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Pharmacological basis for the medicinal use of *Carissa carandas* in constipation and diarrhea

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ABSTRACT

Ethnopharmacological relevance: *Carissa carandas* Linn. commonly known as "Karaunda" (Apocynaceae) is a popular medicinal herb widely distributed in different parts of Pakistan. In addition to other medicinal uses, *Carissa carandas* is popular in indigenous system of medicine for its medicinal use in gut motility disorders like, constipation and diarrhea.

Objective: This study was planned to provide pharmacological basis to the medicinal use of *Carissa carandas* in constipation and diarrhea.

Materials and methods: The crude extract of the leaves of *Carissa carandas* (Cc.Cr) was prepared in methanol and its fractionation was carried out with ethylacetate, petroleum ether and *n*-butanol. *In-vivo* studies were conducted on mice, while isolated rabbit jejunum and guinea-pig ileum preparations were used for the *in-vitro* experiments. The spasmogenic and spasmolytic responses of gut tissues were recorded using isotonic transducers coupled with PowerLab data acquisition system.

Results: The HPLC fingerprints of Cc.Cr, its petroleum (Cc.Pef), ethylacetate (Cc.Eaf) and *n*-butanol (Cc.Baf) fractions showed the presence of oleanolic acid, ursolic acid, stigmasterol and β-sitosterol. Oral administration of Cc.Cr to mice increased fecal output at lower doses (30 and 50 mg/kg), while it showed protection against castor oil-induced diarrhea at higher doses (300 and 600 mg/kg). In isolated guinea-pig ileum and rabbit jejunum, Cc.Cr and Cc.Baf exhibited stimulatory effect at 0.003–3 mg/ml, which was partially sensitive to atropine or pyrillamine or partially/fully sensitive to atropine+pyrillamine, followed by relaxation at higher tested concentrations, being more potent in rabbit tissues. The ethylacetate fraction (0.1–5 mg/ml) exhibited fully atropine-sensitive contractions in both guinea-pig and rabbit tissues, being more potent in guinea-pig while more efficacious in rabbit tissues. However, the petroleum fraction (0.003–1.0 mg/ml) showed only spasmolytic activity in spontaneously contracting rabbit tissues, similar to nifedipine. In guinea-tissue, Cc.Pef did not cause any stimulant effect. When studied against high K^+ (80 mM)-induced contraction, the crude extract and its fractions caused a dose-dependent inhibition, with the following order of potency: Cc.Pef > Cc.Eaf > Cc.Cr ≥ Cc.Baf, similar to nifedipine indicating Ca^{++} channel antagonist like activity, which was further confirmed when the plant extract displaced Ca^{++} curves to the right with suppression of maximum effect similar to that of nifedipine.

Conclusion: This study demonstrates that the crude extract of *Carissa carandas* possesses a gut-stimulatory effect mediated primarily through the activation of muscarinic and histaminergic receptors while its spasmolytic effect was mediated possibly through Ca^{++} antagonist pathway. Thus, this study provides a clear evidence for the dual effectiveness of *Carissa carandas* in constipation and diarrhea, thus validating its medicinal use.

1. Introduction

Carissa carandas Linn. commonly known as "Karaunda" belongs to the family Apocynaceae, is native to India and distributed in Sri Lanka, Indonesia, Malaysia, Myanmar and in different parts of

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Pakistan (Hedge et al., 2009). The plant has a long history of its diverse medicinal utility in traditional system of medicine (Baquar, 1989). Various parts (fruits, leaves, bark and roots) of *Carissa carandas* are popular for their medicinal use in diarrhea, constipation, malaria, epilepsy, neurological disorder, pain, myopathic spams, leprosy (Rahmatullah et al., 2009), anorexia, cough, pharangitis, diabetes, seizures, scabies and fever (Usmanghani et al., 1997).

Phytochemical studies revealed the presence of glycosides (Joglekar and Gaitonde, 1970), terpenoids (Siddiqui et al., 2003; Begum et al., 2013), flavonoids, tannins, saponins, unsaturated sterols, salicylic acid (Baquar, 1989), proteins, vitamin C, phenolic acids, carissol, carissic acid and β-sitosterol (Rastogi et al., 1966; Nizami et al., 1993) as plant constituents. Alkaloids have also been reported to be present in the roots and stem bark (Naing, 2011). It has also been documented that the plant possessed hepatoprotective (Hegde and Joshi, 2009), anticonvulsant (Hedge et al., 2009), cardiotonic (Vohra and Den, 1963), antibacterial (Israr et al., 2012), antihypertensive (Joglekar and Gaitonde, 1970; Shamim and Ahmad, 2012), antihelminthic (John et al., 2007), antidiabetic (Itankar et al., 2011) and antihyperlipidemic (Sumbul and Ahmed, 2012) properties. To the best of our knowledge, there is no report available for its effectiveness in constipation and diarrhea, validating its medicinal claim in such disorders (Morton, 1987; Iyer and Dubhash, 2006; Itankar et al., 2011). This study will provide pharmacological basis to its medicinal use in gastrointestinal motility related disorders like constipation and diarrhea.

2. Materials and methods

2.1. Preparation of the crude extract and fractions

The leaves of *Carissa carandas* were collected in May 2010 from Karachi, Pakistan. The plant was identified by Dr. Ghulam Rasool and deposited (Voucher no. 86465) in the Herbarium of Department of Botany, University of Karachi. Fresh leaves (5 kg) of *Carissa carandas* were repeatedly extracted with methanol at room temperature. The syrupy concentrate (Cc.Cr), obtained on removal of the solvent from the methanolic extract under reduced pressure was partitioned between ethylacetate (EtOAc) and water. The EtOAc layer was washed with water, dried (anhydrous Na_2SO_4), charcoaled, filtered and freed of the solvent under reduced pressure to yield gummy residue of ethylacetate fraction (Cc.Eaf, 311.8 g). It was then treated with petroleum ether, affording petroleum ether soluble fraction (Cc.Pef, 110.3 g). The aqueous layer was extracted with *n*-butanol, which after washing, drying and removal of the solvent yielded a semisolid residue of *n*-butanol fraction (Cc.Baf, 1.67 g) (Williamson et al., 1998).

2.2. HPLC fingerprint analysis

The high performance liquid chromatographic (HPLC) analysis of methanolic extract (Cc.Cr) and its fractions (Cc.Pef, Cc.Eaf and Cc.Baf) was performed using an Agilent Technologies 1260 Infinity Series LC system (USA) equipped with a binary pump, PDA detector and Zorbax SB-C18 column (Agilent Technologies, Santa Clara, CA, USA, 3.0 mm i.d. × 50 mm, 1.8 μm). Around 1 mg of each sample was dissolved in 1.0 ml of mobile phase and filtered through a Millipore (0.45 μm) filter prior to injection. The mobile phase consisting of acetonitrile: ethanol (40:60, v/v), was delivered at a flow-rate of 0.2 ml/min, while column temperature was maintained at 20 °C. UV detection was performed at 203 nm. Ursolic acid (UA), oleanolic acid (OA), β-sitosterol (BS) and stigmasterol (SS) were used as qualitative standards.

2.3. Standard drugs

Acetylcholine perchlorate, atropine sulfate, histamine hydrochloride, pyrillamine maleate, loperamide hydrochloride, nifedipine, ursolic acid, oleanolic acid, β-sitosterol and stigmasterol were purchased from Sigma-Aldrich chemicals, St. Louis, MO, USA. Castor oil was purchased from Karachi Chemical Industries F/25 S. I. T.E., Karachi (Pakistan). All the chemicals used were of analytical grade and dissolved in distilled water/saline. Stock solutions of all chemicals were prepared fresh on the day of experiment.

2.4. Animals

BALB/c mice (weighing 20–25 g, $n=100$), and locally bred rabbits (weighing 1–1.5 kg, $n=12$) and guinea pigs (weighing 400–600 g, $n=13$) of both sexes, were housed at the animal house of the Aga Khan University under controlled environmental conditions (23–25 °C). The animals were kept in plastic cages (47 × 34 × 18) with sawdust (changed at every 48 h) and fasted for 24 h before the experiment, whereas they were given tap water and standard diet routinely. Experiments were performed with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Research Council, 1996) and also in accordance with Institutional guidelines. The study protocol (031-ECACU-BBS-13) was also approved by ECACU (Ethics Committee for Animal Care and Use) of the Aga Khan University.

2.5. In-vivo experiments

2.5.1. Laxative effect

A previously described method (Mehmood et al., 2011) was followed with slight modification. Mice (weighing 20–25 g, $n=36$) of both sexes were deprived of food for 24 h with free access to water before starting the experiment. The animals were placed individually in cages lined with clean filter paper and were divided into six groups (6 animals in each). The first group received saline (10 ml/kg, p.o.) orally serving as negative control. The second and third groups serving as positive controls were administered carbachol (1 mg/kg, i.p.) or histamine (2 mg/kg, i.p.) intraperitoneally. The fourth, fifth and sixth groups received the plant extract at different doses (30–50 and 100 mg/kg, p.o). After 5 h, feces production (total number of feces, total number of wet feces and total number of dry feces) was measured and the percentage of the wet feces was considered as laxative effect.

2.5.2. Antidiarrheal effect

To assess the antidiarrheal activity, a previously described method (Borrelli et al., 2006) was followed. Mice (weighing 20–25 g, $n=24$) of both sexes were fasted for 24 h before the experiment. The animals were housed in individual cages and divided into four groups ($n=6$/group). The first group received saline in vehicle (10 ml/kg, p.o.) and served as a negative control. The second and third groups were administered 300 and 600 mg/kg plant extract, respectively. The fourth group received loperamide (1 mg/kg), serving as positive control. One hour after the treatment, each animal received castor oil (10 ml/kg, p.o.) through a feeding needle. After 5 h, the cages were inspected for the presence of typical diarrheal droppings; the absence was regarded as a positive result, indicating protection from diarrhea.