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Kingiodendron pinnatum, a pharmacologically effective alternative for *Saraca asoca* in an Ayurvedic preparation, *Asokarishta*

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ABSTRACT

Saraca asoca (Fabaceae) is a prime ingredient in *Asokarishta*, a well-known Ayurvedic preparation for gynecological ailments. Due to scarcity, adulteration or substitution of related raw drugs is a common practice in its preparation. The bark of *Kingiodendron pinnatum* (Roxb. ex DC.) Harms, morphologically similar to *S. asoca* (Asoka) is a widely used substitute. The present study aimed to evaluate the pharmacological effectiveness of *K. pinnatum* as an alternative for *S. asoca* in *Asokarishta* by determining the inhibitory effect of estrogen induced uterus endometrial thickening in immature female rats. *Arishta* was prepared using *S. asoca* and with the substitute, *K. pinnatum* as per Ayurvedic Pharmacopeia. Uterus endometrial thickening was induced by the administration of estradiol (20 μg/kg b. wt, i.p) to 8-day-old rats for 5 alternate days. On day 16, following estradiol administration, the serum estrogen level was found elevated to 156.5 ± 8 pg/ml from the normal value 32.4 ± 5 pg/ml and consequently increased the thickness of uterus endometrium from 16.7 ± 1.4 to 75.2 ± 15.3 μm. Upon oral administration of 400 μl/kg b. wt *Asokarishta* (ASA) and *Arishta* made with *K. pinnatum* (AKP), the thickening was reduced to 42.5 ± 12.7 and 47.1 ± 10.5 μm and the estrogen level diminished to 102.6 ± 10 and 97.3 ± 8 pg/ml, respectively. *Arishta* also reduced the chronic/acute inflammations in mice and improved the antioxidant status of rats. No toxic symptom was observed in the animals by the treatment of *Arishta*. The study supports the use of *K. pinnatum* as an alternative to *S. asoca* in *Asokarishta* and gives a scientific validation for *Asokarishta* in gynecological ailments.

1. Introduction

Ayurveda, the most ancient and widely practiced traditional system of medicine in India, is a holistic approach to prevent the incidence or cure of diseases by maintaining homeostasis among the three body control systems, *Vata* (air), *Pitta* (fire) and *Kapha* (water). A balance of these systems is achieved by practising the basic principles of nature and the use of herbal decoctions or medicinal preparations.[1] Because of the pronounced adverse effects of synthetic products, a greater emphasis has recently been received by the herbal systems in disease management. Unfortunately, India has not been able to capitalize the Ayurveda by promoting its use due to various reasons such as loss of ancient knowledge to select the pertinent medicinal plants, non-standardized forms of drug preparations, substitution or adulteration, deforestation, improper harvesting and processing, lack of quality control measures and research and development on herbal drugs etc.[2,3] The unavailability of genuine raw materials is a serious concern in Ayurvedic drug preparations. In most of the Indian systems of medicine, the botanical source of a raw drug is often attributed to one species and the continuous extraction of a particular species lead to its rarity or loss. Thus, many treasured medicinal plants are over-exploited and have become depleted or even lost from their natural habitats.[4] Because of the scarcity of the actual plant materials, the usage of related species as substitutes in many medicinal preparations without proper scientific evaluation is continuing.[5,6] The adulteration in medicinal preparations may reduce the reputation of

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Ayurvedic system of medicine largely, which is a burning problem facing Ayurvedic industry.[7,8] However, the substitution of the herbs is a need of the hour due to the fact that more than 300 valuable medicinal plants are being red listed in India. Hence, the identification of chemical constituents as well as the pharmacological properties of plants is to be evaluated before using as a substitute. These measures can bring the herbal drugs to the standards of WHO[9] and may promote the practice of Ayurveda globally.[10,11]

Among the threatened medicinal plants, *Saraca asoca* (Roxb) de Wilde belonging to the family Fabaceae is used in large quantities in Ayurvedic industry as Asoka[12] and one of the foremost plants utilized from antiquity till to date. Asoka is a sacred tree of India and renowned for its use in treating gynecological disorders especially menorrhagia.[13,14] The bark of *Saraca asoca* is an important raw drug in *Asokarishta*, a fermented formulation and in several other medicinal preparations. The tannins contained in bark are thought to provide the astringent action for halting excessive menstrual bleeding, bleeding hemorrhoids, bleeding ulcers and hemorrhagic dysentery.[15,16] The annual consumption of Asoka in Ayurvedic drug industry in India is estimated to be nearly 850 tonnes/year.[17] Thus, over the years, due to over exploitation of the plant, the size of the population has been largely dwindling or almost depleting from its natural habitat. International Union for Conservation of Nature and Natural Resources (IUCN) has listed this species under the threat category, Globally Vulnerable.[18] Thus the scarcity has led to the substitution with the bark of related trees of the same family including largely *Kingiodendron pinnatum* (Roxb. ex DC.) Harms. *K. pinnatum* is a large tree sparsely distributed in the evergreen forests of Western Ghats of India. Even though the population is less in wild, the species is widely used as substitute due to its giant size and the possibility of getting large volume bark compared to an Asoka tree. Traditionally, an oleo-gum-resin extracted from *K. pinnatum* is being used by tribes for gonorrhoea, catarrhal conditions of genito-urinary and respiratory tracts and in curing sores in elephants. There are no reports on the pharmacological properties of *K. pinnatum* except antioxidant[19] and antibacterial effects. Some biologically active components like phenols, flavonoids, glycosides and diterpenes were also reported in the plant.[20,21] On this perspective, the focus of the study was to identify the suitability of *Kingiodendron pinnatum* as alternative for Asoka in *Arishta* preparation.

2. Materials and methods

2.1. Preparation of Arishta

The bark of *Saraca asoca* was collected from medicinal garden of Kerala Forest Research Institute (KFRI), Thrissur, Kerala and *Kingiodendron pinnatum* from Wayanad forest, Kerala. The voucher specimens of *Saraca asoca* (No. KFRI 4849) and *Kingiodendron pinnatum* (No. KFRI 4725) are deposited in Herbarium of KFRI. *Arishta* with *S. asoca* (ASA) and with *K. pinnatum* (AKP) were prepared as per Indian Ayurvedic Pharmacopeia. Briefly, the chopped bark of the principal component and other 14 plants as minor ingredients were mixed together and boiled in water until the volume got reduced to quarter. The sediments were removed and the filtrate kept for 30 days in an airtight china clay jar with the flowers of *Woodfordia fruticosa* and sugar candy for fermentation. After 30 days, the *Arishta* was filtered and used for the study.

2.2. Animals

Female young Wistar rats (8 day old) and mature Swiss albino mice (8–10 week old, 25–30 g) were purchased from Small Animal Breeding Station, College of Veterinary, Kerala Veterinary and Animal Sciences University (KVASU), Thrissur, Kerala. The animals were maintained under standardized environmental conditions (22–28 °C, 60–70% relative humidity, 12 h dark/light cycle) and fed with standard rat feed (Lipton, India) and water *ad libitum*. All the animal experiments were carried out in Amala Cancer Research Centre with the prior permission of Institutional Animal Ethics Committee (IAEC).

2.3. Experimental design for anti-keratinization study

Eight-day old female Wistar rats were used for the study. Total of 210 animals were distributed in to 7 groups comprising 30 animals each. Keratinization in uterus of all the rats was induced by the i. p. injection of 20 μg/kg b. wt. estradiol in 0.05 ml of propanediol for 5 alternative days[22] except first and second groups of animals, which was kept as normal and vehicle control receiving propanediol, respectively. Among these, the animals of third group were kept as control without drug treatment. Fourth and fifth groups received *Asokarishta* (ASA) at 200 and 400 μl/kg b. wt and sixth and seventh groups received *Arishta* prepared with *K. pinnatum* (AKP) at 200 and 400 μl/kg b. wt orally for 5 alternative days along with the estradiol injection. During the experiment, 6 animals from each group were sacrificed at 8, 16, 24, 32 and 40 days after birth. The uterus was dissected and kept in formalin. The sections of uterus were prepared and stained using hematoxyline-eosin. The pattern of keratinization on endometrium of uterus was analysed microscopically using Leica Application Suit software. On 16th day of treatment (24 days after birth), the serum estrogen level was estimated by radioimmuno assay (RIA).

2.4. Free radical scavenging property analysis

The scavenging activity of *Arishta* on various free radicals was analysed. Superoxide anion radical was determined by light induced superoxide generation with riboflavin and subsequent reduction of nitro blue tetrazolium (NBT).[23] Hydroxyl radical (OH^-) generated by the Fe^{3+}/ascorbate/H_2O_2 system (Fenton reaction) was measured by the thiobarbituric acid reacting substances (TBARS).[24] Lipid peroxidation induced in rat liver homogenate[25] was estimated by thiobarbituric acid reactive substances.[26] ABTS (2,2-azobis-3-ethylbenthiozoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and ferric reducing power (FRAP) of the *Arishta* were also determined. The scavenging of free radicals and inhibition of lipid peroxidation by ASA and AKP was calculated using the formula, % of inhibition = (OD of Control−OD of treated/OD of Control) × 100.

2.5. In vivo antioxidant property analysis

Male Swiss albino mice were used for the study. After the treatment of ASA and AKP orally for 5 alternative days, the animals were sacrificed, liver excised and blood was collected into the heparinized tubes by cardiac puncture for hematological and biochemical analysis. Blood samples were centrifuged at 1000×*g* for 15 min and the upper portion of the centrifuged samples was removed and the packed erythrocytes at the bottom were washed three times with phosphate buffer saline (pH 7.4). A known volume of red blood cells were lysed with hypotonic phosphate buffer. After removing the red blood cell debris by centrifugation of the mixtures (3000×*g* for 15 min), the lysates were recovered. Superoxide dismutase (SOD)[23] and catalase[27] activities and the level of reduced glutathione (GSH)[28] in blood and liver were determined.

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