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Comparison of the effects of mesquite pod and *Leucaena* extracts with phytoestrogens on the reproductive physiology and sexual behavior in the male rat

S. Retana-Márquez [a,*], L. Juárez-Rojas [a], A. Hernández [a], C. Romero [a], G. López [a], L. Miranda [b], A. Guerrero-Aguilera [a], F. Solano [a], E. Hernández [a], P. Chemineau [c], M. Keller [c], J.A. Delgadillo [d]

[a] *Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana-Iztapalapa, San Rafael Atlixco 186, México City C.P. 09340, Mexico*
[b] *Colegio de Posgraduados, Campus San Luis Potosí, Mexico*
[c] *INRA, Physiologie de la Reproduction et des Comportements, UMR 7247 INRA–CNRS–Université F. Rabelais–IFCE, 37380 Nouzilly, France*
[d] *Centro de Investigación en Reproducción Caprina, Universidad Autónoma Agraria Antonio Narro, Torreón, Coahuila, Mexico*

HIGHLIGHTS

- The estrogenic effects of mesquite and *Leucaena* extracts on male were quantified.
- Their effects were compared with those of isoflavones and estradiol.
- Extracts disrupted sexual behavior, sperm quality, and decreased testosterone.
- The effects of the extracts were similar to those of daidzein and genistein.
- Mesquite and *Leucaena* extracts have estrogenic effects on male reproductive variables.

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ABSTRACT

Mesquite (*Prosopis* sp.) and *Leucaena leucocephala* are widespread legumes, widely used to feed several livestock species and as food source for human populations in several countries. Both mesquite and *Leucaena* contain several phytoestrogens which might have potential estrogenic effects. Thus, the aim of this study was to evaluate the effects of mesquite pod and *Leucaena* extracts on several aspects of behavior and reproductive physiology of the male rat. The effects of the extracts were compared with those of estradiol (E_2) and of two isoflavones: daidzein (DAI) and genistein (GEN). The following treatments were given to groups of intact male rats: vehicle; mesquite pod extract; *Leucaena* extract; E_2; DAI; GEN. The results indicate that mesquite pod and *Leucaena* extracts disrupt male sexual behavior in a similar way to DAI and GEN, but less than E_2. The main disruptor of sexual behavior was E_2, however after 40 and 50 days of administration, both extracts and phytoestrogens disrupted sexual behavior in a similar way to E_2. The extracts also increased testicular germ cell apoptosis, decreased sperm quality, testicular weight, and testosterone levels, as phytoestrogens did, although these effects were less than those caused by estradiol. The number of seminiferous tubules with TUNEL-positive germ cells increased in extracts treated groups in a similar way to phytoestrogens groups, and E_2 caused the greatest effect. The number of TUNEL-positive cells per tubule increased only in *Leucaena* extract and E_2 groups, but not in mesquite- and phytoestrogens-treated groups. Spermatocytes and round spermatids were the TUNEL-positive cells observed in all experimental groups. This effect was associated with smaller testicular weights without atrophy in experimental groups compared with control. Testicular atrophy was only observed in estradiol-treated males. Testosterone decreased in males of all experimental groups, compared with control, this androgen was undetectable in E_2 treated males. These results suggest that mesquite pod and *Leucaena* extracts cause effects similar to those of phytoestrogens in male rat reproduction, these effects were lower than those caused by E_2.

1. Introduction

Phytoestrogens, which are found widely in a variety of plants and fodder, can have adverse effects on the reproductive tract of most animal species, including human beings. Phytoestrogens are considered

* Corresponding author at: Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana-Iztapalapa, Mexico City C.P. 09340, Mexico.
E-mail address: rems@xanum.uam.mx (S. Retana-Márquez).

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as endocrine disruptors, since they can induce estrogenic and antiestrogenic effects in mammals by weakly binding to nuclear estrogen receptors (ER) α and β [1,2]. Most phytoestrogens bind both ERα and ERβ and activate ER-dependent gene transcription through both subtypes; however, they generally have a higher relative binding affinity for ERβ than ERα. Most isoflavones bind and activate both ERα and ERβ more readily than synthetic endocrine disrupting chemicals (EDCs), but in the presence of phytoestrogens and other endocrine disruptors, it appears that ERβ is more efficient than ERα at recruiting coactivators (for review see [1,2]). These compounds can also inhibit the interaction of steroids with enzymes; inhibit the binding of steroids to steroid-binding proteins, and compete with estradiol (E_2) for binding to either or both ER subtypes in reproductive organs [3]. Phytoestrogens given in high doses to adult or neonatal rodents can cause decreased testicular weight and spermatogenesis, lower FSH and testosterone levels (for review see [4]), and temporary infertility syndromes in domestic animals (ewes) have been related to high phytoestrogen consumption during grazing [5]. These effects are of concern given the increase in the consumption of phytoestrogens both in animals and humans, due to the increased use of legumes in animal diets as well as the increase in vegetarian diets in some human populations.

In adult mammalian males, estrogens are synthetized by Leydig, Sertoli, and germ cells. ERα and β are present in the testis, brain, in efferent ductules, in prostate, and epididymis of most species, regulating their function [6,7]. ERα is expressed in Leydig cells of rats and mice [8], where it mediates estrogen inhibition of LH effect on those cells, reducing serum testosterone levels [9]. ERα is also present in ductuli efferents where they are essential for luminal fluid absorption from the testis [7]. In epididymis, ERα is related with the acidification of epididymal fluid and sperm motility acquisition [10]. ERβ is expressed by rat and human pachytene spermatocytes, round spermatids and Sertoli cells where it appears to function in cell differentiation and regulation of apoptosis [10,11]. ERβ is expressed in rat prostate and are important for differentiation and anti-proliferative function [12]. ERα and ERβ are located in the hypothalamic preoptic area (POA) [13], where sexual behavior is controlled in the male rat [14]. Both receptor subtypes are required for adequate male sexual behavior [15,1]. Isoflavonoid phytoestrogens can be competitive inhibitors of testosterone and androstenedione in their interaction with cytochrome p450 aromatase [16,3], an enzyme necessary for the conversion of testosterone to estradiol [16]. Both E_2 and ERα are also necessary for fertility in the male, as their absence result in decreased fertility [17]. Therefore, the male reproductive tract potentially can be sensitive to phytoestrogens, and several studies report effects of these compounds alone or contained in legume or extracts on male reproduction. Neonatal exposure of pups through milk of rat dams fed a coumestrol reduce sexual behavior, especially mount and ejaculation latencies, as well as ejaculation rate in adulthood [18]. Feeding adult rams with clover, which is rich in isoflavones, results in decreased sperm counts [19,20]. Daidzein (DAI) administered chronically (12 weeks) to adult rabbits causes erectile dysfunction [21]. The administration of kiwi extract, a fruit rich in isoflavones and flavonoids, is capable of reducing the levels of plasma testosterone, estradiol, and sperm count in adult male rats [22]. Concerning humans, there are very few epidemiological or clinical studies evaluating the effects of phytoestrogens on fertility and reproductive parameters in men, and they show contradictory results. Some studies show no effects on sperm quality or ejaculate volume. Other studies report decreased sperm concentration and motility probably related to phytoestrogen consumption in Asiatic countries such as China and Japan, where the consumption of phytoestrogens is more pronounced than in other parts of the world [23,24,25]. A recent review [26] indicates that sperm numbers, in this part of the world, are about the same level as the lowest ones seen in Europe. Furthermore, epidemiologic surveys demonstrated that erectile dysfunction incidence in men aged >20 years was nearly 10% higher in Chinese than in Americans (28.3% vs 18.4%) [27]. If these effects are due to the diet, other lifestyle related factors and/or genetic diversity cannot be concluded yet.

In regard to testosterone levels, contradictory results are also reported [for review see 4]. Despite this, phytoestrogens have been considered as natural xenoestrogens causing decreased sperm concentration and semen volume observed in men during the last decades [28,29]. Nonetheless, long term, large scale comprehensive human studies are necessary in order to clarify their effects on human reproduction.

Mesquite (*Prosopis* sp.) is a widespread legume in arid and semi-arid areas in Mexico, Africa and Asia, and is considered an important natural resource. Mesquite is used mainly to feed several livestock species due to its high content of protein, carbohydrates, fiber [30,31], minerals and vitamins [32,33]. In addition, mesquite pod is a source of food for human consumption as bread [34], cakes or porridge [32], syrup and beverages [35], desserts, and as a coffee substitute [36]. As a legume, mesquite contains high amounts of some phytoestrogens such as the mesquitol, a flavanol [37], quercetin, luteolin, and isoharmnetin (flavonols), and the flavone vitexin [38], which could have potential estrogenic effects in livestock and other animals, including human beings. No other chemicals seem to be present in mesquite extract that could be considered EDCs [39]. However, to our knowledge, there are no reports on its possible estrogenic activity or harmful effects on male reproductive function.

Leucaena leucocephala is a small, fast-growing leguminous tree [40] native of Southern Mexico and Northern Central America [41], and now is widely spread in many tropical and subtropical regions of the world [42]. In humans, *Leucaena* has been used for contraception [43] and abortion [44], and causes reproductive damage, such as abortion in female goats [45] and pigs [46], and decreased calving percentages in cows [47]. It also causes a decrease in sperm motility and concentration in rabbits [48,49], decreased libido and infertility in male rats, as well as dead fetuses and fetal resorption in rats [50]. *Leucaena* contains several phytoestrogens (flavonols) such as isorhamnetin, kaempferol, quercetin, and luteolin [51], and it is possible that these phytoestrogens could be involved in its harmful effects on reproduction. *Leucaena* also contains mimosine, a non-protein amino acid considered to affect fertility indices. However, its content can decrease by heating *Leucaena* [52].

Considering that mesquite pod and *Leucaena* are used as a feed for livestock and food for humans, the aim of this study was to quantify the effects of mesquite pod and *Leucaena* extracts on masculine sexual behavior, testicular cell death, sperm quality, and testosterone levels in male rats. The effects of the extract were compared with those of E_2 and of two isoflavones (Daidzein and Genistein) whose effects on reproduction are well known.

2. Materials and methods

2.1. 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's Guide for the Care and Use of Laboratory Animals, and Mexican Official Regulation (NOM-062-ZOO-1999).

Adult male Wistar rats (three months of age) weighing 300–350 g were housed, five per cage (50 × 30 × 20 cm), under standard vivarium conditions. The colony room was maintained on a 12:12 reversed 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 [53]. The effects of