Journal of Ethnopharmacology 150 (2013) 298–307

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Eriosema laurentii De Wild (Leguminosae) methanol extract has estrogenic properties and prevents menopausal symptoms in ovariectomized Wistar rats

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ARTICLE INFO

Article history:
Received 27 May 2013
Received in revised form
18 July 2013
Accepted 23 August 2013
Available online 5 September 2013

Keywords:
Aryl hydrocarbon receptor agonistic activity
Atherogenic index
Eriosema laurentii De Wild
Estrogenic activity
Osteoporosis

ABSTRACT

Ethnopharmacological relevance: *Eriosema laurentii* De Wild (Leguminosae) is a medicinal plant used in West and Central Africa for different diseases. In Cameroon, this plant is used as a treatment for infertility, and various gynecological and menopausal complaints. However, despite this use as a natural remedy, the biological activity of *Eriosema laurentii* has not been studied until now.
Aim of study: In order to determine the potential use of this plant in gynecological conditions/disorders, we evaluated the estrogenic properties of a methanol extract of its aerial parts and its ability to prevent different menopausal health problems induced by bilateral oophorectomy.
Material and methods: Two approaches were used. *In vitro*, recombinant yeast systems were applied, featuring either the respective human receptors (ERα, AR, and PR) or into chromosome III integrated human aryl hydrocarbon receptor (AhR) and the respective reporter plasmid. *In vivo*, the investigation was carried out using the 3 days uterotrophic assay and 9 weeks oral treatment in ovariectomized rats.
Results: The results showed that the methanol extract of the aerial parts of *Eriosema laurentii* transactivated the estrogen receptor-α and displayed AhR agonistic activity but was neither androgenic nor progesteronic. In rats, the extract did not induce endometrium proliferation either in the 3-day or the 9-week treatment regimens, but induced vaginal stratification and cornification, prevented loss of femur bone mass, increased high density lipoprotein cholesterol (HDL-C), and reduced total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), TC/HDL-C ratio, LDL-C/HDL-C ratio and the atherogenic index of plasma (AIP).
Conclusion: These results suggest that the methanol extract of the aerial parts of *Eriosema laurentii* does not seem to have an undesirable influence on the endometrium but might prevent vaginal dryness and bone mass loss and improve the lipid profile.

1. Introduction

Estrogens play a vital role in growth regulation and function in numerous female target organs such as uterus, vagina, and skeletal and cardiovascular systems (Couse and Korach, 1999; Korach et al., 1995). Therefore, estrogen deficiency resulting in ovarian production cessation is associated with many complaints experienced by menopausal women (Mikkola and Clarkson, 2002; Turner et al., 1994; Versi et al., 2001). For decades, the hormone replacement therapy (HRT) has been used as an alternative for managing menopause-induced complaints (Greendale et al., 1998; Nichols et al., 1984). Although HRT is presumably a safe treatment

Abbreviations: AEL, methanol extract of the aerial parts of *Eriosema laurentii* De Wild; AhR, aryl hydrocarbon receptor; AIP, atherogenic index of plasma; AR, androgen receptor; b-NF, β-naphthoflavone; CE, cholesteryl ester; CHD, coronary heart disease; CVD, cardiovascular disease; E2, 17β-estradiol; E2B, estradiol benzoate; E2V, estradiol valerate; ERα, estrogen receptor alpha; HDL-C, high density lipoprotein cholesterol; HPLC-DAD, high performance liquid chromatography-diode array detector; LDL-C, low density lipoprotein cholesterol; ONPG, *o*-nitrophenyl-β-galactopyranoside; OVX, bilaterally ovariectomized rats; PR, progesterone receptor; Rt, retention time; Sham, sham operated animals; TC, total cholesterol; TG, triglycerides; UEH, uterine epithelial height; UWW, uterine wet weight; VEH, vaginal epithelial height; VLDL, very low density lipoprotein.

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http://dx.doi.org/10.1016/j.jep.2013.08.050

for short-term therapy, it is implicated in adverse outcomes after long-term use such as increased risk of endometrial and breast cancer, stroke and pulmonary thromboembolism (Beral et al., 1999; Million Women Study Collaborators, 2003, 2005; Rossouw et al., 2002; Tavani and La Vecchia, 1999). Due to these reports women turned to natural health remedies and thus, the interest in plant derived phytoestrogens raised significantly in the last decades.

Even in developing countries a regained interest in herbal medicine is observed. In the last two decades traditional medicine has become very popular in Cameroon, mainly due to the long unsustainable economic situation in the country, the high cost and undesirable side effects of synthetic drugs, and increase in drug resistance to common diseases (Fokunang et al., 2011). In this context and similar to several other *Eriosema* species over the world (Bryant, 1983; Hutchings, 1996; Morton, 1981), decoctions of *Eriosema laurentii* De Wild (Leguminosae) are used in Cameroon for the treatment of infertility and various gynecological and menopausal complaints and several other uses have been mentioned (Burkill, 1985). Given its use and the lack of any scientific evidence about its efficacy we decided to investigate this plant according to the strategic platform for promoting traditional medicine research, development and practice put in place by the World Health Organization (WHO) in collaboration with the Cameroon Government (Fokunang et al., 2011). Traditional uses of *Eriosema laurentii* suggest that it could have estrogenic properties and prevent several menopausal complaints. To verify this hypothesis, we evaluated the estrogenic potential of *Eriosema laurentii* using recombinant yeast systems featuring either the respective human receptors (ERα, AR, and PR) or into chromosome III integrated human aryl hydrocarbon receptor (AhR) as these receptors are involved in female reproductive physiology (Baba et al., 2005; Conneely et al., 2002; Couse and Korach, 1999; Traish et al., 2002; Tsuchiya et al., 2005; Wormke et al., 2003). *In vivo*, we used a 3-day uterotrophic assay in ovariectomized adult rats, a classical tool for the prediction of estrogenicity of chemicals (OECD, 2007). The 9-week assay was also performed in order to evaluate the preventive effects of *Eriosema laurentii* on some menopausal health problems. The investigation focused on histological (uterine and vaginal epithelial height) and morphological (uterine wet weight, vagina stratification and cornification) endpoints, bone mass, biochemical parameters and lipid profile.

2. Material and methods

2.1. Chemicals and media

Estradiol, estradiol benzoate, 5α-dihydroxytestosterone, progesterone, and β-naphthoflavone were obtained from Cayman Chemicals (Ann Arbor, Michigan) or Sigma-Aldrich (St. Louis, Missouri, USA). Estradiol valerate (Progynova®) was purchased from Delpharm (Lille, France). Buffer reagents, dimethyl sulfoxide (DMSO), and *o*-nitrophenyl-β-galactopyranoside (ONPG) were obtained from Fluka (Buchs, Switzerland), Sigma-Aldrich or Merck (Darmstadt, Germany). For yeast media preparation, yeast nitrogen base was obtained from Difco (Franklin Lakes, New Jersey, USA) and amino acids were purchased from Serva Feinbiochemica (Heidelberg, Germany) and drop out medium without tryptophan from Sigma-Aldrich.

2.2. Yeast transactivation screens

Saccharomyces cerevisiae strains were used to perform assays. The strain 188R1, a hyperpermeable derivative of RS188 N, was used for ERα and AR transactivation assays, and BJ3505 and YCM3 strains were used for PR and AhR assays, respectively. The assays are mostly two-plasmid systems containing expression plasmids with the respective human receptor gene (ERα, PR or AR) and a LacZ reporter plasmid. For the aryl hydrocarbon receptor (AhR), the yeast construct contains the human AhR and aryl hydrocarbon receptor nuclear translocator (ARNT) genes integrated in chromosome III. The assay performance and data evaluation have been described previously (Medjakovic and Jungbauer, 2008; Reiter et al., 2009).

2.3. Plant material and HPLC analysis

Aerial parts of *Eriosema laurentii* were collected in July 2010 in Bazou, Cameroon West Region. The plant was identified and authenticated by Mr. Victor Nana, botanist at the Cameroon National Herbarium. A voucher specimen has been deposited at the Cameroon National Herbarium in Yaounde under the number 24480/SRF/Cam. Dried and pulverized aerial parts of *Eriosema laurentii* (2.5 kg) were extracted with 95% methanol at room temperature (5 L of solvent × 3, 48 h per extraction). The combined solutions were evaporated to dryness using a rotary evaporator at 40 °C to afford 130 g of a methanol extract named AEL.

The HPLC-UV analysis was performed on a Shimadzu CTO-20AC model apparatus with LC-20AC HT autosampler and LC-20AD pump. The SPD M20A diode array detector was set to measure the range 180–600 nm. A Luna® 5 μm C18 (2) 100 Å (250 × 4.60 mm^2 I.D.) column was used. The injection volume was 10 μl and flow rate was 1 ml min^{-1}, the oven temperature was controlled at 25 °C; the binary mobile phase consisted of water acidified with 0.1% (v/v) acetic acid (eluent A) and ACN with 20% (v/v) eluent A (eluent B). The following elution profile was used: 0–5 min, isocratic at 20% (v/v) B; 5–10 min, linear gradient from 20–30% (v/v) B; 10–30 min, linear gradient from 30–34% (v/v) B; 30–50 min, linear gradient from 34–50% (v/v) B; 50–55 min, isocratic elution at 50% (v/v) B; 55–80 min, linear gradient from 50–100% (v/v) B; 80–85 min isocratic elution at on 100% (v/v) B. The wavelength of detection was set at 254 nm.

2.4. Animals

Juvenile female Wistar rats, 10- to 12-week-old, were supplied by the production 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*. Animals were handled and *in vivo* experiments carried out in conformity with the European Union on Animal Care (CEE Council 86/609) guidelines adopted by the Institutional Ethic Committee of the Cameroon Ministry of Scientific Research and Technology Innovation.

2.5. Estrogenic effects in vivo

2.5.1. The 3-day uterotrophic assay

Twenty five rats were ovariectomized (OVX) under diazepam/ketamine (10 and 50 mg/kg body weight, respectively) anesthesia and 14 days after endogenous hormonal decline, they were randomly allocated in five groups ($n=5$). The first group or OVX group received vehicle only (corn oil) and the second group received 2 μg/kg estradiol benzoate (E2B). The three further groups were treated with the methanol extract of *Eriosema laurentii* (AEL) at 50, 100 and 200 mg/kg for 3 days by subcutaneous administration. Twenty four hours after the last administration animals were sacrificed after light anesthesia. Uteri and vagina were removed. Prior to fixation of uteri and vaginas in 10% formaldehyde solution for histological analysis, uterine wet weight was determined.