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Effects of heart of palm (Palmito) extract on reproductive system of adult male rats

Masoomeh Dokhanchi[1*], Hojatollah Karimi Jashni[2], Nader Tanideh[3], Negar Azarpira[4]

[1]*Transgenic Research Center , Department of Biology, Shiraz University of Medical Science, 7134873985 Shiraz, Iran*

[2]*Department of Anatomy, Jahrom University of Medical Science, 7414846199 Jahrom, Iran*

[3]*Transgenic Research Center, Department of Pharmacology, Shiraz University of Medical Science, 7134873985 Shiraz, Iran*

[4]*Transplant Research Center, Department of Pathology, Shiraz University of Medical Science, 7193711351 Shiraz, Iran*

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ABSTRACT

Objective: To evaluate the toxicity effects of aqueous extract of *heart of palm (Palmito)* on plasma testosterone, estradiol, LH and FSH hormone levels and testicular tissue in male rats. **Methods:** A total of 70 male Sprague-Dawley rats were divided into seven groups. The control group was given 1 mL of distilled water, and the experimental groups were given 25, 50, 100, 150, 200, and 250 mg/kg/day of extract for 56 days. The effect of the treatment on the male reproductive system was evaluated by assessing plasma testosterone, estradiol, LH, and FSH levels, as well as spermatogenesis process. **Results:** A significant decrease in testosterone and estradiol levels were observed in rats treated with different doses of palmito. The extract consumption provided no remarkable change in the serum levels of LH and FSH, and weights of bodies, and testes in the six treated-groups compared to those in the control group. Also, treatment with the palmito resulted in a decrease of the mean seminiferous tubular diameter and the number of spermatogonia, preleptoten and pachytene spermatocytes, round spermatids, and Leydig cells. **Conclusion:** The ethanol extract of palmito has anti-androgenic properties, which affected spermatogenetic activity in all experimental groups.

1. Introduction

The status of herbal medicine has been fast gaining ground all over the world during the last few decades, mainly due to the general belief that herbal drugs are without any side effects besides being cheap and locally available [1,2]. Plants are extensively used to relieve sexual dysfunction [3]. Many recent studies have been conduced to evaluate the actual efficacy and adverse effects of plants on reproductive organs and functions [4].

Phoenix dactylifera L. commonly known as the date palm is one of such plant within many localities in arid and semi-arid regions of the world, especially in southwest Asia and North Africa [5-7]. The information accrued in the past four decades suggest that dates possess diverse medicinal uses including antihyperlipidemic, anticancer, gastroprotective, hepatoprotective, nephroprotective and aphrodisiac activities [5]. Besides fruit, the date palm over the centuries has also provided a large number of other products which have been extensively used by man in all aspects of daily life[8]. The palmito, also known as "heart of palm", is one of such products of palm which has composed of the apical meristem of the palm plus part of the young or immature leaves emerging from the meristem. This edible meristem is frequently consumed as a fresh vegetable in areas where the palms are grown. It ranks among the favorite salads, soups and other gourmet dishes of South and Central America and also Southeast Asia[9-11].

Although several palms produce edible palmitos, some

*Corresponding author: Masoomeh Dokhanchi, Transgenic Research Center, Department of Biology, Shiraz University of Medical Science, 7134873985 Shiraz, Iran.
Tel.: +98 917 714 0938
Fax: +98 711 646 2793
E-mail: dokhanchi.m@gmail.com

species provide most of the palmitos produced. *Euterpe edulis* is the most important species which locally known as Juçara [9].

Juçara is considered the reference product in terms of quality, as it is more tender, sweet and light in color than the other species. Phenolic compounds in palmito from the *Euterpe edulis* (Juçara) occur as phenols, flavonoids, and tannins [9,11]. Flavonoids and phenols have been related to the occurrence of adverse effects on the endocrine and reproductive systems [12,13]. Previous studies have shown that flavonoids produced antiandrogenic activities in male dogs [12,14].

Several investigations have been done on the effects of date palm parts, such as date extracts [15], gemmule [16], pollen [17-19] and date pits [20-22], on the reproductive organs. However, until now there was no published data about *in vivo* influence of palmito on the reproductive systems. Considering the growing popular use of palmito and the presence of two substances with antifertility activity among its constituents, scientific evidence is needed to determine and evaluate the androgenic or anti-androgenic activity of this part of plant. Therefore the purpose of the present study was to investigate the effect of palmito on the male reproductive organ *in vivo* by assessing plasma testosterone, estradiol, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels, as well as spermatogenesis process.

2. Materials and methods

2.1. Palmito extract preparation

Fresh palmito was obtained from agricultural research center of Jahrom (28° 57N′, 53° 57E′), Iran. This location represents the major production areas in this region and has subtropical climate conditions with hot dry summers and mild winter. The palmito was kept refrigerated (4 ℃) for future reference. In order to prepare dry extract, 200 g of palmito was grated into small pieces and then 9 mL of ethanol/distilled water solution (50/50 %w/w) was added. After 72 h, the suspension was placed in a percolator system. Then, the solution was filtered and excess solvent was removed by rotary. Finally, dry matter was prepared using a desiccator. Before each experiment, dried extract was dissolved in 1 mL distilled water and vortexed for 2 min at room temperature.

2.2. Animals and experimental design

After obtaining approval from the institutional animal ethical committee, 70 healthy adult Sprague-Dawley male rats were acquired from Animal House of Shiraz University of Medical Sciences, Iran. The animals were maintained under conditions of controlled temperature (23±3 ℃) with 12-hour day-night cycle. They were fed standard rat pellet diet and were given access to water *ad libtium*.

The rats were randomly divided into seven groups of 10 rats each. The first group (group 1) was fed with 1 mL of distilled water and used as the control. In groups 2-7 the palmito extract was administrated by gavage technique to rats at the doses of 25, 50, 100, 150, 200, and 250 mg/kg/day for 8 weeks. This period of time corresponds to the duration of the spermatogenic cycle of this species [23]. After eight weeks, the animals were killed with ether anesthesia. The following parameters were evaluated: terminal body weight, testes weight, hormone levels, and histology of the testes.

2.3. Hormonal analysis

At the end of the study, blood samples were collected from cardiac puncture and centrifuged at 3 000 rpm for 10 min. Plasma was separated and then stored at −20 ℃ until biochemical and hormonal analyses. The hormone levels were determined from plasma using the double-antibody radioimmunoassay (RIA). The total testosterone, estradiol, FSH, and LH levels were measured using RIA kits (Beijing Beimian, East Asia) following the manufacturer's protocols for serum samples.

2.4. Histological analysis

Blocks of testicular tissue were immediately fixed in Bouin's fluid, and embedded in paraffin. Sections (5 μm) were cut and stained with hematoxylin-eosin. These specimens were examined under bright-field optical microscopy using a light microscope at ×200 magnification power. Corresponding digital images were captured for later analysis. The evaluation of the cell population was based on the calculations made for each cell type per cross-section of the seminiferous tubule. The Leydig cells, spermatogonia, preleptoten and pachytene spermatocytes, and round spermatids were counted. The group counts of these cell types were designated as crude counts and these crude counts were corrected by using Abercrombie's formula [24]. Seminiferous tubular diameter was measured by an ocular micrometer under light microscope. The mean diameter of 100 seminiferous tubules randomly selected in each cross-section, overall 30 cross-sections per each experiment was selected for microscopic evaluation using Olysia Bio-report software (Olympus, Tokyo, Japan).

2.5. Statistical analysis

The results were expressed as means ± standard deviation. All data were done with the Statistical Package for Social Sciences (SPSS 11.0 for windows). The results were analyzed using one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) for comparison between different treatment groups. Statistical significance was set at $P<0.05$.

3. Results

3.1. Body and reproductive organ weights

No animals died during the study and no clinical and behavioral changes were observed in any of the animals after administration of the palmito extract. Fifty six days after treatment with palmito extract, there were no significant differences in the weights of bodies, and testes in the six treated-groups of rats compared to those in the control group or all comparisons (Table 1).

Table 1
Effect of palmito extract on the weights (mean±S.E) of bodies, and testes(g).

Treatment	Weights of bodies	Weights of testes
Control	276.0 ± 7.6	1.25 ± 0.02
Group 2	294.0 ± 12.7	1.23 ± 0.04
Group 3	303.0 ± 10.0	1.19 ± 0.04
Group 4	300.0 ± 13.3	1.27 ± 0.01
Group 5	298.0 ± 8.6	1.24 ± 0.02
Group 6	306.0 ± 11.5	1.29 ± 0.02
Group 7	292.0 ± 9.7	1.22 ± 0.03

3.2. Biochemistry

The plasma total testosterone and estradiol levels of the experimental groups were significantly decreased compared with the control group ($P<0.05$; Figure 1a, b). When the 250 mg/kg of extract is given (group 7), the serum level of testosterone is 0.607 ng/mL, which is about 72% lower than control group. This result also suggests that increasing the extract dose in group 7, leads to a decrease (about 48%) in estradiol. Rats treated with palmito extract had little increase in plasma LH and FSH levels compared with those of the control group ($P<0.05$; Figure 1c, d). However, no significant change was observed compared to the control group.

3.3. Histopathology

The testicular section of control group showed normal seminiferous tubule and the luman were filled with spermatids (Figure 2a). At highest dose, the number of spermatogonia, preleptoten and pachytene spermatocytes were reduced by 32%, 31% and 33% respectively compared to control group. In addition, the production of round spermatids was decreased by 44% in the group 7. Also, the total number of Leydig cells reduced significantly ($P<0.01$) when compared to controls. Although no interstitial edema, hemorrhage, and necrosis were observed in testes of treated rats, palmito extract exposure markedly decreased the mean diameter of seminiferous tubules in comparison with the control group (Figure 2b).

Table 2 shows the results obtained from the histopathological evaluations following palmito extract treatment.

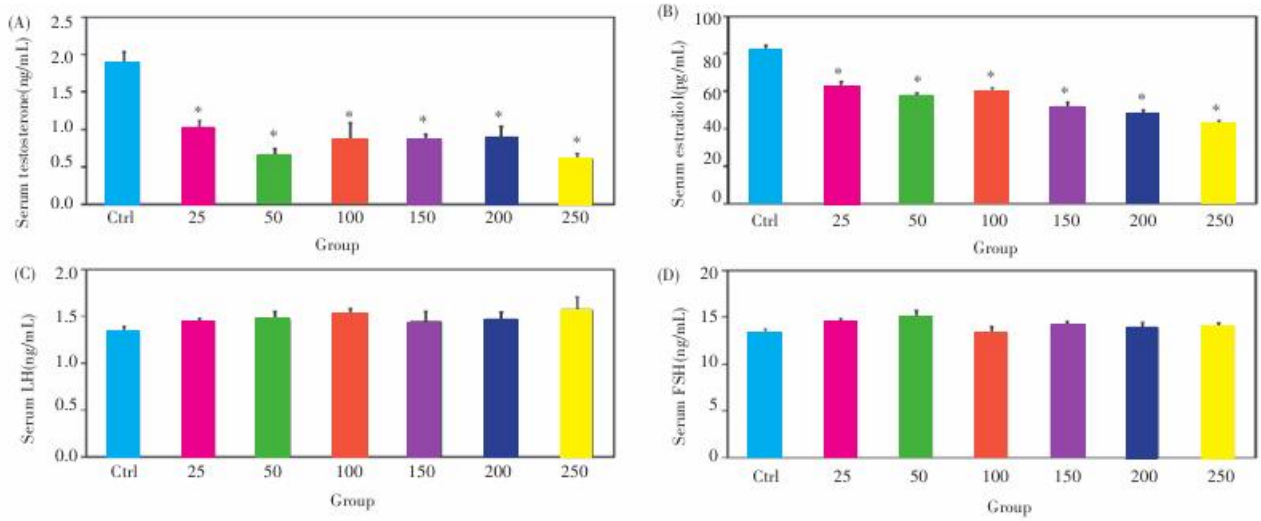

Figure 1. Effect of palmito on plasma (A) testosterone, (B) estradiol, (C) LH, and (D) FSH in control and treated rats for 56 days ($^*P< 0.05$).

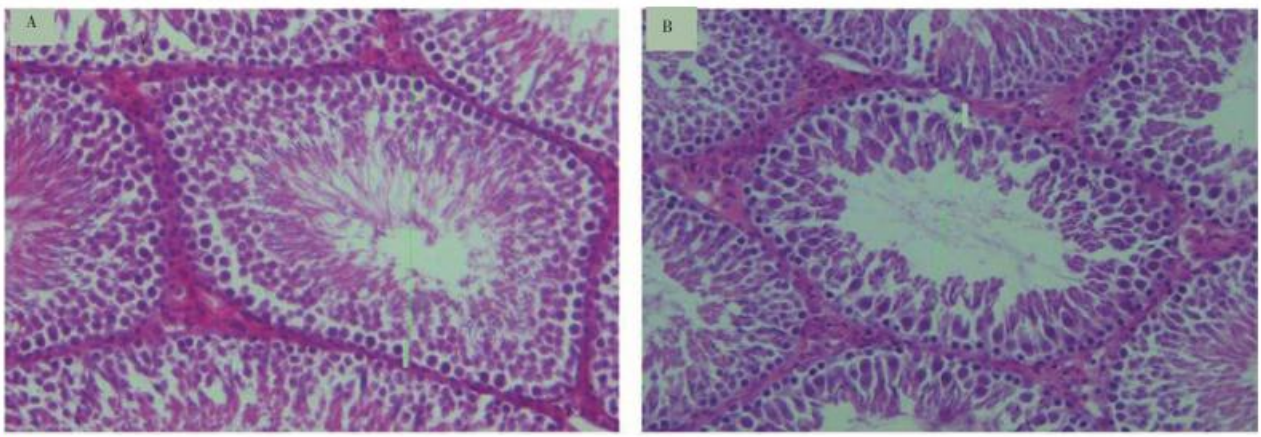

Figure 2. (A) Control group: histological section of testis showing normal spermatogenetic activity. (B) Experimental group: given high dose (250 mg/kg) of palmito extract which provided marked decreases in all the number of spermatogonia, preleptoten and pachytene spermatocytes, round spermatids, and Leydig cells. Also, the mean diameters of the seminiferous tubules remarkably were reduced.

Table 2

Comparison of the number of spermatogonia, preleptoten and pachytene spermatocytes, round spermatids, Leydig cells, and seminiferous tubular diameter between control and experimental groups.

Treatment	Number of spermatogonia (per tubule)	Number of preleptoten spermatocytes (per tubule)	Number of pachytene spermatocytes (per tubule)	Number of round spermatids (per tubule)	Number of Leydig cells	Seminiferous tubular diameter (μ m)
Control	6.14±0.64	18.97±2.11	22.72±1.52	53.23±1.57	29.00±2.15	317.43±4.29
Group 2	5.36±0.23*	16.83±1.47*	19.36±2.66*	49.61±1.77*	26.00±1.64*	311.97±2.77*
Group 3	4.70±0.18*	16.24±2.32*	18.57±2.19*	42.37±1.40*	25.00±2.07*	310.43±5.31*
Group 4	5.12±0.37*	14.77±2.17*	18.30±1.73*	39.14±1.73*	23.00±2.21*	310.28±4.14*
Group 5	4.67±0.21*	15.50±2.15*	16.14±2.23*	36.88±1.47*	23.00±2.19*	308.33±2.82*
Group 6	4.85±0.66*	13.61±1.43*	15.97±2.11*	31.17±1.48*	20.00±1.88*	307.92±3.27*
Group 7	4.19±0.52*	13.14±2.13*	15.15±1.44*	29.92±1.19*	18.00±2.11*	307.12±2.53*

*Significantly different from the control ($P<0.01$).

4. Discussion

In the present study, the administration of palmito extract was carried out for 56 consecutive days at different dose levels. Despite the long-term and high-dose treatment, the results show nontoxicity of the palmito extract as evidenced by unaltered body and organ weight. To assess the potential androgenic or anti-androgenic effect of the plant extract on the reproductive system, spermatogenesis and hormone production were investigated.

Normal functioning of the reproductive system is essential for normal sexual development, behavior, spermatogenesis, *etc*[25]. The testicle is one of the highly specialized gonadal organs, which has two important functions in the adult male: the production of germ cells and the synthesis of testosterone and other hormones. Control of both functions is guided by the central nervous system (CNS) in a classic endocrine feedback loop with follicle stimulating hormone (FSH) and luteinizing hormone (LH) as the key hormonal signals. In males, LH can stimulate Leydig cells to synthesize and secrete testosterone (T), while FSH mainly functions on the sertoli cells and germ cells to facilitate spermatogenesis [26,27]. Hormones such as testosterone, FSH and LH are known to influence the germ cell fate. In addition, Pentikainen *et al.* [28] have demonstrated that estradiol acts as a germ cell survival factor in the human testis *in vitro* [29]. Any chemical interference to steroidogenesis, *e.g.*, altering enzymatic activity or hormone production, can cause adverse effects to the reproductive system[30].

The statistically significant decrease in spermatogenesis, plasma total testosterone and estradiol levels in the experimental groups may suggest that phytoestrogen compounds such as flavonoids in palmito may disrupt normal reproductive performance. Similar results have been reported in some investigations [31,32]. No significant changes in gonadotropin were observed in the experimental groups. This can be attributed to the fact that the phytoestrogens can produce inhibitory effects on gonadotropin secretion in both animals and humans [33].

Observation of the testicular histology showed a consequent decrease in seminiferous tubular diameter. Recent studies demonstrated that decreased seminiferous tubule diameter may indicate germ cell loss[34]. Additionally, a positive relationship usually exists between the tubular diameter and the spermatogenic activity of the testis[35]. Also, treatment with the extract resulted in a decrease of the number of spermatogonia, preleptoten and pachytene spermatocytes, and round spermatids cells. This may be derived from a hormonal imbalance including serum levels of testosterone and estradiol as described above [36]. The mechanism of spermatogenic abnormalities was more likely a result of the direct effect on germinal epithelium, and the hormonal deficit appeared to be a result of reduction in the number of Leydig cell [37]. These findings suggest that palmito may also adversely affect reproductive function. This deterioration increased with increasing of the dosage of extract. In conclusion, the results obtained from this pilot *in vivo* study have demonstrated that the ethanol extract of palmito has anti-androgenic properties, which affected spermatogenetic activity in all experimental groups. Further studies are warranted to determine and clarify the extract mechanisms involved.

Declare of interest statement

We declare that we have no conflict of interest.

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