

Anti-fertility effects of physalis alkekengi alcoholic extract in female rat

Azadeh Montaserti, M.Sc., Maryam Pourheydar, M.Sc., Mozafar Khazaei, Ph.D., Rostam Ghorbani, Ph.D.

Reproduction Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

Received: 19 June 2006; accepted: 22 September 2006

Abstract

Background: Physalis alkekengi (P. alkekengi) has been used as an abortive plant in Iranian traditional medicine for many years.

Objective: To investigate the effects of P. alkekengi on the fertility rate in female rats.

Material and Methods: In this experimental study, 40 female albino rats were divided randomly into two groups; group 1/for investigating the implantation sites and group 2/ for investigating the number and weight of neonates. In both groups, treated animals received plant extract at dose of 150 mg/kg on days 1-5 of pregnancy. In group 1, treated animals were euthanized at 7th days of pregnancy and number of implantation sites were counted. In group 2, treated animals maintained till delivery time and after delivery, the number and weight of neonates were investigated.

Results: Data showed that administration of P. alkekengi extract on days 1-5 of pregnancy significantly decreased the number of implantation sites, number and weight of neonates.

Conclusion: These results suggest that the extract produced anti-fertility effect probably by inhibiting implantation.

Key words: Physalis Alkekengi, Rat, Implantation, Anti-fertility

Introduction

Physalis alkekengi (P. alkekengi or ground cherry) is an indigenous herb in Iran and many other regions in the world (1). In Iranian herbal medicine the plant extract has been used for treatment of wide range of diseases including difficult urination, kidney and bladder stone, febrile diseases, inflammation, constipation, general edema, arthritis and rheumatism (1,2).

Chemical studies have demonstrated the presence of physalin, citric acid and Vit C as the major compounds of the extract of P. alkekengi (3-6).

In folk medicine it is also claimed that P. alkekengi exhibits contraceptive and abortive effects (1,4,5). However there is no documented study to clarify the effects of this plant on the fertility rate. Fertility control is an issue of global and national public health concern. Current methods of contraception result in an unacceptable rate of unwanted pregnancies.

Regarding to the importance of fertility control and side effects of the existing contraceptive methods, the usage of biologically active botanical substances or fertility-regulating agents of plant origin which are ecofriendly in approach and interfere with the natural patterns of reproduction becomes necessary (8, 9).

Previous studies demonstrated that P. alkekengi can affect some reproductive factors such as estrus cycle. It was shown that intraperitoneally injections of an aqueous extract of P. alkekengi to adult normal cycling female rats produced 100% diestrus and diminished uterine glucose 6-P dehydrogenase activity (an estrogen-induced protein) by 52% (10). This resulted in the diminution of the pituitary lysyl-aminopeptidase (Lys-AP) activity. They report that P. alkekengi aqueous extract is an estrogen antagonist and can inhibit the Lys-AP enzymes (estrogen-induced proteins) (11).

Many plant extracts have been reported to affect fertility in rodents. Gebri *et al* (2005) reported that methanolic extract of Rumex steudelii decreased the number of implantation sites significantly. They also showed that the extract of this plant did not affect the

Correspondence Author:

Azadeh Montaserti, Reproduction Research Center, Kermanshah University of Medical Science, Kermanshah, Iran.

E-mail: Montaserti_azi@yahoo.com

serum estrogen-progesterone ratio (12). Nivsarkar *et al* (2005) showed that *Hibiscus rosa-sinensis* flowers has antifertility and abortifacient activity. They also showed that this extract exhibit antiestrogenic activity, as judged by increase in uterine weight (13). Kulkarni *et al* (2005) reported that the alcoholic extract of lemon seeds exerted reversible anti-fertility effect in female mice by virtue of its anti-zygotic action (14).

There is no documented evidence referring to the anti-fertility effects of *P. alkekengi* extract. Thus the present study was an attempt to investigate the effects of *P. alkekengi* alcoholic extract on the implantation sites, number and weight of neonates in female rat.

Materials and methods

Plant material

P. alkekengi (ground cherry) was obtained from the local market. The plant was authenticated by a botanist.

The extract was prepared according to WHO protocol CG-04 for the preparation of an alcoholic extract (15). Briefly 100 gr of fruit plant were shed-dried, powdered and added to 1000 ml of 70% ethanol (v/v) and were left to macerate at room temperature for 20 hours. The basin was slowly rotated during this time. After filtration, ethanol was evaporated at low pressure at 30 centigrade degree. The extract was dissolved in normal saline and was immediately administered intraperitoneally (i.p) to rats expressed as mg of extract per Kg body weight.

Acute toxic dose

The intraperitoneally acute toxicity (LD50) of the extract was evaluated in Swiss albino mice as described by Miller and Tainted (16). Briefly, the method involved the administration of 5 different doses of the extract to 5 groups of mice (5 mice/group). After 1 week there were no deaths in animals that received the plant extract at dose of 1, 10, 100, 500 and 1000 mg/kg.

We tested the effect of 3 different doses of plant extract on fertility rate. The 50 and 100 mg/kg/day showed no significant change in fertility rate, therefore the dose was changed to 150 mg/kg/day.

Animals

In total 40 female albino rats [Razi Institute of Iran] (160-180 gr) were housed with adult male

albino rats in the ratio of 3:1 in each cage, under controlled environmental conditions with 12/12 hr light-dark cycle, animals were fed with pelleted standard rat feed and water provided ad libitum.

Fertility test

Prior to the mating, the females isolated for one month to rule out pre-existing pregnancy. As described above, female and male rats were housed in the ratio of 3:1 per cage and in the next morning the vaginal smears were checked to observe the presence of sperm which indicated positive mating (8). This day was considered day one (D1) of pregnancy. Then the positive vaginal smear rats separated and classified as 2 groups: The animals in each group (n=20) subdivided as experimental and control subgroups. Experimental group (n=10) received *P. alkekengi* alcoholic extract at dose of 150 mg/kg/day (50 mg/0.3 ml of distilled water) intraperitoneally on days 1-5 of pregnancy and control group (n=10) received distilled water respectively.

1. The effect of *P. alkekengi* extract on implantation sites: Animals were maintained on normal diet and at 7th day of pregnancy, they were euthanized. The numbers of implantation sites in each uterine horn were studied under stereomicroscope (Leica zoom 2000) in both subgroups.

2. The effect of *P. alkekengi* extract on the number and weight of neonates: These animals were maintained on normal diet till delivery time, after delivery the number and weight of neonates were counted and compared in both subgroups.

Statistical analysis

The number of implantation sites and neonates were analyzed by Mann-Whitney test and the weight of neonates analyzed by independent-T test. Differences with $p < 0.05$ between experimental groups at each point were considered statistically significant.

Results

Number of neonates

The average number of the neonates in experimental rats was 3.3 while this was 7.6 in control group ($p < 0.05$) (Figure 1).

Weight of neonates

The average of neonates weight was 3.1 gr in experimental and 5.51 gr in the control group ($p < 0.05$) (Figure 2).

Number of implantation sites

The average number of the implantation sites in experimental rats was 2.5 but in the control group was 7.3 ($p < 0.05$) (Figure 3).

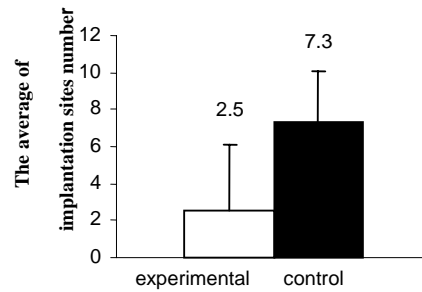


Figure 3. Comparison between the number of implantation sites in experimental and control groups

		volume	Average of the number of implantation sites	Average of the number of neonates	Average of the weight of neonates
Group 1 (Control of implantation sites)	experimental	N=10	2.5	-	-
	control	N=10	7.3	-	-
Group 2 (Control of the number and weight of neonates)	experimental	N=10	-	3.3	3.1
	control	N=10	-	7.6	5.51

Table 1: result of administration of *p. alkekengi* extract in 2 groups.

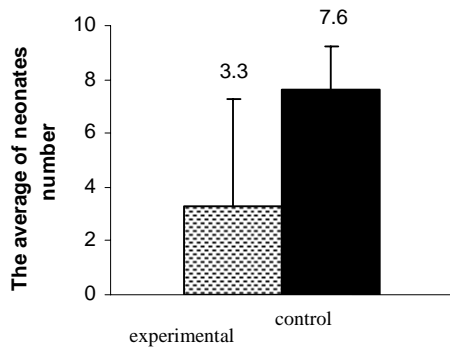


Figure 1. Comparison between the number of neonates in experimental and control groups

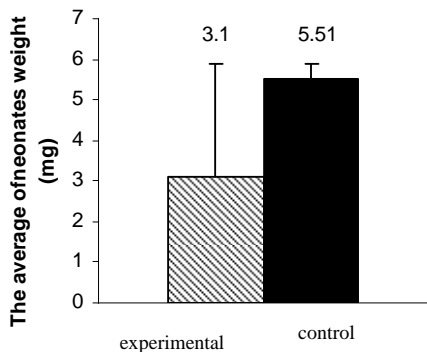


Figure 2. Comparison between the weight of neonates in experimental and control groups

Discussion

The present study demonstrated that administration of *P. alkekengi* alcoholic extract can produce significant decrease in implantation sites, weight and number of neonates. Plant products as contraceptive will be more acceptable for economic reasons and less side effects than chemical agents. As described in previous studies, aqueous extract of *P. alkekengi* can affect some reproduction factors in female rats (10, 11).

Vessal *et al* (1991) showed that intraperitoneal injections 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% to 82% (17). Similarly many plant extracts have been reported to affect fertility in rodents (12-14).

According to the importance of progesterone and estrogen hormones in the maintenance of implanted embryo, the anti-fertility activity of this plant seems to be due to this fact that *P. alkekengi* is an antagonist for this hormones and can interfere with fertility. But

the exact mechanism(s) of this observed anti-fertility activity of the plant extract remains unclear. The present study elucidates the fact that the *P. alkekengi* extract has no toxic effect on vital organs such as liver, spleen and kidney by parallel manifestation of toxicity test.

Conclusion

The results of the present study showed that administration of *P. alkekengi* demonstrates anti-implantation activity and reduces the number of neonates that is consistent with its use in folk medicine as an anti-conceptual agent. Further studies are recommended for understanding the exact mechanism(s) of this plant and the probable changes in hormonal levels and anti-fertility effect in male rats due to its administration.

References

1. Amini A. Dictionary of therapeutic plants. Tehran University, Tehran, 2004; 157-159.
2. Zargari A. Medical plants. Tehran University, Tehran, 2003; 3595-3596.
3. Heber D. Herbal medicine. 2005; 3: 885.
4. Duke JA. Handbook of Medicinal herbs, 2002; 2: 787-788.
5. Mirheydar H. Herbal information: usage of plants in prevention and treatment of diseases. 2003; 5: 39-42.
6. Bown D. Encyclopedia of herbs and their uses. 2002: 313-314.
7. Henshaw SK. Unintended pregnancies in United States. *Fam plan prospect* 1998; 30: 24-29.
8. Dehghan M, Martin T, Dehghanan R. Anti-fertility effect of Iranian neem seed alcoholic extract on epididymal sperm of mice. *IJRM* 2005; 3: 83-89.
9. Dixit VP. Plant products/non-steroidal compounds affecting fertility in the Indian desert gerbil, *Meriones hurrianae* jerdon. In: Parkash I & Ghosh PK (editor), Rodents in Indian Agriculture. Scientific publishers. Jodhpur, India 1992; 1: 595-604.
10. Vessal M, Yazdani M. Comparison of the effects of an aqueous extract of *Physalis alkekengi* fruits and/or various doses of 17-beta-estradiol on rat estrous cycle and uterine glucose 6-phosphate dehydrogenase activity. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 1995; 112: 229-236.
11. Vessal M, Rasti M, Kooshesh F. Modulation of the pituitary and basomedial hypothalamic lysyl-aminopeptidase activities by beta-estradiol and/or an aqueous extract of *Physalis alkekengi* fruits. *Comp Biochem Physiol B Biochem Mol Biol* 1996; 115: 267-271.
12. Gebri E, Makonnen E, Zerihun L, Debella A. The possible mechanisms for the antifertility action of methanolic root extract of *Rumex studelii*. *Afr Health Sci* 2005; 5: 119-125.
13. Nivsarkar M, Patel M, Padh H, Bapu C, Shrivastava N. Blastocyst implantation failure in mice due to "nonreceptive endometrium": endometrial alterations by *Hibiscus rosasinesis* leaf extract. *Contraception* 2005; 71: 227-230.
14. Kulkarni TR, kothekar MA, Matheenuddin M. Study of anti-fertility effect of lemon seedas (*Citrus limonum*) in female albino mice. *Indian J Physiol Pharmacol* 2005; 49: 305-312.
15. WHO: protocol CG-04. Preparation of alcoholic extract for bioassay and phytochemical studies (APJF/IP, 1001A). Geneva, *World Health Organization* 1983a.
16. Miller LC, Tainter ML. Estimation of ED₅₀ and its error by means of log probit graph. *Proceedings of the Society of Experimental Biology and Medicine* 1944; 57: 261-264.
17. Vessal M, Mehrani HA, Omrani GH. Effects of an aqueous extract of *Physalis alkekengi* fruit on estrus cycle, reproduction and uterine creatine kinase BB-isozyme in rats. *J Ethnopharmacol* 1991; 34: 69-78.