. Isotonic and isometric transducers coupled with Power Lab data acquisition system was used to record the responses of isolated tissues. Results: M. champaca extract relaxed the spontaneous and high K⁺ (80 mmol/L)-induced contractions of isolated jejunum preparation of rabbit showing a Ca²⁺ channel blocking mechanism. Moreover, extract shifted calcium concentration response curves towards right like standard calcium channel blocker verapamil. In rabbit tracheal preparation, M. champaca relaxed both carbachol (1 µmol/L) and high K⁺-induced contractions, likewise verapamil. In rabbit aorta preparation, M. champaca relaxed phenylephrine (1 µmol/L) and high K⁺-induced contractions similar to verapamil. Conclusion: M. champaca possesses spasmylytic, airways relaxant and vasodilator actions mediated perhaps due to blocking of Ca²⁺ channels, hence validating its therapeutic usage in diarrhea, asthma and hypertension.

1. Introduction

Gastrointestinal, respiratory and cardiovascular diseases are increasing globally. Complementary and alternative medicines are gaining momentum again for treatment of smooth muscle related diseases like asthma, diarrhea, constipation and hypertension. In Pakistan, tribal and rural areas are far from cities, so local people traditionally rely on medicinal plants to cure common diseases. Medicinal plants are valuable because of their safety, efficacy and immunity enhancing effects. Moreover, herbal medicines are free from side-effects as compared to allopathic medicines. Michelia champaca L. (M. champaca; Magnoliaceae), frequently known as Champak or Golden champa, is a widely used medicinal plant in Pakistan because of its diversity of folk medicinal uses. It is a large evergreen tree widely distributed in Pakistan, Sri Lanka, Nepal, Burma, India, China, Bangladesh, Thailand and Malaysia[1]. M. champaca L. has traditionally been used to treat diarrhea, cough, bronchitis, hypertension, dyspepsia, fever, rheumatism, abscesses, dysmenorrhea and inflammation. It is also used as purgative, expectorant, cardiotonic, digestive, carminative, stomachic, stimulant, diuretic, diaphoretic, antipyretic and astrigent. The seeds...
and fruits are claimed to be useful in psoriasis and for healing cracks in feet[1,2].

The plant is scientifically stated to exhibit antipyretic, anti-inflammatory[3], antioxidant[4,5], antimicrobial[6], cytotoxic[7], antidiabetic[8], and analgesic[5] activities. *M. champaca* contains various bioactive constituents like alkaloids, saponin, palmitic acid, oleic acid, carbonyl acid and ester[2], oliveroline, lysicamine, norruciferine, cyperone, ficaprenolepi-yangambin, pheophytin, aristophyll, michephylla and formylanonaine[9]. *M. champaca* has great medicinal value as proved by various *in vitro* and *in vivo* studies[10-14]. The plant has folkloric reputation of having remedial effects in hypertension, asthma and diarrhea, but scientific evidence for its conventional medicinal usage is lacking so far. As part of our research group continuous studies regarding rationalization of ethnopharmacological potential of extracts of medicinal plants of Pakistan[15-18], effects of crude extract of *M. champaca* were assessed in isolated tissues of rabbit (aorta, trachea and jejunum) to validate its usage in gastrointestinal, respiratory and cardiovascular diseases.

### 2. Materials and methods

#### 2.1. Plant material and extraction

*M. chamapca* (whole plant) was collected in August 2012 from Pattoki, Pakistan and was authenticated by well-known botanist with Voucher Specimen No. Fl. Pak.283. The plant material was extracted as per reported methods[19]. About 950 g of plant substance was drenched with aqueous ethanol (70%) at 37 °C in closed amber colored bottle for 10 d with 3 or 4 times mixing. The soaked substance was first filtered from muslin cloth subsequent from Whatmann-1 filter paper. The filtrate was vaporized to get a thick, semisolid mass which was then transferred to Petri dish and placed at room temperature to remove remaining solvent. Triple maceration was followed by the same method as described above.

#### 2.2. Drugs and chemicals

Phenylephrine, magnesium chloride, potassium chloride, verapamil hydrochloride, ethylene diamine tetra-acetic acid (ETDA) and carbachol (CCh) were purchased from Sigma Chemicals Co. (St Louis, MO, USA). Supplier of potassium di hydrogen phosphate, glucose, magnesium sulphate, calcium chloride, and other required chemicals as reported in our previous findings[15-18] was Merck (Germany) and BDH (England).

#### 2.3. Rabbits

The white albino rabbits (male and female) of weight (1.0–1.8 kg) and 6–7 months in age were used in these experiments. The conditions for animals were the same as described in our previous research papers[15-18]. Experiments done fulfilled the verdicts of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council[20] approved by institutional Ethical Committee of B.Z.U, Multan with EC/09/2011 dated 16-02-2011.

#### 2.4. Preliminary phytochemical investigation

Crude extract of *M. champaca* was initially tested for the existence of alkaloids, coumarins, flavonoids, tannins, phenols, sterols, saponins, terpenes and anthraquinones[21]. High-performance liquid chromatography (HPLC) studies of extract were performed to get fingerprint of various chemical constituents present. *M. champaca* extracts were hydrolyzed by a previously reported method[22]. Separated phenolic acids were detected by UV-visible detector (SPD-10AV) at 280 nm and were identified by comparing their retention time with standards.

#### 2.5. Isolated tissue preparations

Rabbit jejunum, trachea and thoracic aorta were dissected out and prepared for experiments as discussed as previously reported by our research group[23-26]. The standard drug used was verapamil hydrochloride for trachea detection. Sustained contractions were produced by phenylephrine in an aggregate approach in isolated aortic tissues[25].

#### 2.6. Mechanism of calcium channel blockade

Calcium antagonist activity was evaluated as per our previously reported methods[23-26]. Positive control used was verapamil hydrochloride.

#### 2.7. Statistical analysis

The results for bronchorelaxant, spasmolytic, and vasorelaxant potential are shown as mean ± standard error of mean (SEM; *n*=number of experiments) and median effective concentration (EC50) with 95% confidence interval (95% CI). Concentration-response curves were calculated by applying non-linear regression sigmoidal response curve with variable slope using software Graph Pad Prism program version 5.0 for Windows.

### 3. Results

#### 3.1. Chemical analysis of *M. champaca* extract

Chemical analysis of *M. champaca* extract showed existence of saponins, alkaloids, terpenes, sterols, anthraquinones, tannins, phenols and absence of coumarins, flavonoids and cardiac glycosides among aqueous:ethanolic extractable constituents. Phenolic acid (mg/g of plant extract) determined by HPLC are: chromotropic acid (3.09), quercetin (0.29), gallic acid (1.21), caffeic acid (0.78),
3.2. Effect of *M. champaca* extract on jejunum

*M. champaca* inhibited normal impulsive contraction of the jejunum at dose range of 0.1–3.0 mg/mL with relevant EC$_{50}$ of 0.290 mg/mL (95% CI: 0.183–0.458 mg/mL). It completely calmed K$^+$-induced shrinkage at 3.0 mg/mL with relevant EC$_{50}$ of 1.410 mg/mL. Verapamil (Standard drug) showed an analogous outline of repression of the spontaneous and K$^+$-induced shrinkages with relevant EC$_{50}$ of 0.266 µmol/L (95% CI: 0.179–0.397 µmol/L, n=5) and 0.180 µmol/L (95% CI: 0.129–0.252 µmol/L, n=5) (Figure 1), respectively. Moreover, *M. champaca* extract (0.3–1.0 mg/mL) tilted the Ca$^{2+}$ concentration-response curves likewise verapamil towards right (0.1–0.3 µmol/L) (Figure 2).

3.3. Effect of *M. champaca* extract on trachea

*M. champaca* relaxed CCh-induced contraction of tracheal preparation at dose of 0.03–3.0 mg/mL with EC$_{50}$ of 0.457 mg/mL (95% CI: 0.319–0.653 mg/mL) and K$^+$-induced contraction at similar dose (0.03–3.0 mg/mL) with relevant EC$_{50}$ of 0.931 mg/mL (95% CI: 0.617–1.405 mg/mL, n=5) respectively. Likewise verapamil produced relaxation of CCh- and K$^+$-induced contractions with relevant EC$_{50}$ of 0.133 µmol/L (95% CI: 0.089–0.197 µmol/L) and 0.133 µmol/L respectively (Figure 3).

4. Discussion

Currently, functional foods and herbal supplements are focus of research due to their proved effects on human health. The grey area
between plants and medicine is vanishing rapidly and it is becoming impossible to draw a line between plants and medicines. In both developing and developed countries, a large number of medicines derived from natural sources are used in health care systems. The traditional uses of medicinal plants in healthcare practices provide tips for new areas of research and become popular in curing disease. The medicinal and food plants of Pakistan have shown significant benefits to treat the gastrointestinal, respiratory and cardiovascular diseases[23-26]. M. champaca has a traditional fame to provide relief in hyperactive condition of gastrointestinal system such as diarrhea, hence aqueous-ethanolic extract of M. champaca was checked on spontaneous rhythmic contractions of isolated jejunum preparation to determine possible antispasmodic action.

HPLC method is one of the most important, authentic, fast and reliable methods for identification of phenolic compounds. Therefore in present study, crude extracts were analyzed for presence of various phenolic compounds. The results suggest that chromotropic acid is present in highest amount while syringic acid is present in lowest amount in sample.

The plant extract showed antispasmodic effect by preventing spontaneous contractions. The contraction in smooth muscle is because of increased quantity of free calcium in cytoplasm. This increased concentration in the cell is by virtue of either through opening of L-type voltage- reliant-Ca"+" channels or discharged from intracellular reservoirs in sarcoplasmic reticulum[27-30]. The impulsive rhythmic movements of the jejunum are coordinated by action potential that is because of uninterrupted depolarization and repolarization. Depolarization shows that the action capacity produced due to inflow of calcium by L-type Ca"+" channels[31]. The subsequent stoppage of the impulsive contractions in jejunum can be because of hindrance either with entry of Ca"+" via voltage dependent Ca"+" channels or Ca"+" discharged from intracellular supplies. To investigate the mode of this finding, crude extract of M. champaca was tried on high K"+"-induced (80 mmol/L) contraction in jejunum. Contact with K"+" (80mmol/L), caused persistent depolarization with high conductance of Ca"+" towards cell through voltage dependent Ca"+" channels, raised intracellular Ca"+" and greatly depolarized cell membrane resulted in spasmogenic contraction[31,32]. The M. champaca repolarized the membrane potential by obstruction of Ca"+" current inflow, hence showing its relaxant or spasmolytic activity. Substances competent to prevent K"+" (80 mmol/L)-induced contraction are thought to be calcium channel blockers. It was expected following series of events were inhibited, i.e., i) reduced cytosolic Ca"+" ions concentration, ii) inhibition of Ca"+" attachment on calmodulin, thus calcium-calmodulin complex did not materilize, iii) reduced instigation of myosin light chain kinase, so phosphorylation of myosin light chain could not happen, and iv) reduced contact between actin and myosin so contractile response was not created[32,33]. The Ca"+" antagonistic action of M. champaca was further verified due to shifting of the Ca"+" concentrations response curves rightward on jejunum preparation which is analogues to verapamil (a standard calcium channel blocker).The calcium channel blockers are proven remedial class, the mutual property of which is dose-dependent impediment of the sluggish flow of Ca"+" and U-turning of the action to Ca"+"[34]. M. champaca also has traditional uses also in respiratory disorders, i.e., bronchitis, cough and expectoration. Its aqueous-ethanolic extract demonstrated relaxant action on carbachol (1 μmol/L)- and high K"+"-induced contractions in trachea analogous to verapamil. Carbachol belongs to class of cholinergic drugs and act on G₂-coupled muscarinic M₁ receptors which facilitates signaling transduction for discharge of intracellular calcium ions from calcium stores, and causes contraction by different pathways[35]. The M. champaca exerts its bronchodilator action by antagonized G₂ coupled muscarinic M₁ receptor and blockades the signal transduction of muscarinic receptor pathway of contractile response[36].The relaxation of high K⁡⁺-induced contraction shows its Ca²⁺ channel blockade effect. Hence the relaxant result observed is probably referred as Ca²⁺ channel inhibiting mechanism. The Ca²⁺ channel inhibitors are very important in airway hyperactivity and indication of this activity can justify the conventional use of the plant in airways diseases.

The airways diseases are etiologically related to vasospastic disorders, so extract of M. champaca was assessed for its potential vasorelaxant capacity. It indicated relaxation of phenylephrine and high K⁡⁺-induced contractions in rabbit aorta comparable to verapamil. The phenylephrine-induced contraction of aorta is because of enhanced cytosolic calcium via both Ca²⁺ entry through receptor operated channels and calcium released from intracellular reservoirs. Earlier it was discovered that membrane depolarization causes high K⁺-induced contraction in vascular smooth muscle resulting in increased calcium entry perhaps through T- or L-category of calcium channels[37,38]. Hence, it may be opined that relaxation of both high K⁺-induced and phenylephrine contractions by crude extract of M. champaca is due to calcium channel blocking mechanism. The M. champaca displayed calcium channel blocker capacity in-vitro on aorta, trachea and jejunum that may be credited to the existence of alkaloids[39], flavonoids[40] and tannins[41] amongst the constituents of M. champaca noticed in chemical screening.

The crude extract of M. champaca has shown spasmyloic, airways-relaxing and vasodilating potential which can be ascribed to presence of calcium channels blocking activity. The study therefore rationalizes its traditional usage in hypertension, diarrhea, and asthma in conventional systems of medicine.

Conflict of interest statement

The authors declare that they have no competing interests.

References
