Journal of Ethnopharmacology 161 (2015) 156–162

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Research Paper

Effect of the aqueous extract of *Senecio biafrae* (Oliv. & Hiern) J. Moore on some fertility parameters in immature female rat

L.L. Lienou [a], P.B. Telefo [a,*], J.R. Njimou [b], C. Nangue [c], B.R. Bayala [d], S.C. Goka [a], P. Biapa [a], M.D. Yemele [a], N.J. Donfack [e], J.T. Mbemya [a], S.R. Tagne [a], A.P.R. Rodrigues [e]

[a] University of Dschang, Faculty of Science, Department of Biochemistry, P.O Box: 67 Dschang, Cameroon
[b] University of Yaounde I: Analytical Chemistry Laboratory Inorganic Chemistry Department, Faculty of Sciences, P.O Box 812, Yaounde, Cameroon
[c] Pathological Analysis Department of the Central Hospital of Yaounde, Cameroon
[d] University of Ouagadougou, UFR/SVT, Laboratory of Animal Physiology, 03P.O. Box 7021 Ouagadougou 03, Burkina Faso
[e] State University of Céara, Faculty of Veterinary Sciences, LAMOFOPA, Fortaleza, Brazil

ARTICLE INFO

Article history:
Received 18 June 2014
Received in revised form
16 October 2014
Accepted 5 December 2014
Available online 17 December 2014

Chemical compounds studied in this article:
Apigenin CID 5280443
Quercetin CID 5280343
Eugenol CID 3314
Theobromine CID 5429
Caffeic acid CID 689043
Hydroxy-tyrosol CID 216200
Catechin CID 9064
Rutin CID 5280805
Phenol CID 996

Keywords:
Aqueous extract
Senecio biafrae
Fertility
Ovary
Uterus

ABSTRACT

Ethnopharmacological relevance: *Senecio biafrae* is a plant from the huge family of Asteraceae used in the African pharmacopoeia for the treatment of many ailments among which is infertility.
Material and methods: The aqueous extract, which was primarily subjected to polyphenol analysis, has been administered to immature female rats for 20 days at 8, 32, 64 and 128 mg/kg of body weight. The day following the treatment, the animals were sacrificed; their serum, ovary and uterus were retained respectively for reproductive hormones, ovarian and uterine proteins, and ovarian cholesterol assays.
Results: Light body weight gain variation of treated animals was observed during the experimental period. A significant increase ($p<0.05$) in serum estradiol and proteins as well as in uterine weight ($p<0.01$) of all *Senecio biafrae* treated animals was noted. No significant variation was noticed in the ovarian weight and follicle numbers.
Conclusion: The various biochemical and physiological parameters of fertility were significantly improved with the aqueous extract of *Senecio biafrae*, thus attesting some of its traditional usage.
© 2014 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Medicines derived from plants play an important role in traditional health care systems all over the world and particularly in Cameroon (Jiofack et al., 2008). *Senecio biafrae*, a plant of the Cameroonian pharmacopoeia, is used in traditional localities, especially in western part of the country, to cure many diseases among which are infertility and reproductive tract ailments (Focho et al., 2009; Telefo et al., 2011, 2012). Previous studies on the ethanolic and aqueous extracts of the plant, prepared under the guidance of the traditional healers, revealed their inducing effect after 30 days of oral administration on precocious puberty attainment and enhancement of fertility (Lienou et al., 2010, 2012; Telefo et al., 2011). These promising results prompted us to more investigations on their mode of action. Phytochemical characterization of many plant derived compounds is effective. The majority of those acting on the reproductive tracts of mammals are polyphenolic secondary metabolites and they have been grouped as phytoestrogens (Cos et al., 2003). The duration of 30 days used in the previous studies was chosen according to the main treatment duration used in Baham subdivision (Western Cameroon) for the treatment of infertility by *Senecio biafrae* extracts (Telefo et al., 2011). However, that duration matched with the beginning of the puberty period of the animals which normally occurs between the 40th and 50th day following their birth (Leibowitz et al., 2009). However, the apparition of the estrous cycle is linked to a high variability in the

* Corresponding author. Tel.: +237 77 20 42 67; fax: +237 33 45 19 35.
E-mail address: bphelix@yahoo.co.uk (P.B. Telefo).

http://dx.doi.org/10.1016/j.jep.2014.12.014
0378-8741/© 2014 Elsevier Ireland Ltd. All rights reserved.

biochemical and physiological parameters of reproduction in female rat according to the cycle phases. A reduction in the duration of the treatment and increase in the dose were obviously a better solution for an evaluation of these parameters with a lot of efficacy because it allowed us to study the effect of the extract before their puberty. Additive parameters such as follicle count in the ovarian cortical region and the hormonal evaluation have been considered in view of having a better understanding of the mode of action of the active compounds in the plant.

2. Material and methods

2.1. Preparation of the extract

The fresh leaves and stems of *Senecio biafrae* were collected in October 2012 in Baham subdivision (Western Region of Cameroon) and identified in the National Herbarium of Cameroon under voucher specimen code 32999/SRF/Cam. These parts were washed and dried at room temperature. The dried plants were ground in a mortar and the powder obtained was macerated in water for 24 h. The solution was then filtered with the Whatman paper and dried in a ventilated oven at 45 °C. The powder obtained was further resuspended in distilled water to prepare the extract at different concentrations for their daily administration to the animals at the needed doses i.e 8, 32, 64 and 128 mg/kg body weight.

2.2. Polyphenol analysis

The phenolic compounds were quantified in the aqueous extract of *Senecio biafrae* using a High Liquid Pressure Chromatography (HPLC). The sample was dissolved in pure water at a 3% concentration and centrifuged at 2647*g* for 10 min. The supernatant was filtered through a cellulose acetate membrane filter (0.20 μm or 0.45 μm, Schleicher & Schuell) and used for analysis. A 25 μL portion of the filtrate was injected into the HPLC system and eluted. The analysis was performed on an Agilent Technologies 1200 HPLC system fitted with a SUPELCOSIL LC-18 column (length 250 mm, diameter 4.6 mm, packaging size 5 μm). The column temperature was settled equal to 20 °C. The mobile phase consisted of an aqueous solution of 0.5% volume acetic acid ("A") and acetic nitrile ("B"). At start and lasting for the first 2 min of the run, 100% of A was used. From 2 min to 60 min after run starts, a linear ramp was used, targeting 40% of A and 60% of B. The flow rate settled equal to 1 mL/min. Polyphenols were detected by a UV detector (280 nm). Beforehand, the retention times of the polyphenolic compounds of interest were measured by using single polyphenol standard solutions at a concentration of 1%.

2.3. Animals

The animals used were immature female albino Wistar rats, 21–23 days old, weighing 25–35 g. They were obtained from the animal house of the Biochemistry Department (University of Dschang), housed under uniform husbandry conditions of light (12-h cycle) and temperature (22 ± 2 °C) and fed a standard laboratory diet and tap water ad libitum. Experimental protocols used in this study were accepted by the local ethical committee of our Faculty (Faculty of sciences, University of Dschang, Cameroon) and were designed in strict concordance with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986 (EEC, 1986).

2.4. Treatments

A total of 25 immature female rats were randomized into 5 groups of five animals each: four groups were receiving the extract at the doses of 8, 32, 64 and 128 mg/kg of body weight and the fifth group, which was the control, was receiving distilled water by oral gavage during 20 days.

At the 21st day, the animals were sacrificed by intra-abdominal injection of thiopenthal sodium. Their blood was removed by cardiac puncture; their ovaries and uteri were also removed, blotted and weighed. The left ovary of each animal was conserved with the uterus at −20° C until use. They were then homogenized in Tris–Sucrose buffer (0.25 M Sucrose, 1 mM EDTA and 10 mM Tris–HCl, pH 7.4) at 1% and 2% respectively. After centrifugation (4000*g*, 15 min), their supernatants were collected and used for protein (Bradford, 1976) and cholesterol (Trinder, 1969; Richmond, 1973; Roeschlau, 1974) assays. The right ovary was fixed in Bouin's liquid and conserved for two days for histological analysis and counting of the follicles at different growing stages. The blood was centrifuged (2500*g*, 15 min) and the serum collected was stored at −20 °C for the dosages of proteins (Gornall et al., 1949) and hormones (FSH, LH, estradiol and progesterone).

2.5. Hormonal evaluation

The FSH, LH, estradiol and progesterone assays were performed using the direct (for FSH and LH) and indirect (for estradiol and progesterone) competitive binding techniques (ELISA). The reagents used to perform it were obtained from GBC (General Biological Corporation, Hsin Chu, 30077, Taiwan, R.O.C) and the hormonal levels were obtained by absorbance reading to Microtiter (well reader LabSystems Multiskan RC, 351, FIN-00881, Helsinki, Finland) at 450 nm.

2.6. Ovarian histology

The right ovary of each rat was removed from Bouin's liquid and progressively dehydrated with ethanol (70%, 80%, 90% and 100%) and after with xylene (100%). Each tissue block was further embedded in paraffin wax, serially sectioned at 7 μm thickness every 60 μm using Leica rotary microtome (Leica RM 2125, Leica Microsystems Nussloch GmbH, Deutschland) and strips of sections were gently lowered into the surface of a warm water bath at 40 °C. The floated sections were mounted on microscopic slides, and put in an oven maintained at 60 °C for 30–40 min to fix the tissue firmly on the slide. They were colored with haematoxylin and eosin. Following the staining stage, all sections were examined microscopically at 100 × magnification (Fig.1) and the mean number of primary, secondary and antral follicles in the ovarian cortex was calculated for each specimen.

2.7. Statistical analysis

The data from biological assays were registered as Mean ± s.e.m (standard error on the mean). The statistical differences between the values were shown by ANOVA (Analysis of Variance) test. The Fisher LSD test was used for the comparison of means and the significance of the differences was established at the 5% level ($p < 0.05$) (Schwartz, 1991).

3. Results

3.1. HPLC analysis for phenolic compounds

The quantitative evaluation of phenolic compounds in the aqueous extract of *Senecio biafrae* showed the following quantities

Download English Version:

https://daneshyari.com/en/article/5835564

Download Persian Version:

https://daneshyari.com/article/5835564

Daneshyari.com