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Original Research Paper

Effect of Alcoholic Extract of *Phoenix dactylifera* Spathe on Pituitary-Gonad Hormones in Adult Male Rat

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ABSTRACT

The chemical composition of *Phoenix dactylifera* spathe including proteins, fibres, moisture, reducing sugar, none-reducing sugar, three kinds of coumarin, organic compounds of camphor family, phytosterols, and 1,2-dimethoxyl 4 methyl benzene. These chemical compounds have biological effects mainly antimitotic, estrogenic, anticancer, antidiabetic, antiobesity and antimutation. In the present paper effect of extract of *Phoenix dactylifera* spathe on spermatogenesis, and concentrations of LH, FSH and testosterone and histological changes in testis of rat were studied. The concentration of testosterone showed a significant decrease to different dosage of extract 0.5, 0.1 (g/kg) in comparison to the control and sham groups. In addition, histological studies showed a significant effect caused by the extract such as decreased density of spermatozoa in seminiferous tubules and disorders in spermatogenesis. So body weight and testis showed significant decrease in experimental groups relative to control and sham groups. Serum levels of LH and FSH did not show a significant difference in experimental groups relative to control and sham groups.

INTRODUCTION

Date tree or *Phoenix dactylifera* is a bicuspid plant with flowers forming a large inflorescence called spadix. Spadixes are produced among top new leaves. There are a large number of flowers on numerous branches of inflorescence. Inflorescences aggregate in an oval-shape long woody structure called spathe. Spathes protect flowers against harsh environmental conditions. Spathes are never produced in bright colour but instead are hard and woody. As flowers mature and outgrow, spathe is torn apart and remains as a covering at the bottom of the inflorescence.

There are a number of different chemicals in the date spathe which include proteins, fibres, moisture, diminishing sugars, non-reducing sugars, three kinds of coumarins, organic compounds of camphor family, phytosterols, 1, 2-dimethoxyl 4 methyl benzene and inorganic materials (Mikki et al. 1989). Spathe coumarins have various properties such as antiaromatase, antiedema, estrogenic, anticancer, antiandrogenic and inhibitor of reductase activities (Shiuam et al. 2004). In addition, phytosterols can inhibit cytochrome p 450 scc and cause atrophy in somniferous tubules (Moghadasian et al. 1999). Furthermore, presence of furfural causes stimulation in mucus membrane of skin and respiratory tract and acts as a toxin for nervous system, liver, kidney and blood. Exposure of rats to furfural via mouth or under skin injection causes paralysis, epilepsy attacks, coma and changes in liver, kidneys, blood and bone marrow (Hathway et al. 1991). In humans, these compounds induce headache, throat etching, tearing and redness in eyes (Grant 1989). Finally, plant fibres inhibit angiotensin and beta receptors, and have antidiabetic antiobesity and antitumor properties and can prevent cancer, protect heart, diuretic, reduce cholesterol, anticonstipation and vasodilator as well (Malini & Vanithakumari 1991).

The aim of this research was to investigate the effect of *Phoenix dactylifera* spathe extract on spermatogenesis, and concentration of LH, FSH and testosterone, and changes in testis and body weight. In this way, the possible results could be used by endocrine and reproductive centres.

MATERIALS AND METHODS

The animals used in this study were 50 male Wister rats, each weighting 282±10g. These animals were supplied by animal house of Islamic Azad university of Kazeroun and their average age was about 2.5-3 months at time of the experiment. The rats were kept in polycarbonate cages at about 20±2°C and 12 hours light and 12 hours dark cycle. Their drinking water was from city water supply and they were fed dry concentrated rat food made by Fars animal food company. Cages were disinfected twice a week and cleaned once every three days. In order to adapt to new laboratory conditions, the experiments began one week after stabilization of animals in the laboratory.

Animals were divided into five groups of tens: A control group receiving normal food, a sham group receiving 1 mL distilled water for 14 days, and three experimental groups receiving 0.05, 0.1, and 0.2 g/kg extract of spathe respectively. The dosages of distilled water and extracts were injected intra peritoneally for 14 days. During this time, all groups were kept in the same environmental condition. After two weeks, animals of each group were weighted, and then were anaesthetized by ether, their chests were opened up, and blood samples were taken from their hearts. These samples were centrifuged for 15 minutes at 3000 RPM and the separated sera were frizzed and kept at -20°C to be tested later for different hormones. The concentrations of LH, FSH and testosterone were measured by routine laboratory tests, i.e., radio immunoassay (RIA). The results were evaluated by SPSS and Tukey test and significance level was set at $p \leq 0.05$. In addition, testis were removed, fixed, weighed and thin sections were prepared. After staining by haematoxylin-eosin method, testicular sections were studied by light microscope.

RESULTS

The mean body and testis weight, and the concentrations of LH, FSH and testosterone were analysed statistically by Tukey test. The results and statistical analysis were plated by Microsoft excel. The comparison of control and sham groups with experimental groups, which received different dosages of *Phoenix dactylifera* spathe extract, shows a significant fall in body weight (Table 1). In respect to control and sham groups there is a significant reduction in testis weight in groups receiving date spathe extract (Table 1). Different dosages of used alcohol extract in experimental groups show no significance effect on concentration of LH and FSH. There is a significant reduction in testosterone concentration of groups receiving 0.05 and 0.1 g/kg spathe extract of *Phoenix dactylifera* while the reduction caused by 0.2 g/kg extract is not significant. In addition, histological study of testis shows a considerable reduction in sperm cells in seminiferous tubules of experimental groups.

DISCUSSION

The effect of alcoholic extract of *Phoenix dactylifera* spathe on body weight is probably due to presence of compounds such as phytosterols, which increase hepatic lipases and lipoproteins as well as diminishing activities of 3-hydroxil, 3-methyl glutei coal reducates (HMG-coA) that cause a reduction in total cholesterol, LDL cholesterol and triglyceride levels in rats (Hendriks et al. 1999). It seems that fibres present in extract decrease food intake and increase energy consumption both by diminishing cholesterol level and by impairing lateral parts of hypothalamus as well as feeding centre at bed nucleus and medial ventral nucleus. The decline in testicular weight is probably due to the

presence of phytosterols and coumarin which have antiandrogenic effects and can decrease concentration of serum testosterone. Reduction of testosterone concentration causes a decrease in testis weight by limiting protein synthesis and elevation of T3 hormone and biolysis and proteolysis. Malini & Vanithakumari (1991) have also reported that phytosterols diminish testis weight in rats. It seems that spathe phytosterols lower testis weight, seminal vesicle and epididymis by reducing activity of 5-alpha-reductase (Wilt & McDonald 1991).

The results show that serum concentration of LH did not change significantly among various groups. Since testosterone concentration is decreased by spathe extract, an increase in LH concentration was expected due to a negative feedback mechanism, but there are a number of active compounds in date extract such as phytosterols which lower gonadotropins, including LH and exert their modulatory effects (Khan et al. 2004). In addition, the presence of β-cytosterol, which is a phytosterol, has no effect on pituitary responses and size of dimorphic sexual nucleus in castrated rats.

According to the results serum concentration of FSH did not change significantly among various groups. This means that feedback mechanisms are not initiated only by testicular steroids, but inhibin, activin and follistatin also play a role in FSH concentration by central effect on GnRH production, and lack of a significant change in FSH concentration may due to modulatory effects of these factors.

The study shows that alcohol extract of *Phoenix* spathe causes a significant decrease in testosterone concentration. Different studies indicate that spathe extract has inhibitory effect on 5-α-reductase activities due to the presences of phytosterols. This reduction causes a decline in concentration of dehydrotestosterone hormone (the active form of testosterone in tissues). In addition, phytosterols diminish tissues sensitivity to androgens and activities of androgens such as testosterone by inhibiting 5-α-reductase and aromatase enzymes (Khan et al. 2004). It has been shown that phytosterols and fibres reduce testosterone constituents by decreasing cholesterol level and thereby diminish testosterone concentration (Sugano et al. 1976). The presence of phytosterols in date extract cause a reduction in conversion of cholesterol to pregnenolone in mitochondria by reducing the activity of cytochrome p 450 scc (cholesterol desmolase enzyme) and reduction of pregnenolone decreases steroids synthesis including testosterone (Hathway et al. 1991).

The fibres present in extract also have β-blocker properties and inhibit β-adrenergic receptors. The study of Khan et al. (2004) shows that β-adrenergic receptors antagonists, one of which is plant fibre, lower production of testosterone in Leydig cells in rats by inhibiting cyclic AMP, but have no effect on enzymes involve in steroid synthesis. The presence of organic compounds of camphor family in date extract can inhibit activities cytochrome p 450 2B1 which is necessary for the function

Table 1: Body weight, testes weight changes and concentration of LH, FSH and testosterone in different groups on 14th day.

Groups	Body weight (g)	Testes weight (g)	Concentration of LH(IU/L)	Concentration of FSH(IU/L)	Concentration of testosterone (nmol/L)
Control	311±4.4	1.58±0.05	0.28±0.04	0.21±0.03	7.68±2.5
Sham	300±5.05	1.47±0.03	0.32±0.06	0.23±0.02	6.47±2.3
0.05(g/kg)	281.2±3.98*	1.44±0.04*	0.3±0.06	0.20±0.02	2 .13±0.3*
0.1(g/kg)	274.7±8.52*	1.36±0.06*	0.23±0.04	0.17±0.04	2.36±1.31*
0.2(g/kg)	274±8.86*	1.34±0.08*	0.31±0.05	0.30±0.03	3.88±1.3*

The average ± Standard Error of Mean (x ± SEM). The average amounts marked by * have a significant difference with the control group. The number of samples in each group is: n=10.

of some enzymes involved in androgen, including desmolase and 17-hydroxylases. Thus, reduction in the activity of this cytochrome causes a reduction in the activities of these enzymes and this leads to a decrease in testosterone synthesis.

According to the histological results, density of sperms in experimental groups receiving *Phoenix* extract showed a fall which may be due to presences of phytosterols in the extract (Khorasani & Cheung 2000). It seems that phytosterols diminish sperms via inhibition of acrozomic reaction induced by progesterone in sperms. Furthermore, in female rats, it has been shown that β-cytosterol, which is a phytosterol, increases the activity of glucose 6-phosphate dehydrogenase in uterus, which increases uterus weight and conforms high estrogenic effect of phytosterols. It is likely that phytosterols cause a decrease in sperm cells in male rats by their estrogenic effects (Malini & Vanithakumari 1993). In addition, the presence of coumarins in the date extract has estrogenic effects which cause interference in spermatogenesis and a decline in the density of sperms.

CONCLUSION

According to the results of the present study it can be concluded that *Phoenix dactylifera* spathe extract can reduce plasma concentration of testosterone, body and testis weight and sperm, and may possibly be used for treating sexual disorders in male persons.

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