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Emmenagogue properties of *Milicia excelsa* (Welw.) C.C. Berg (Moraceae) based, at least in part, on its ability to correlate the activity of the hypothalamic-pituitary axis to that of the ovaries

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ABSTRACT

Ethnopharmacological relevance: *Milicia excelsa* (Welw.) C.C. Berg (Moraceae) is a medicinal plant recommended over tropical Africa as a cure for several ailments including amenorrhea. This is the hallmark of an ovarian lesion or a dysfunction of the hypothalamic-pituitary-ovarian axis which may lead to infertility, osteoporosis or endometrial cancer. However, regardless these traditional claims, no scientific report dealing with emmenagogue properties has been reported yet.

Aim of the study: To bring scientific evidence to the curative action of the plant, we proposed evaluating the effects of a root aqueous extract of *Milicia excelsa* on female Wistar rat sexual maturation.

Material and methods: The study was performed in immature (45 days old) female Wistar rats orally treated with the root aqueous extract of *Milicia excelsa* at doses of 14, 77 and 140 mg/kg BW/day for 7 and 15 consecutive days. Genistein (10 mg/kg BW) served as the reference substance. Negative control animals, treated with the vehicle, were followed up for 7, 15, 30 and 45 days and rats were aged 52, 60, 75 and 90 days at these respective days. This allowed setting the nubile age of experimental animals and to determine the impact of treatments with genistein or the aqueous extract of *Milicia excelsa* on the age of rat sexual maturation. Since female rats do not have menstruation and that the normal menstrual cycle occurs because of changing levels of hormones made and secreted by the ovaries in response to hormonal signals from the pituitary gland, the effects of treatments were evaluated on the pituitary production of gonadotropins, the ovarian production of estradiol and progesterone, and uterine and vaginal growths.

Results: The sexual maturation of untreated rats was set at 90 days old. This sexual maturation was indicated by the simultaneous elevation of gonadotropins (FSH and LH ($p < 0.01$)) and ovarian hormones (estradiol ($p < 0.001$) and progesterone ($p < 0.05$)) in animals aged 90 days. Uterine and vaginal growths ($p < 0.001$) observed in these animals appear as the result of elevated level of estradiol. The root aqueous extract of *Milicia excelsa* displayed genistein-like effects and increased FSH and estradiol serum levels following both treatment periods (7 and 15 days). Estradiol serum concentration significantly increased following a 7-day treatment at the dose of 14 mg/kg BW ($p < 0.001$). This resulted in an increase in the uterine wet weight, uterine and vaginal epithelial heights ($p < 0.05$). These results suggest that the root aqueous extract of *Milicia excelsa* reduced rats' sexual maturation from 90 to 52 days. Moreover, animals' body weight was not affected following treatment with *Milicia excelsa*.

Conclusion: The root aqueous extract of *Milicia excelsa* may solve the problem of amenorrhea by synchronizing the activity of the hypothalamic-pituitary axis to the ovarian production of estradiol and progesterone. The unaltered body weight following treatments justifies at least in part, the traditional use of *Milicia excelsa* for primary and secondary amenorrhea.

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1. Introduction

Amenorrhea is the medical term for the absence of menstrual periods, either on a permanent or temporary basis. It can be classified as primary or secondary. In primary amenorrhea, menstrual periods have never begun (by age 16), whereas secondary amenorrhea is defined as the absence of menstrual periods for three consecutive cycles or a time period of more than six months in a woman who was previously menstruating. Indeed, the normal menstrual cycle occurs because of changing levels of hormones made and secreted by the ovaries. The ovaries respond to hormonal signals from the pituitary gland which in turn, is controlled by hormones produced in the hypothalamus (Marieb and Hoehn, 2010). Disorders that affect any component of this regulatory cycle can lead to amenorrhea.

Primary amenorrhea is typically the result of a genetic or an anatomic condition in young females that never developed menstrual periods and are not pregnant. The main causes of primary amenorrhea include delay of growth and puberty, Müllerian agenesis, Turner syndrome, androgen insensitivity syndrome and gonadal dysgenesis or premature ovarian failure (Folch et al., 2000; Seldmeyer and Palmert, 2002; Simpson and Rajkovic, 1999). Pregnancy is the most common reason for secondary amenorrhea. However, further causes are varied and may include conditions that affect the ovaries, uterus, hypothalamus, or pituitary gland. These causes may be of peripheral origin and include ovarian failure (premature ovarian failure or early menopause, polycystic ovary syndrome, the most common cause of hyperandrogenic chronic anovulation) (American College of Obstetricians and Gynecologists, 2002) and uterine anomaly (Asherman's syndrome: intrauterine synechiae and scarring, usually from curettage or infection) (Speroff and Fritz, 2005). Causes of central origin include hypothyroidism (Arojoki et al., 2000), pathologies that affect the pituitary gland (Sheehan's syndrome, tumors or other diseases of the pituitary gland that lead to elevated levels of prolactin) (Kiningham et al., 1996; Pickett, 2003; Speroff and Fritz, 2005) and hypothalamic dysfunction often associated with extreme weight loss, emotional or physical stress and rigorous exercise (Sowińska-Przepiera et al., 2015). Complications of amenorrhea include infertility and osteoporosis. Moreover, amenorrhea is considered as an indicator for anovulation and a chronic anovulation increases the risk of endometrial cancer (American College of Obstetricians and Gynecologists, 1989; Croteau and Bérubé, 2011; Kalantaridou et al., 1998; Master-Hunter and Heiman, 2006). This condition therefore requires special treatment at every stage of life in women in reproductive age.

Treatments of primary and secondary amenorrhea are determined by the specific cause. These include inter alia, surgery (in cases in which genetic or anatomical abnormalities are the cause of amenorrhea, typically primary amenorrhea) and hormone therapy recommended in premature ovarian failure to avoid the unpleasant symptoms of estrogen depletion as well as prevent the complications of low estrogen level such as osteoporosis. Due to the invase nature of surgery and risks of hormone-dependant cancers and cardiovascular events associated with hormone replacement therapy (Beral, 2003; Hulley et al., 1998), there is an urgent need for the development of innovative and safe actives substances.

The alternative use of natural medications has greatly increased within the past decades. *Milicia excelsa* (Welw.) C.C. Berg (Moraceae) for instance is a large deciduous fast-growing forest tree species native to tropical Africa and distributed from Senegal and Gambia in West Africa, through Central and East Africa to Mozambique where aqueous extracts of roots and bark are widely used against female sterility, dysmenorrhoea, and as aphrodisiac and galactagogue, and for several others medicinal purposes (Ofori, 2007). Commonly known as “Iroko” or “Abang” in Ewondo and “Chou-lak” in Bagangte (two Cameroonian vernacular languages), aqueous extract of roots of this plant is traditionally recommended in Cameroon as a cure for primary and secondary amenorrhea (Adjanohoun et al., 1996; Njamen et al., 2013). The phytochemical analysis of the roots of *Milicia excelsa* revealed the presence of flavonoids (Ouete et al., 2013), one of the most prevalent classes of phytoestrogens (Veitch, 2007). These are plant-derived chemicals with structural similarities with 17β-estradiol, the most potent endogenous estrogens (Gruber et al., 2002; Knight and Eden, 1996; Price and Fenwick, 1985). They have diverse biological activities resulting from their ability to mimic endogenous estrogen actions, to inhibit hormone action, to modulate hormone production, or to alter hormone receptor populations (Sonnenschein and Soto, 1998; Whitten et al., 1995). The presence of such compounds in *Milicia excelsa* roots may account for its traditionally reported emmenagogue properties. However, till now no scientific report dealing with these properties has been reported yet. To bring scientific evidence to the healing power of the plant, we proposed evaluating the effects of an aqueous extract of *Milicia excelsa* on female Wistar rats. Since female rats do not have menstruations, we investigated the effects of the aforesaid plant on rat sexual maturation, especially on the pituitary production of gonadotropins, the ovarian production of estradiol and progesterone, and uterine and vaginal growths, as the dysfunction of the hypothalamic-pituitary-ovarian axis has been involved in the onset of amenorrhea.

2. Materials and methods

2.1. Plant material and preparation of the aqueous extract

The roots of *Milicia excelsa* were collected in February 2014 in Yaounde, Cameroon center region. The plant was identified and authenticated by Mr. Victor Nana, botanist at the Cameroon National Herbarium, where a voucher specimen has been deposited under the number 57069 HNC.

The root aqueous extract of *Milicia excelsa* was prepared following the recommendations of the traditional practitioners consulted for treating amenorrhea. Slight modifications were applied to improve the yield of extraction. One kilogram of fresh and clean roots was boiled in 5 L of potable water for 30 min, and then filtered with Whatman paper number 3. The filtrate was freeze-dried and a total dry mass of 8.3 g of the aqueous extract was obtained. This extract was kept at 4 °C in an airtight container till use.

Three doses of this aqueous extract were administered to animals: 14, 77 and 140 mg/kg BW. After lyophilizing a little volume (200 mL) of the decoction, obtained from the traditional practitioners, a dose of 14 mg/kg BW was extrapolated from the traditional posology (two glasses (200 mL) of the decoction of *Milicia excelsa* roots twice a day for 14 days). The highest dose was obtained by multiplying the extrapolated dose (14 mg/kg BW) by 10. The median dose (77 mg/kg BW) represents the average of 14 and 140 mg/kg BW.

2.2. Animals

Immature female Wistar rats aged 45 days were obtained from the breeding facility of the Animal Physiology Laboratory, University of Yaounde I (Cameroon). They were bred and kept under a standard soy-free rat diet in order to eliminate exposure to exogenous estrogenic compounds. All rats were given free access to diet and water ad libitum. Animal handling and in vivo experiments were carried out in conformity with the European Union on Animal Care (CEE Council 86/609) guidelines adopted by the Institutional Ethics Committee of the Cameroon Ministry of Scientific Research and Technology Innovation.

2.3. Study design

Sixty female Wistar rats were randomly distributed into 5 groups as follows: negative control animals (n=20) receiving the vehicle (distilled water), positive control animals (n=10) treated with genistein at dose of 10 mg/kg BW/day; the three remaining groups were made up of 10 animals each. They were treated with the root aqueous extract of