

EFFECTS OF ALCOHOLIC EXTRACT OF PHYSALIS ALKEKENGII ON THE REPRODUCTIVE SYSTEM, SPERMATOGENESIS AND SEX HORMONES OF ADULT NMRI MICE

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Summary

There is a rapidly growing trend in the consumption of herbal remedies in the developing countries. Plant therapy can be useful for the control of fertility. *Physalis alkekengi* has a large history of herbal use because of pharmacological characteristics. Therefore, the objectives of this study were to determine the effects of alcoholic extract of *Physalis alkekengi* on testis structure, sperm characteristics and hormones levels in adult NMRI male mice. Healthy, adult male NMRI mice were divided randomly, into three groups of 10 mice each (control, experimental and recovery group). The effect of alcoholic extract (300 mg/kg/day intraperitoneally injection, for 30 days) of *P. alkekengi* on testis structure, sperm characteristics and levels of testosterone, FSH and LH was studied. We also fulfilled recovery observation for this study. This study indicated decrease in testes weights and created disorganized germinal epithelium, degenerated and necrotic cells in seminiferous tubules, exfoliated germ cells and presence of a large number of metaphasic cells in their germinal epithelium. The plant extract had a antispermatogenic action demonstrated by decrease in sperm count, motility and increase in sperm abnormalities. The hormonal profile was also influenced by the *Physalis alkekengi* extract. The testosterone level was significantly decreased. There was an increase in the blood level of LH in experimental group. However, this increase was not significant. Results indicated normal level of FSH in three groups. We concluded that alcoholic extract of *P. alkekengi* has temporary antispermatogenic properties in adult male mice caused by chemical compounds in *P. alkekengi* and it may be useful to regulate spermatogenesis and male fertility. Key words: Testis; spermatogenesis; antispermatogenic; NMRI male mice; *Physalis alkekengi*.

Introduction

The use of herbal medicine has become increasingly popular worldwide. The population of developing countries is increasing at alarming rate leading to poverty. Therefore finding safe effective contraceptive materials can be useful for this aim. Fertility control through natural products is being given great attention by WHO these days [1]. The plant therapy's literature is related to that of humanity, because in most cultures man has always been depended on the curative values of medicinal herbs to cure some illnesses. In some cultures namely in Persia and India as well as in Europe and North America, the plant therapy is more and more appreciated especially for its holistic approaches [2]. A large number of medical plants are known to have antifertility activities [3]. *Physalis alkekengi*, belongs to the family Solanaceae. It is distributed in Asia (Iran, India, Japan and China) and Europe (Spain, Italy and Turkey) has a large history of herbal use, and an interesting chemistry but it is seldom used in modern practices [4]. Chemical studies have demonstrated the presence of physalin, citric acid and vit C as the major components of *P. alkekengi* extract. Physalin is the most chemical compound with various pharmacological characteristics including, anti bacterial, anti leishmanial and anti tumor [5-7]. The whole plant is anti phlogistic, anti pyretic, anti tussive and expectorant [8-10]. It is used in treatment of urinary and skin diseases [11]. Its extract has been used for treatment of wide range of diseases, including kidney and bladder stone, febrile diseases, inflammation, general edema, arthritis and rheumatism [10,11]. The anti-fertility properties of *P. alkekengi* have been vastly described in the Persian traditional medicine. It is claimed that it exhibits contraceptive and abortive effects [9,12,13]. The results from recent studies have shown that administration of *P. alkekengi* has anti implantation activity and reduces the number of neonates that is consistent with its use in folk medicine as an anti conceptional agent [14]. In this regard, no research have been done its effect on male reproductive system, the present work is an attempt to find if it could be used as male fertility regulating agents.

Materials and Methods

Physalis alkekengi was collected from Guilan province, and then was identified by a botanist. Its leaves and fruits were dried under shade and powdered. The extract was prepared by the maceration method (80% ethanol in 300 gr/lit for 48 hours), filtered with filter paper. After filtration ethanol was removed by rotary evaporator. The extract was dissolved in normal saline and administered intraperitoneally into mice.

Healthy, adult male NMRI mice were housed in plastic cages, at ambient room temperature, with a controlled light and dark period of 12 hours. The mice were fed with a standard laboratory food and water provided ad libitum. They were weighted before and after of study.

Male mice were divided, randomly, into three groups of 10 mice each (control, experimental and recovery group), with equal average weight, age and their fertility were proven. Experimental and recovery group received 300 mg/kg/day alcoholic extract of *P. alkekengi* for 30 days. This dose was selected base on paper of Montaserti and his colleagues [14], and control group received normal saline for the period of 30 days.

At the end of treatment period, the mice (control and experimental) were killed by cervical dislocation. The reproductive organs were removed, testes were weighted and the weights were expressed in terms of 100g of body weight. For sperm characteristics the epididymis was exposed by scrotal incision, and sperms were expressed out by cutting the distal end of the caudal epididymidal tubule. Sperms with epididymal fluid was diluted with physiological saline and sperm motility and morphology were studied. For sperm count, spermatozoa were counted as per the method of Zaneveld and Polakoski [15]. Sperm suspension was placed on both sides of Neubauer's hemocytometer and allowed to settle in a humid chamber (wet) for 1 hour. The number of sperms in the appropriate squares of the hemocytometer was counted under the microscope at 100x magnification. Testes were fixed in formalin 10% and after tissue processing were stained with H&E (Hematoxylin & Eosine) for histological studies under light microscope.

After cervical dislocation, blood collected in dry tubes. The blood samples were centrifuged and the serum was immediately stored in the freezer (-20°C) for the measurement of testosterone, follicle stimulating hormone (FSH), and leutinizing hormone (LH). The hormones were measured by means of a radioimmunoassay coat-A-count kit (Diagnostic products corporation, Los Angeles, Calif) using a Packard Cobra gamma-counter.

After completion of the treatment, 10 animals were kept for recovery observation for a period of 60 days. Their sperm characteristics and testosterone level were examined.

Mean and standard error of mean [SEM] were calculated and the significance of difference was analyzed by applying student's 't' test. Level of significance difference was $P < 0.05$.

Results

The results recorded in table 1, show that there is no significant reduction in body weight between three groups, but there is a significant reduction in testes weight of experimental group compared with control and recovery groups. Also results showed that there is no significant difference in body and testicular weight between control and recovery groups. Results about sperm characteristics showed, treatment of mice with *P. alkekengi* reduced sperm count and motility, and increased sperm abnormalities in experimental group in compare with control and recovery groups. Statistical analysis showed that reduction in sperm count and motility in experimental group is significant in compare with control and recovery groups, but increase in sperm abnormalities in experimental group is not significant in compare with control and recovery groups. Also there is no significant difference in sperm characteristics between control and recovery groups.

Measurement of hormones exhibited a significant decrease in the testosterone level of experimental group in compare with control and recovery groups. There was an increase in the blood level of LH in experimental group. However, This increase was not significant. Results indicated normal level of FSH in three groups (Table2).

Table1: The effects of *Physalis alkekengi* alcoholic extract on body weight, testicular weight and sperm characteristic of NMRI mice.

Parameters	Control	Experimental	Recovery
Initial body weight	36.63 ± 0.17	36.43 ± 0.14	36.48 ± 0.15
Final body weight	36.68 ± 0.18	36.22 ± 0.14	36.59 ± 0.14
Testicular weight(mg/100g)	326.98 ± 0.49	302.31 ± 1.59*	326.46 ± 0.63
Sperm count 10 ⁶ /ML	16.43 ± 0.27	10.27 ± 0.41*	16.2 ± 0.25
Sperm motility (%)	71.66 ± 0.66	63.97 ± 3.27*	72.12 ± 0.60
Sperm abnormalities (%)	31.82 ± 0.93	34.53 ± 0.97	31.98 ± 0.93

Values are mean ± SEM (n=10); *P < 0.05 (significantly different) vs. control.

Table 2: Effect of 300 mg/kg/day alcoholic extract of *P. alkekengi* (for 30 days) on testosterone level in NMRI mice

Parameters	Control	Control	Recovery
Testosterone level (ng/ml)	0.944 ± .0064	0.834 ± .0034*	.9426 ± .0062
FSH (MIU/M)	0.189 ± .0073	0.188 ± .0046	0.185 ± .0072
LH (MIU/M)	0.442 ± .021	0.450 ± .124	0.443 ± .014

Values are mean ± SEM (n=10); *P < 0.05 (significantly different) vs. control.

Data from histological studies showed normal seminiferous tubules in control group (Figure1). In animals that received 300mg/kg/day alcoholic extract of *P. alkekengi* for 30 days, there were seen disorganized germinal epithelium in the most of seminiferous tubules. Degenerated and necrotic cells were observed in some seminiferous tubules (Figure 2). Some seminiferous tubules in treated animals had exfoliated germ cells (Figure 3). There was also large number of metaphasic cells in germinal epithelium (Figure 4).

Discussion

Interestingly, although some study demonstrated the effects of *Physalis alkekengi* on female in rats and mice, no attempt appears to have been made so far to determine the effects of this plant on fertility of male mice and rats. This study demonstrated the antispermatogenic properties of *P. alkekengi* alcoholic extract in adult male mice. *P. alkekengi* has a large history of herbal use. The anti fertility of *p. alkekengi* have been described in the Persian traditional medicine, having contraceptive and abortive properties [9,12,13]. Recent researches have shown anti implantation activity and reduction the number of neonates of *P. alkekengi* [14]. Vessal et al. (1991) showed that intraperitoneal injection of aqueous extract of *P. alkekengi* to the female rats had no effect on body weight, uterus weight and plasma total creatine kinase activity. However the level of plasma progesterone was diminished by 44%. They demonstrated that, uterine creatine kinase BB-isozyme (an estrogen-induced protein) showed a time-dependent inhibition of activity from 55%-82% [13]. We designed this animal model to investigate the efficacy of *P. alkekengi* alcoholic extract on testis structure, sperm characteristics and testosterone level.

Figure 1: Normal seminiferous tubules of control group (H&E, 100x).

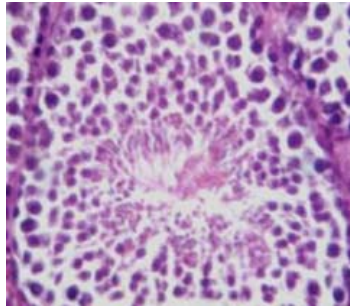


Figure 2: Cross-section of seminiferous tubule of treated group, in which degenerative changes and disorganization of germinal epithelium were observed (arrows, H&E, 400x).

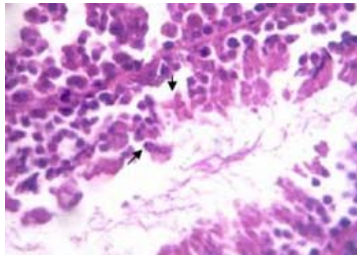


Figure 3: Cross section of a seminiferous tubule of treated group, in which necrotic cells (➔) and exfoliation (⌈⌋) were observed (H&E, 400x).

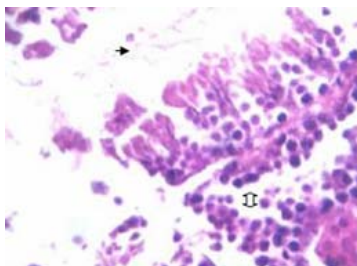
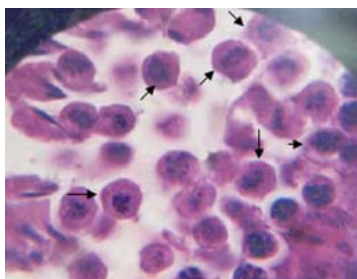


Figure 4: Seminiferous tubule of treated group, having large number of metaphasic cells.



Results indicated that extract of *P. alkekengi* caused reduction in testes weights, sperm count and motility, increase in sperm abnormalities and disorganized in testes structures. Testicular size and weight is the best primary assessment of spermatogenesis. Reduction in testicular weight, which is known to be mostly related to the number of spermatozoa present in the tissue and tissue condition. The number of sperm is decreased and we saw some histological change in testis structure that can reduce the testicular weight. Data from sperm count and motility showed a significant decrease ($P < 0.05$) in treated group compared with the control group. This could be due to the influence of the extract on the cell cycle or cell division. Also it is possible that, these changes might be due to an alteration in the microenvironment in the cauda epididymis. The extract can make a toxic microenvironment with its chemical compounds, thus it influences sperm count and motility. A large number of metaphasic cells were observed in the germinal epithelium of treated group that might be caused by cell cycle blockage. Pervious studies showed that physalin is the most chemical compound in *P. alkekengi* that exhibit cytotoxicity against tumor cells [8]. It is probable that reduction in testes weight, sperm count and motility and increase in sperm abnormalities performed by chemical compounds in *P. alkekengi* specially physalin. Also there was found an alteration in the spermatogenesis process, such as disorganized germ epithelium, degenerated and necrotic cells and exfoliated germ cells. These alterations might be caused by cytotoxicity of *P. alkekengi* alcoholic extract. These results were also reported with *Achillea millefolium*, gossypol and *Trypterygium wilfordii* [16-18]. In the present study hormonal measurement showed a significant reduction in testosterone level. Testosterone is synthesized from cholesterol in the leydig cells. The secretion of testosterone is under the control of LH, and the mechanism by which LH stimulates the leydig cells involves increased formation of cyclic AMP via the serpentine LH receptore [19]. Testosterone exerts an inhibitory feedback effect on pituitary LH secretion [19]. It appears that reduction in testosterone level cause a reduction in inhibitory action of testosterone on LH secretion, thus the secretion of LH increase. The level of FSH was normal in three groups. Along with testosterone, FSH is responsible for the maintenance of gametogenesis. FSH acts on the sertoli cells to facilitate the spermatogenesis [19]. Given the results, it seems that alcoholic extract of *Physalis alkekengi* by creation of cytotoxicity in testes of NMRI mice causes these changes in testes structure, spermatogenesis and hormonal levels. Study of recovery group proves that the effect of *Physalis alkekengi* is temporary. It appears that these effects caused by substances present in *P. alkekengi* extract, which leads to its antispermatogenic effect. Given the results, we concluded that alcoholic extract of *P. alkekengi* has antispermatogenic properties in adult NMRI

male mice caused by chemical compounds in *P. alkekengi*. Therefore, it may be useful to regulate spermatogenesis and male fertility.

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