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Mesquite pod extract modifies the reproductive physiology and behavior of the female rat

S. Retana-Márquez [a,*], F. García Aguirre [b], M. Alcántara [a], E. García-Díaz [a], M. Muñoz-Gutiérrez [a], M. Arteaga-Silva [a], G. López [a], C. Romero [a], P. Chemineau [d], M. Keller [d], J.A. Delgadillo [c]

[a] Departamento Biología de la Reproducción, Universidad Autónoma Metropolitana-Iztapalapa, San Rafael Atlixco 186, México City C.P. 09340, Mexico
[b] Departamento de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa, San Rafael Atlixco 186, México City C.P. 09340, Mexico
[c] Centro de Investigación en Reproducción Caprina, Universidad Autónoma Agraria Antonio Narro. Torreón, Coahuila, Mexico
[d] INRA, Physiologie de la Reproduction et des Comportements UMR INRA-CNRS-Univ F. Rabelais- Haras Nationaux 37380 Nouzilly, France

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ABSTRACT

Phytoestrogens are non steroidal compounds that can bind to estrogen receptors, mimicking some effects of estradiol (E_2). These compounds are widespread among legumes, which are used as pasture, and their importance in animal agriculture has increased. Mesquite (*Prosopis* sp) is a widespread legume, widely used to feed several livestock species in Mexico. The main product of mesquite is the pod, which is considered high quality food. As a legume, it could be assumed that mesquite contains some amounts of phytoestrogens which might induce potential estrogenic effects. However, to our knowledge, there are no reports regarding the possible estrogenic activity of this legume either in livestock or in animal models such as the rat. Therefore, in this study, we evaluated the potential estrogenic effects of mesquite pod extract on several aspects of behavior and reproductive physiology of the female rat. The effects of the extract were compared with those of E_2 and two isoflavones: daidzein (DAI) and genistein (GEN). The following treatments were given to groups of intact and ovariectomized (OVX) female rats: vehicle; mesquite pod extract; E_2; GEN; DAI. Compared to vehicle groups, mesquite pod extract, DAI, GEN, and E_2 increased uterine weight and induced growth in vaginal and uterine epithelia. In intact rats, mesquite pod extract, GEN and DAI altered estrous cyclicity, decreased lordotic quotient and intensity of lordosis. In OVX rats, mesquite pod extract, DAI and GEN induced vaginal estrus, increased vaginal epithelium height, and induced lordosis, although its intensity was reduced, compared with intact rats in estrus and E2-treated rats. These results suggest that mesquite pod extract could have estrogenic activity. However, the presence of phytoestrogens in this legume remains to be confirmed.

Introduction

Phytoestrogens are non-steroidal compounds present in plants. They have structural similarity with natural estrogens such as 17β-estradiol, allowing them to bind with an estrogen receptor and thereby induce biologically detectable effects (Setchell et al., 1984; Navarro, 2005). These compounds are widespread among legumes such as alfalfa (*Medicago sativa*) (Bora and Sharma, 2010), white clover (*Trifolium repens*), subterranean clover (*Trifolium subterraneum*) (Sakakibara et al., 2004) and red clover (*Trifolium pretense*) (Sabudak and Guler, 2009), as well as soybean (*Glycine sp.*) (Setchell et al., 2002). The most important estrogenic compounds in legumes are coumestanes (coumestrol) and isoflavones: genistein (GEN), and daidzein (DAI), whose estrogenicity relative to estradiol-17β is approximately 1/1000 and 1/10,000, respectively (Adams, 1995). Although phytoestrogens can mimic the effects of estradiol (E_2), their effects are not necessarily identical to estrogens, since there are no reliable reports of phytoestrogens causing behavioral estrus in ovariectomized females. Phytoestrogens may also have antiestrogenic activity (Yildiz, 2005), competing with endogenous steroids, so that the balance between estrogenic and antiestrogenic activity is determined by the ratio of phytoestrogen to estrogen (Folman and Pope, 1966). This may explain why estrogenic effects of these compounds predominate in the sheep, but anti-estrogenic effects are mainly reported in humans, in which circulating concentrations of steroidal estrogens are relatively high (Adlercreutz et al., 1991). Cattle have relatively low circulating concentrations of E_2, so it may be expected that estrogenic effects would dominate in this species.

Several studies have evaluated the effect of isoflavones on female physiology and sexual behavior in animal models such as the rat. High doses of GEN increase uterine weight, induce mitosis in endometrial epithelium, and enlargement of endometrial glands. In the vagina, GEN induces hyperplastic epithelium compared with the atrophic OVX controls (Rimoldi et al., 2007; Santos et al., 2010). In

* Corresponding author. Fax: +52 55 5804 4930.
E-mail address: rems@xanum.uam.mx (S. Retana-Márquez).

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other studies, it has been observed that the administration of resveratrol combined with estradiol benzoate, but not resveratrol alone, induces lordosis in ovariectomized rats (Henry and Witt, 2002).

Chronic administration of GEN also causes altered estrous cycles, reduction in litter size, and aberrant cycles, consisting in 3 or more consecutive days of estrus or 4 or more consecutive days of diestrus (Delclos et al., 2009)

On the other hand, pasture legumes containing phytoestrogens is widely used in animal agriculture throughout the world (Adams, 1995; Rachuonyo et al., 2005; Sabudak and Guler, 2009), and reproductive abnormalities due to phytoestrogens have been described in cows and sheep (Bennetts et al., 1946). Estrogenic syndromes attributed to phytoestrogens have been described both in cows fed with alfalfa, containing coumestrol, and in ewes grazing on clover containing isoflavones. These syndromes are characterized by repeated estrus, abortions, endometriosis, cystic ovaries, pseudoestrus, swollen uterus (Adler and Trainin, 1960) and decreased gestation index with normal levels of E_2 in plasma (Romero-R et al., 1997) in cows. In ewes, very low lambing rates, prolapse of the uterus and dystocia, severe metritis, pyometron and *hydrops uteri*, reduced ovulation and conception rates, as well as permanent infertility have been described (Lightfoot et al., 1974).

Soy is a legume with many proven estrogenic effects observed in both animal models and human beings treated either with soy extracts or soy isoflavones. In animal models, it has been shown that soy and isoflavones increase uterine weight, and the height of endometrial and vaginal epithelium (Gallo et al., 1999; Rimoldi et al., 2007; Santos et al., 2010).

Mesquite (*Prosopis* sp) is a widespread legume in arid and semi-arid areas and is widely used to feed several livestock species in Mexico. The main product of mesquite is the pod, which is considered high quality food for livestock. The crop is abundant from May to September, which is important since drought decreases seasonal crops, as well as the fodder of the summer pasture season (http://www2.ine.gob.mx/publicaciones/libros/72/usos.html). As a legume, mesquite could contain some quantity of phytoestrogens that might have potential estrogenic effects in livestock. However, to our knowledge, there are no reports on the possible phytoestrogen content in this plant and its possible estrogenic activity or the possible harmful effects on reproductive function. For this reason, we evaluated the effects of mesquite pod extract on several aspects of behavior and reproductive physiology of the female rat used as an animal model. The effects of the extract were compared with those of E_2 and two isoflavones (DAI and GEN).

Materials and methods

Animals

All experimental procedures used in this study were approved by the Universidad Autónoma Metropolitana's Institutional Animal Care and Use Committee, in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, Mexican Official Regulation (NOM-062-ZOO-1999).

Adult female Wistar rats, weighing 200–230 g were housed, four per cage (50×30×20 cm), under standard vivarium conditions. The colony room was maintained on a 12:12 reverse light cycle (lights off: 09:00) and under controlled temperature (23±1 °C). Food and water were available ad libitum throughout the experiments. The rodent diet used was "2018 Teklad global" from Harlan Laboratories. It should be noted that this diet contains phytoestrogens DAI and GEN (range from 150 to 250 μg/g, www.harlan.com). However, all animals in this study were exposed to the same diet ad libitum regardless of their treatment group. Moreover, there are no reports confirming any estrogenic effects of this rodent diet (Naciff et al., 2004).

The effects of mesquite extract and isoflavones were tested under two physiological situations: in intact and in ovariectomized (OVX) females. Bilateral OVX was performed under ketamine anesthesia (0.9 mg/g of body weight) using standard surgical procedures, at the age of 3 months. OVX animals were used after two months of recovery (5 months of age), a delay which is sufficient to ensure that levels of ovary hormones were completely depleted. The following treatments were given to the experimental groups (n=8/group): 1) Intact/vehicle group: females received only vehicle injections (0.2 ml) sc; 2) intact/mesquite extract group: rats received mesquite pod concentrated extract (4 g/kg); 3) intact/E_2 group: females received E_2 (reference E1024-1G, Sigma; 40 μg/kg); 4) intact/GEN; intact rats received GEN (reference G6649, Sigma; 1.6 mg/kg); 5) intact/DAI group: intact females received DAI (reference D7802, Sigma; 1.6 mg/kg); 6) OVX/vehicle group: ovx females received only vehicle injection (0.2 ml); 7) OVX/extract group: ovx females received concentrated extract (4 g/kg); 8) OVX/E_2 group: ovx females received E_2 (40 μg/kg); 9) OVX/genisten group (1.6 mg/kg) sc; and 10) OVX/DAI (1.6 mg/kg). All the treatments were administrated subcutaneously daily during 30 days. The volume of injection was 0.2 ml in all cases.

The dose of mesquite pod extract was selected according to daily consumption in ewes (140 g/day), which is equivalent to 3.5 g/kg/day.

The E_2 dose used in this study is known to induce receptive behavior in female rats (Retana-Márquez et al., 2003); DAI and GEN doses were selected according to those used to elicit physiological responses, such as disrupted estrous cyclicity, reduced body weight, ovarian hypertrophy, and some non-lasting effects on socio-sexual behavior (Henry and Witt, 2002).

Mesquite extract. Mesquite pod extract was obtained by macerating 500 g of pod in 200 ml of water:ethanol/85:15 during 15 days. The extract was then filtered and concentrated with a freeze-drier. The concentrated extract was then re-dissolved in saline and administered (1000 mg/0.2 ml) to intact and OVX females.

Measures

Vaginal cytology and estrous cyclicity

Estrous cycles were monitored by daily evaluation of vaginal smears which were stained with hematoxilin–eosin and evaluated with an optic microscope (Olympus, model CX41RF). Vaginal smears were obtained 2 h after the onset of the dark period, under red light (40 W). Estrous cycles were classified as follows: (a) 3-day cycle, an irregular shortened cycle usually resulting from a condensed or absent diestrus period. In OVX females, a 3-day cycle was rated whenever proestrus was absent, with the remaining periods present; (b) 4-day cycle, a normal length cycle consisting of full estrus, metestrus, diestrus, and proestrus periods; (c) 4–5-day cycle, also a normal length cycle that includes an additional 24 h of diestrus, called diestrus II; (d) constant estrus, an irregular estrous cycle defined by the persistence of cornified cells beyond 2 days (modified from Henry and Witt, 2002).

Behavioral testing

Female sexual behavior was assessed in a Plexiglas arena (40×40×50 cm) with sexually experienced males (n=40). Males were alternated during test days; 10 different stimulus males were used each test day, and rested for 3 days. All testing was done during the first 3 h of the dark phase, under red light. Female behavior was assessed through receptivity and proceptivity. Receptivity for each female was determined as a lordosis quotient [LQ=(number of lordosis/10 mounts)×100]

The intensity of lordosis (extent of dorsiflexion) was quantified according to the lordosis score proposed by Lehmann and Erskine (2004). The rating of lordosis intensity (LR) was established based on the degree of spinal dorsiflexion and the extent to which the sagittal ridge of the head lined up in a vertical plane. The rating was based on a scale of no vertebral dorsiflexion (0), slight dorsiflexion coupled with slight movement of the head toward the vertical plane (1), moderate