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Estrogen-like effect of a *Cimicifuga racemosa* extract sub-fraction as assessed by *in vivo*, *ex vivo* and *in vitro* assays

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Abstract

Black cohosh (*Cimicifuga racemosa*) is used in the treatment of painful menstruation and menopausal symptoms. Data about the nature of the active compounds and mechanism(s) of action are still controversial, chiefly with respect to its estrogenic activity.

This work aimed to assess the possible estrogenic activity of a commercial dry hydro-alcoholic extract of *C. racemosa* and its hydrophilic and lipophilic sub-fractions on *in vivo*, *ex vivo*, and *in vitro* assays.

In a yeast estrogen screen, only the lipophilic sub-fraction was able to activate the human estrogen receptor α, with a lower potency but comparable efficacy to that of 17 β-estradiol.

Neither the total extract nor the lipophilic sub-fraction showed an *in vivo* uterotrophic effect in 21-day-old rats. Uterine tissues obtained *ex vivo* from *C. racemosa* treated animals were generally much less sensitive to oxytocin, prostaglandin $F_{2\alpha,}$ and bradykinin than tissues obtained from estradiol valerate treated rats.

The lipophilic sub-fraction, instead, induced a dose-dependent inhibitory activity on the *in vitro* response to oxytocin, prostaglandin $F_{2\alpha,}$ and bradykinin of uterine horns from naïve 28-day-old rats, with a potency rate close to 1:30 of that of 17 β-estradiol.

Reported results confirm the effectiveness of *C. racemosa* in menstrual distress and further emphasize the possibility that lipophilic constituents bind to an as yet not identified estrogen receptor, likely inversely involved in inflammation.

Keywords: *Cimicifuga racemosa*; Yeast estrogenicity assay; Oxytocics; Rat uterus

1. Introduction

Cimicifuga racemosa, also known as *Actaea racemosa* (L.) Nutt. (Ranunculaceae), is native to North America. Over two centuries ago, North American Indians discovered that the root and rhizome of the black cohosh plant helped to relieve menstrual cramps, pain during labour, and climacteric complaints, including hot flashes, irritability, mood swings, and sleeping disorders [1,2]. Today, black cohosh, present in different licensed and unlicensed medicinal herbal products, is still often used for these purposes and has been widely used for over 40 years in Europe as an effective alternative to hormone replacement therapy for menopause [3].

Black cohosh contains triterpene glycosides, considered to be the major secondary compounds, including actein, 27-deoxyactein, and cimicifugoside. Aromatic acids (ferulic and isoferulic acid), alkaloids, and tannins were also detected. An isoflavone called formononetin has been identified in a single report as binding to estrogen receptors in rat uterus [4], but there are conflicting data as to whether formononetin is in black cohosh [5,6].

Both *in vivo* and *in vitro* studies on different black cohosh extracts demonstrate that the hormonal active component(s) is (are) in the lipophilic fraction [4,7]. Endocrine effects of *C. racemosa* extracts were studied *in vitro*, in ovariectomized rats, or in patients suffering from physical and psychological menopausal symptoms. According to some authors, the *in vivo* effects are mostly due to a hypophyseal action [8]. Extracts had no stimulatory effect on the uterus of ovariec-

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tomized rats, whereas showed marked central effects in lowering body temperature and in ketamine induced sleep enhancement. These effects were abolished by sulpiride, a D2 receptor antagonist [2,9]. In other experiments, an alcoholic extract significantly reduced LH levels and estrogen receptor expression in the central nervous system and in bones [10] and had an osteoprotective effect. These results led to the conclusion that constituents of this plant behave as selective estrogen receptor modulators (SERMs) both in the central nervous system and in bones [11]. Testing for estrogenic and antiestrogenic effects of ethanolic and isopropanolic *C. racemosa* extracts on proliferation of human breast cancer cells (MCF-7) and on gene expression, Zierau et al. [12] found an antagonism of estradiol-induced activities, which has been attributed to an antiestrogenic effect. However, *C. racemosa* constituents do not seem to bind to either of the known estrogen receptors (α and β) [13]. Thus, the hypothesis is that the effects of *C. racemosa* are mediated by mechanisms not involving these receptors but the hormonal regulation [14].

Considerable research on this plant has been reported, including phytochemical studies, bioassays, and a number of placebo and/or treatment-controlled clinical trials [6,15–18]. Although, in folk medicine, *C. racemosa* is used both to reduce menstrual pain and to facilitate parturition, so far no effects have been demonstrated on the uterus. Aim of this work was to assess the activity of a commercial dry hydro-alcoholic extract of *C. racemosa* and of its hydrophilic and lipophilic sub-fractions, on a number of *in vivo*, *ex vivo* and *in vitro* assays, mostly on rat uterus.

The ability of the three samples to interact with the estrogen receptor was first assessed using a yeast assay expressing the human estrogen receptor α.

Subsequently, estradiol valerate, the total extract of *C. racemosa* and its lipophilic sub-fraction, were administered to 21-day female rats to evaluate their effect on uterine weight (*in vivo* uterotrophic assay), and the sensitivity of isolated uterine horns from the treated animals to known oxytocic compounds, namely oxytocin, prostaglandin $F_{2\alpha}$, and bradykinin (*ex vivo* assay).

Finally, the effects of the total extract and of the lipophilic sub-fraction were compared to that of 17 β-estradiol on the response of *in vitro* uterine horns of 28-day naïve rats to the abovementioned oxytocic compounds.

2. Materials and methods

2.1. Chemicals

17 β-Estradiol (E2), estradiol valerate (EV), raloxifen (R), 4-hydroxytamoxifen (70% *trans*, 30% *cis*) (OHT), oxytocin (OXY), prostaglandin $F_{2\alpha}$ methyl ester ($PGF_{2\alpha}$), bradykinin (BK) and other reagents, were all obtained from Sigma–Aldrich (Milan, Italy).

Saccharomyces cerevisiae lac Z strain was kindly supplied by Prof. S. Ottonello (Parma University, Italy) on behalf of Prof. J. Sumpter (Brunel University, Uxbridge UK). Chlorophenol red-β-D-galactopyranoside (CPRG) was purchased from Roche Diagnostics (Monza, MI- Italy).

The commercial dry hydro-alcoholic extract of *C. racemosa* root and rhizome was kindly supplied by Res Pharma (Trezzo sull'Adda, MI-Italy). The hydrophilic (98.88% yield; C) and lipophilic (1.12% yield; D) sub-fractions were obtained by extraction with water/ethyl acetate; the sub-fractions were evaporated to dryness and dissolved in the appropriate medium just before use.

3. Recombinant yeast estrogenicity assay

The yeast estrogenicity assay has been previously described by Routledge and Sumpter [19]. In brief, the yeast transfected with the DNA sequence of the human estrogen receptor α (hER α), also contains expression plasmids carrying oestrogen-responsive sequences controlling the expression of *lac Z* reporter gene. This gene is responsible for the encoding of β-galactosidase enzyme following the influence of an estrogenic compound. Thus, this enzyme breaks down the chromogenic substrate, CPRG, added to the medium, turning from yellow into a red product that can be measured by absorbance (540 nm).

The commercial dry hydro-alcoholic extract of *C. racemosa* root and rhizome was dissolved in minimal medium [19], serially diluted, and tested at the concentration range of 1–40 mg/ml (total extract). The hydrophilic (C) and lipophilic (D) sub-fractions were dissolved in aqueous medium and ethanol (96%, v/v), respectively, and tested at the following concentration ranges: 1687.5–6750.0 μg/ml for the hydrophilic and 0.336–448.0 μg/ml for the lipophilic fraction. 17 β-Estradiol (0.005–20 nM), 4-hydroxytamoxifen (0.5–2500 nM), and raloxifen (1–5000 nM) were dissolved in ethanol (96%, v/v). Each sample (10 μl) was transferred in 4 wells of 96-well optically flat-bottom microtiter plates. When ethanol was used for dilution, the solvent was allowed to evaporate to dryness. A suspension in growth medium (190 or 200 μl) containing CPRG (0.01%) and a yeast concentration of 0.16×10^6 cells/ml was added in each well.

Each plate contained blank (assay medium and vehicle without yeast suspension) and negative (assay medium and vehicle with yeast suspension) controls.

All the experiments were carried out in sterile conditions. After 48 h of incubation at 32 °C, plates were shaken to resuspend the cells and the colour development of the medium was measured at an absorbance of 540 nm. The values were corrected for turbidity, and t_0 values subtracted.

In order to eliminate the possibility that the results obtained were due to direct conversion of the CPRG by the different components of the tested samples, these were also assayed without *S. cerevisiae* in the medium.

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3.1. In vivo uterotrophic assay

Impuberal female Sprague Dawley rats (Harlan, Milan, Italy), 21-day-old, with body weight of 45–50 g, were used [20]. Animals were housed four per cage, in a multiple rat rack. Temperature (21 ± 2 °C) and humidity (55 ± 15%) were controlled and a 12 h light/dark cycle, was maintained. Water and food were *ad libitum*. All animals had been acclimatized for three days in the animal room prior the first treatment.

Estradiol valerate and D sub-fraction were dissolved in the minimum amount of ethyl alcohol and diluted in tap water; the same amount of ethyl alcohol was added to tap water (control group) and to *C. racemosa* total extract dissolved in water.

Animals were administered the tested samples (0.5 or 1.0 mg/10 ml/kg estradiol valerate; 300 or 600 mg/10 ml/kg total extract; 20 or 40 mg/10 ml/kg D sub-fraction) for 3 days by oral gavage. The body weight of each animal was recorded daily, and detailed clinical observation carried out simultaneously. Animals were sacrificed 24 h after the final dose by inhalation of CO_2. Uteri were excised, trimmed free of any fat and adhering non-uterine tissue, pierced and blotted to remove excess fluid. The body of the uterus was cut just above its junction with the cervix and at the junction of the uterine horns with the ovaries. The uteri were then weighed (wet weight) and absolute uterine weight was used as an index of uterine growth [21]. Data are reported as mean ± S.E.M. of % variation (*versus* control group) of uterine weight.

3.2. Ex vivo assay

Both uterine horns from the animals used for the uterotrophic assay were used. From each horn a 1.5 ± 0.2 cm piece was excised and the two pieces placed together in 10 ml isolated organ baths containing Tyrode solution of the following composition (g/l): NaCl, 80.0; KCl, 2.0; $CaCl_2{\cdot}2H_2O$, 2.0; $CaCl_2{\cdot}2H_2O$, 2.0; $MgCl_2{\cdot}6H_2O$, 1.0; NaH_2PO_4, 0.5. The solution was maintained at 32 °C and continuously bubbled with an oxygen:CO_2 (95:5) mixture [22]. The tissues, connected to a Basile High Sensitivity (Type D10) transducer, were stretched to a passive tension of 2 g, allowed to equilibrate for 1 h and thereafter tested for the response to oxytocin (0.2–10 nM), prostaglandin $F_{2\alpha}$ (14–28 nM), bradykinin (1–5 nM). Changes in tension were recorded on a Basile 7050 Unirecord. The under the curve areas (UCAs) were obtained by carefully cutting and weighing (R160D analytical balance, Ditta Blasi Giorgio, Rome, Italy) the paper response traces. Data are reported as mean ± S.E.M. of the UCAs.

3.3. In Vitro assay

Prepuberal female Sprague Dawley rats (Harlan, Milan, Italy), 28-day-old, with body weight of 90–105 g, were used. Housing and feeding conditions were the same described in the *In vivo* uterotrophic assay section. Animals were sacrificed by inhalation of CO_2. The uterine horns were excised as formerly described and weighed. From each horn, a 1.5 ± 0.2 cm piece was excised and placed in tyrode solution in 10 ml isolated organ baths, connected to a Basile High Sensitivity (Type D10) transducer, stretched to a passive tension of 2 g, allowed to equilibrate for 1 h [22] and thereafter tested for the response to oxytocin (0.2 nM), prostaglandin $F_{2\alpha}$ (14 nM) and bradykinin (0.5 nM) without (control), and in the presence of 17 β-estradiol (0.05–2.72 μg, corresponding to 0.02–1.00 μM), total extract (100 and 500 μg), or D sub-fraction (9, 18, 54 and 90 μg). Changes in tensions were recorded on a Basile 7050 Unirecord. The UCAs were obtained by carefully cutting and weighing (R160D analytical balance, Ditta Blasi Giorgio, Rome, Italy) the paper response traces. Data are reported as mean ± S.E.M. of % variation of the UCAs.

3.4. Statistical analysis

Statistical comparisons were performed using analysis of variance, followed by the tests suggested by the SigmaStat 3.0 (SPSS, Chicago, IL, USA) software. P value < 0.05 was considered statistically significant.

4. Results

4.1. Recombinant yeast estrogenicity assay

C. racemosa total extract and hydrophilic C sub-fraction had a very slight estrogenic activity (data not shown), whereas lipophilic D sub-fraction induced a consistent activation of the hER α. The efficacy in activating the estrogen receptor subtype α was higher for D sub-fraction than for 4-hydroxytamoxifen and raloxifen, and comparable to that of 17 β-estradiol, though with a lower potency, as highlighted by the EC50 values (EC50 = 0.193 ng/ml for 17 β-estradiol [0.7 nM/l] and 29.323 μg/ml for D sub-fraction) (Fig. 1).

4.2. In vivo uterotrophic assay

Total extract or D sub-fraction had no uterotrophic effect (data not shown) whereas estradiol valerate (0.5 or 1.0 mg/10 ml/kg) dose-dependently increased uterine weight (+57.4 ± 11.4%; +91.2 ± 15.2%, respectively; $p < 0.001$ Holm–Sidak method). No significant behavioural modification or ponderal increase difference was noticed after the 3-day treatment.

4.3. Ex vivo assay

Uterine tissues coming from rats treated with water, *C. racemosa* total extract and D sub-fraction were generally poorly sensitive to the tested oxytocic compounds, feebly responding to oxytocin (5 and 10 nM/l), bradykinin (1 and

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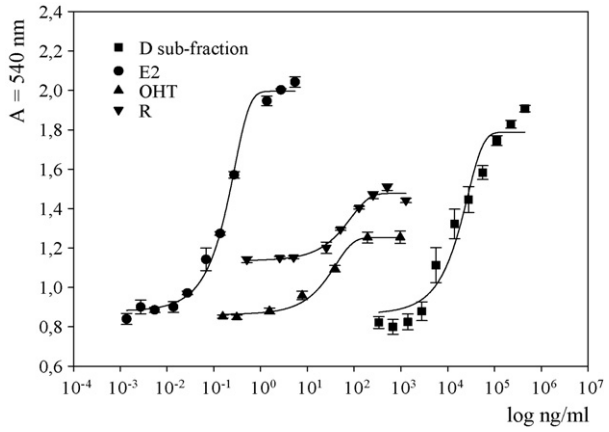

Fig. 1. Recombinant yeast estrogenicity assay. Concentration–response regression curves describing the ability of 17 β – estradiol (E2), 4-hydroxytamoxifen (70% *trans*, 30% *cis*) (OHT), raloxifen (R) and *C. racemosa* D sub-fraction (D sub-fraction) to activate the human estrogen receptor α (hER α). Points are the mean ± S.E.M. of four replicates in three independent experiments.

5 nM/l) and prostaglandin $F_{2\alpha}$ (14 and 28 nM/l). Higher responses were obtained in uterine horns from rats treated with estradiol valerate, in which the response to oxytocin (0.2 and 0.5 nM/l) was between +390 and +820% ($p < 0.001$ Holm–Sidak method) (Fig. 2), and that to bradykinin (5 nM/l) was between +170 and +370% higher than in control group ($p < 0.05$ and < 0.001 Holm–Sidak method) (Fig. 3). The response to prostaglandin $F_{2\alpha}$ (14 and 28 nM/l) was markedly increased (range +120 to +300%) (Fig. 4).

4.4. *In vitro assay*

On naïve uterine tissues, 9–90 μg of D sub-fraction induced a significant, dose-dependent, inhibitory activity on 0.2 nM/l oxytocin (up to −45%) (Fig. 5), 0.5 nM/l bradykinin (up to −64%) (Fig. 6), and 14 nM/l prostaglandin $F_{2\alpha}$ (up to −50%) (Fig. 7) evoked response. This inhibition was comparable to that induced by 1 μM/l 17 β-estradiol (−78% for oxytocin; −67% for bradykinin and −65% for prostaglandin $F_{2\alpha}$) and with an activity rate of nearly 30:1. The total extract only had a very slight, not significant, activity.

5. Discussion

Reported *in vivo* results showed that, unlike 17 β-estradiol, neither *C. racemosa* total extract nor the lipophilic sub-fraction have uterotrophic effect. Most of the published results on this topic have been obtained in ovariectomized rats treated up to 3 months with *C. racemosa* extracts [11,23]. The immature uterotrophic assay we used, more sensitive than the ovariectomized uterotrophic assay [24], confirmed that with black cohosh this effect is absent.

Ex vivo results showed that the uterine horns from control and *C. racemosa*-treated impuberal rats are nearly insensitive to the oxytocic compounds used in our experiments. The 3-day treatment with estradiol valerate, dose-dependently sensitized uteri to oxytocin and, to a lesser extent, to bradykinin and prostaglandin $F_{2\alpha}$. In the uterus, bradykinin is known to be a potent inducer of smooth muscle contraction, which is mediated by the bradykinin B2 receptor subtype, localized to both the circular and longitudinal smooth muscle layers. In rats, B2 receptor levels are up-regulated by estrogen [25].

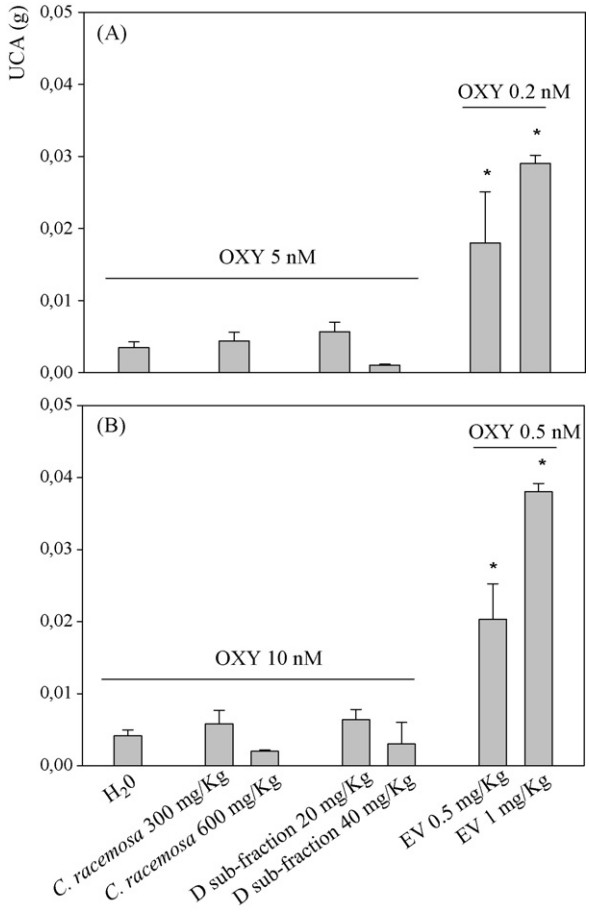

Fig. 2. Mean ± S.E.M. of the under the curve area (UCA), of the response to: 0.2 and 5 nM (panel A), 0.5 and 10 nM (panel B) oxytocin (OXY) in isolated uterine horns from rats orally treated, once a day for 3 days, with: water (H_2O, 10 ml/kg), *C. racemosa* extract (*C. racemosa*, 300 or 600 mg/10 ml/kg), *C. racemosa* D sub-fraction (D sub-fraction, 20 or 40 mg/10 ml/kg), estradiol valerate (EV, 0.5 or 1 mg/10 ml/kg). $N = 4$ animals/group. *$p < 0.001$ *vs.* H_2O group, Holm–Sidak method

Oxytocin receptor number is up-regulated by estrogens in the uterus [26–28]; less is reported about 17 β-estradiol and prostaglandin $F_{2\alpha}$ receptor correlation, especially in the immature uterus. Several lines of evidence support the theory that estrogens increase prostaglandin $F_{2\alpha}$ receptor number [29]. Gordan et al. [30] found a lower prostaglandin $F_{2\alpha}$ evoked response in uterine tissues obtained from ovariectomized rats treated with 17 β-estradiol, but from our results it might be hypothesized that, in young animals, much before

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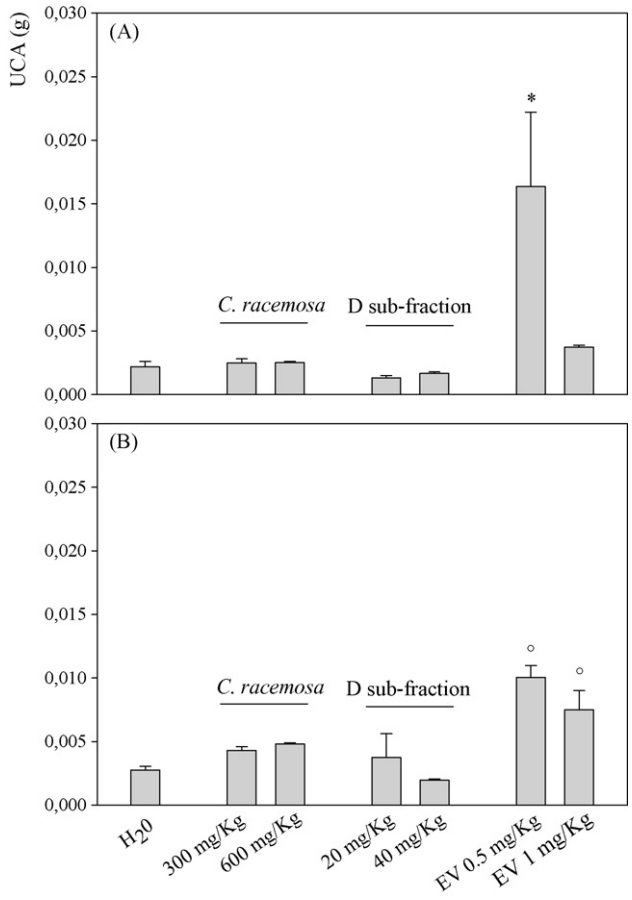

Fig. 3. Mean ± S.E.M. of the under the curve area (UCA), of the response to: 1.0 nM (panel A); 5.0 nM (panel B) bradykinin in isolated uterine horns from rats orally treated, once a day for 3 days, with: water (H_2O, 10 ml/kg), *C. racemosa* extract (*C. racemosa*, 300 or 600 mg/10 ml/kg), *C. racemosa* D sub-fraction (D sub-fraction, 20 or 40 mg/10 ml/kg), estradiol valerate (EV, 0.5 or 1 mg/10 ml/kg). $N = 4$ animals/group. $^*p < 0.001$; $^\circ p < 0.05$ *vs.* H_2O group, Holm–Sidak method.

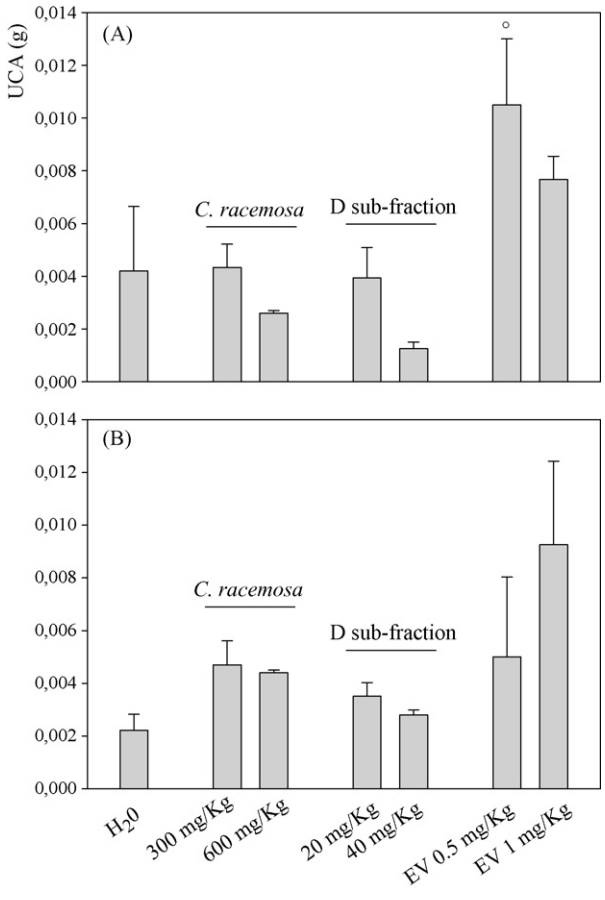

Fig. 4. Mean ± S.E.M. of the under the curve area (UCA) of the response to (panel A) 14 nM, (panel B) 28 nM prostaglandin $F_{2\alpha}$ in isolated uterine horns from rats orally treated, once a day for 3 days, with: water (H_2O, 10 ml/kg), *C. racemosa* extract (*C. racemosa*, 300 or 600 mg/10 ml/kg), *C. racemosa* D sub-fraction (D sub-fraction, 20 or 40 mg/10 ml/kg), estradiol valerate (EV, 0.5 or 1 mg/10 ml/kg). $N = 4$ animals/group. $^\circ p < 0.05$ *vs.* H_2O group, Holm–Sidak method.

sexual development, 17 β-estradiol stimulates prostaglandin $F_{2\alpha}$ receptor synthesis.

In prepuberal 28-day-old rats, starting from concentrations lower than 500 nM/l, 17 β-estradiol had a significant dose-dependent inhibitory effect on the response to all the oxytocic compounds used. D sub-fraction showed an inhibitory activity comparable, especially for bradykinin, to that induced by 17 β-estradiol, with potency rate close to 1:33. D sub-fraction seemed to have lower efficacy then 17 β-estradiol only on the oxytocin evoked response, where the inhibitory effect came to a plateau at about 50% of the effect induced by 17 β-estradiol.

Much is found in literature about an inhibitory activity of estrogens on *in vitro* uterine basal tone and response to oxytocin and prostaglandins in mammals [30–34]. This effect, attributed to an inhibitory action on Ca^{2+} influx and Ca^{2+} release from intracellular Ca^{2+} store [35,36], is not specific for the uterine smooth muscle but is also reported in vasal smooth muscle and it is supposed to be at the basis of the protective effect of estrogens on coronary atheroscle-

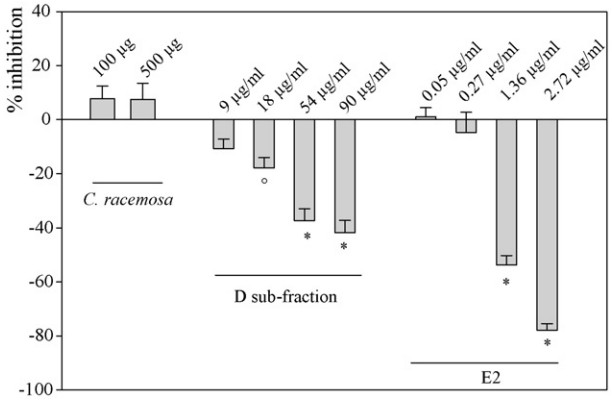

Fig. 5. Mean ± S.E.M. of % inhibition of 0.2 nM oxytocin-evoked response of isolated uterine horns from 28-day-old rats after injection, in the bath chamber, of *C. racemosa* extract (*C. racemosa*, 100 or 500 µg), *C. racemosa* D sub-fraction (D sub-fraction, 9, 18, 54 or 90 µg), or 17 β-estradiol (E2, 0.05, 0.27, 1.36 or 2.72 µg, corresponding to 0.02–1.00 µM). $N = 6$ animal/group. $^\circ p < 0.05$; $^*p < 0.001$ Holm–Sidak method.

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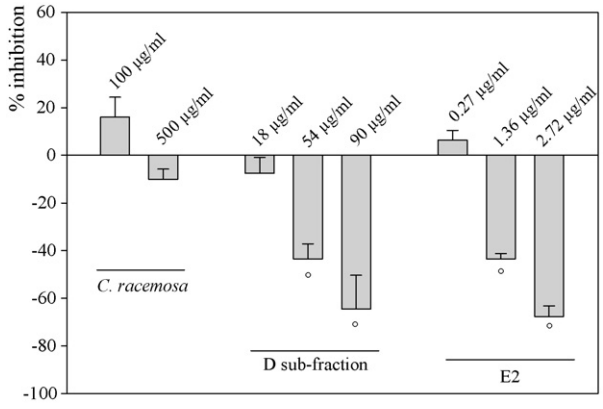

Fig. 6. Mean ± S.E.M. of % inhibition of 0.5 nM bradykinin-evoked response in isolated uterine horns from 28-day-old rats after injection, in the bath chamber, of *C. racemosa* extract (*C. racemosa*, 100 or 500 µg), *C. racemosa* D sub-fraction (D sub-fraction, 18, 54 or 90 µg), or 17 β-estradiol (E2, 0.27, 1.36 or 2.72 µg, corresponding to 0.10–1.00 µM). $N = 6$ animal/group. $^{\circ}p < 0.05$ Dunn's method.

rosis [32,37]. In human myometrium and uterine arteries, ovarian steroids seem to cause a more pronounced inhibition of receptor-mediated than of voltage-dependent Ca^{2+} channels [38]. On the other side, oxytocin, bradykinin and prostaglandin $F_{2\alpha}$ act by increasing intracellular Ca^{2+} concentrations [32,39].

Oxytocin, prostaglandins and, to a certain extent, bradykinin, are the major regulators of uterine contractility; prostaglandins are also involved in other constitutive physiological functions as well as in the inflammatory process. Bradykinin has been implicated, among others, in pain and inflammation and it is involved in follicular contraction at ovulation [40] and uterine smooth muscle contraction at parturition [25]. The results obtained on *in vitro* experiments confirm the validity of *C. racemosa* use to treat dysmenorrhoea.

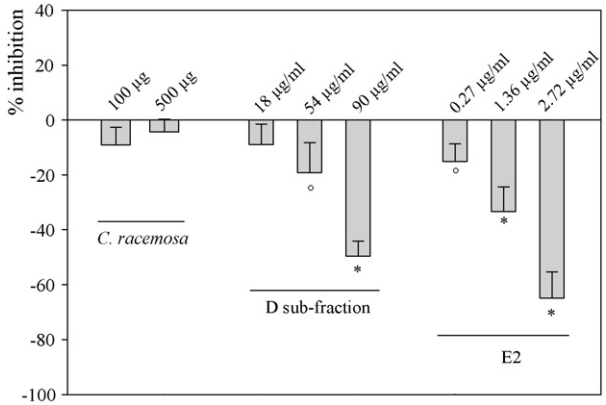

Fig. 7. Mean ± S.E.M. of % inhibition of 14 nM $PgF_{2\alpha}$-evoked response in isolated uterine horns from 28-day-old rats after injection, in the bath chamber, of *C. racemosa* extract (*C. racemosa*, 100 or 500 µg); *C. racemosa* D sub-fraction (D sub-fraction, 18, 54 or 90 µg); or 17 β-estradiol (E2, 0.27, 1.36 or 2.72 µg, corresponding to 0.10–1.00 µM). $N = 6$ animal/group. $^{\circ}p < 0.05$; $^{*}p < 0.001$ Holm–Sidak method.

On the *S. cerevisiae lac Z* strain, D sub-fraction seemed to act as a pure agonist on the hERα with an efficacy comparable to that of the natural ligand 17 β-estradiol but with a very poor potency. This result, along with the results obtained on uterine strips *in vitro*, re-opens the discussion on the possible estrogenic activity of *C. racemosa*.

Lipophilic compounds present in black cohosh could act on estrogen receptor subtypes and this, together with central activity [11,41], could explain the positive results obtained in clinical reports on menopausal complaints [2], bone resorption [17,42], and dysmenorrhoea [43]. Jarry et al. [44] found that compounds present in the *C. racemosa* standardized BNO 1055 extract bind to an estrogen-binding protein contained in human endometrium cytosol, this could either be the so-called estrogen-binding site type II, or a third estrogen receptor type, the γ receptor.

Current uses of black cohosh primarily tend toward the treatment of symptoms of menopause, such as hot flashes, and menopausal anxiety and depression [9]. Extracts have also been shown to be useful for younger women suffering hormonal deficits following ovariectomy or hysterectomy, as well as for juvenile menstrual disorders [3,43,45]. From the therapeutic point of view, *C. racemosa*, lacking uterotrophic activity, might be preferred to hormone replacement therapy. With the exception of a possible interaction with tamoxifen, there are no known interactions between black cohosh extract and any medications [45]. The safety profile of black cohosh seems positive, with low toxicity, few and mild side effects, and good tolerability [43,45]. Nevertheless, the Australian Adverse Drug Reactions Advisory Committee has recently reported warnings of the possible hepatotoxicity of black cohosh, [46]; more knowledge about the causative agents is needed.

In conclusion, our results, obtained from different experimental models, support previous data indicating that the active components of black cohosh are concentrated in the lipophilic fraction, confirm the effectiveness of *C. racemosa* in menstrual distress, further emphasize the possibility that lipophilic constituents bind to an as yet not identified estrogen receptor, which is likely to be inversely involved in inflammation, and highlight the need for further studies in order to identify the compounds responsible for the activity of black cohosh.

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