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Effects of alcoholic extract of *Achilea mellefolium* flowers on fertility parameters of male rats

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Abstract: Bachground and Objective: Fertility regulation with plant preparations has been reported in ancient literature of indigenous systems of medicine. In this research the effects of administration of alcoholic extract of *Achilea mellefolium* flowers on fertility indices, body weight and reproductive organs weight was evaluated in male rats. **Materials and Methods:** 18 rats were randomly divided into 3 groups, control, group A and group B. Each group comprises of six rats. Animals in control group received 1 ml of distilled water (vehicle) and test groups (A and B) received graded doses of 200 and 400 mg/kg body weight of alcoholic extract of *Achilea mellefolium* flowers respectively on daily basis for 50 days. At the end of 50 days of treatment period, fertility indices such as body and reproductive organs weight, sperm motility and viability, epididymal sperm reserve (ESR), daily sperm production (DSP), blood testosterone concentration and fertility percentage were measured. **Results:** There was a significant decrease in the reproductive organs and body weight, sperm motility and viability, ESR, DSP, blood testosterone concentration and fertility percentage especially in the group which received the higher dose of alcoholic extract of *Achilea mellefolium* flowers.
Conclusion: The results of this study showed that alcoholic extract of *Achilea mellefolium* flowers in higher doses could decrease fertility in male rats.
Key words: *Achilea mellefolium*, Fertility, Testosterone, male Rat

Introduction

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (1,2).Nearly 80% of the world populations rely on traditional medicines for primary health care, most of which involve the use of plant extracts (3). A variety of plants have been used for the treatment of ulcer, hypertension, diabetes and male reproductive function(4).

Previous studies have shown the anti-fertility effects, fertility-enhancing properties and spermatogenic effects of some plant extracts. *Melia Azadrach*, *Sarcostemma acidum*, *Malvaviscuss conzattii*, *piper linn*, *Boswellia thurifera*, *Lepidium meyenii*, *Colebrokia oppostifolia* and *Morinda lucida* have mentioned effects (5-14).

Achillea millefolium L. (Asteraceae) is a plant known as Yarrow, it is belonging to the steraceae family (formerly Compositae), is native to Europe, North America, Southern Australia and Asia (15, 16). *A. millefolium* L, a dark green perennial with tough

stems, 30 – 90 cm high with abundant, long, pinnate leaves and pink or white flowers, has been used, mainly in Herbal and Homeopathic medicine, for a wide variety of purposes: diaphoretic, anti-swelling, antitumoral, antibacterial, antihypertensive (17, 18), respiratory infections, fever, rheumatic pains (15) contraceptive and abortifacient(19, 20).

The previous studies showed that *Achillea millefolium* and different variety of *Achillea santolina* have an antispermatogenic and degenerative changes on mice testes (21, 22).

The objective of this investigation was to determine the Effects of alcoholic extract of *Achilea mellefolium* flowers on fertility parameters of male rats.

Materials and methods

Plant material: Flowers of *Achillea millefolium* were collected freshly from Golestan province in north of Iran. The plant was identified and authenticated by the Department of biology, Payame Noor University. The flowers were homogenized by a blender and dried for 48h at 40°C. Air dried powder (100 gram) was extracted by percolation at room temperature with70% hydroalcoholic solution. The extract was concentrated in vacuum desiccators and the residue was dissolved in 45% hydroalcoholic solution.

Animals

18 Adult male Wistar rats (220-250 g body weight and 3- 4 months age) were provided by the animal house of Science Faculty of Razi University. Animals were maintained in plastic cages, under controlled temperature (24 ± 2C) and light (12L, 12D).

Treatments

Eighteen Male rats of proven fertility were divided randomly into 3 groups of 6 animals each. Group A: treated 200 mg/kg extract alcoholic for 50 days. Group B: treated 400 mg/kg extract for 50 days. Control group: received 1ml distill water for the same duration. All animals received alcoholic extract and distill water through oral administration (gavage).

Evaluation of parameters: After the last administration, each male rat was caged separately with 2 coeval females of proven fertility in the evening for 6 days(mating test). After the evaluation of fertility by mating test, the animal were sacrificed by decapitation and testes and epididymis removed and weighted.

Body weight: Body weights of animals were recorded every week during treatment and before the experiments.

GSI (Gonadosomatic index): This index indicates the testes weight/body weight ratio.

Sperm motility and sperm viability: To determine these parameters, 100 mg of cauda epididymides was minced into 5 ml of 0.9% NaCl. One drop of evenly mixed sample was applied to a Neubauer's counting chamber under coverslip. Quantitative motility expressed as percentage was determined by counting motile and immotile spermatozoa per unit area and quantitative viability expressed as percentage was determined by counting viable and imviable spermatozoa per unit area. Viable spermatozoa can't absorb the Negrosin stain but imviable spermatozoa can absorb the Negrosin stain. Cauda epididymal sperm counts were performed by routine procedure and expressed as percentage(6 and 23-24).

ESR and DSP Epididymal sperm reserve (ESR) and daily sperm production (DSP) were assessed by method of Ribb *et al.* Each epididymis was divided into the caput, corpus, and cauda, and each part was processed separately. After mincing the tissue with a pair of scissors, it was transferred quantitatively to a Waring Blendor using 20 ml of homogenizing solution. This solution consisted of physiological saline with 0.05% Triton X-100 added. The tissue was homogenized for 1 min, and the homogenates were transferred quantitatively to glass jars. Additional homogenizing solution was added to dilute sperm concentrations to convenient levels for accurate hemacytometer counting (400-600 sperm per chamber). Sperm concentrations were determined by counting sperm present in 10 large squares of each of 8 hemacytometer chambers. Each chamber was filled with a different pipette, and the homogenates were mixed for 2 min with a magnetic stirrer just prior to filling pipettes. The DSP was determined from quantitative testicular histology. Testes after the weighting, transferred to 50 ml of solution consisted of physiological saline. The tissue was homogenized for 5 min, and the homogenates were transferred quantitatively to glass jars, then sperm concentrations were determined by counting of ESR method. Sperm counts were performed by routine procedure and expressed as million(6 and 25).

Fertility test(mating test): This index was determinated by Oberlander and *et al.* In each stages, each male rat was caged separately with 2 coeval females of proven fertility in the evening for 6 days. Presence of sperms in the vaginal smears examined on the next day morning indicated that the females had mated to the particular male and the day of mating was taken to be days 1 of pregnancy. Fertility test was considered positive when implantation sites were present(26).

Concentration of testosterone: After the evaluation of fertility by mating test, the animals were sacrificed by decapitation and blood was collected by cardiac

puncture and serum was separated. Concentration of testosterone was determined by Radio immunoassay(RIA)(27).

Statistical analysis: Data are expressed as mean ± ESM differences between control and test groups were analyzed using either Student's't' test and INSTANT software, $P<0.05$ was considered as significant difference.

Results

Body and reproductive organs weight: The results showed that administration of alcoholic extract of *Achilea mellefolium* flowers, at the doses of 200 and 400mg/kg/day, for 50days, caused no significant deference of body weight ,in addition, The results presented in table 1 shows that oral administration of alcoholic extract of *Achilea mellefolium* flowers at dose 200 mg/kg body weight(group A) for 50 days to male rats had significant decreased on Epedidymis weight ($P<0.05$). The results also presented significant decreased in GSI($P<0.05$) and Epedidymis weight ($P<0.01$), between group B(400 mg mg/kg body weight) in compared with control group (table 2).

Sperm motility and Sperm viability: The results showed that administration of alcoholic extract of *Achilea mellefolium* flowers, at the doses of 200 and 400mg/kg/day, for 50days, caused no significant deference of sperm motility and Sperm viability. (table 1).

ESR, DSP, Testosterone concentration and fertility: The results presented in table 2 shows that oral administration of alcoholic extract of *Achilea mellefolium* flowers at dose 200 mg/kg body weight(group A) for 50 days to male rats had significant decreased on DSP($P<0.05$) and Testosterone concentration($P<0.05$). The results also presented significant decreased in ESR($P<0.05$), DSP($P<0.001$), Testosterone concentration($P<0.001$) and fertility percentage($P<0.05$) between group B(400 mg mg/kg body weight) in compared with control group (table 2).

Discussion

Present investigation demonstrate that oral administration of alcoholic extract of *Achilea mellefolium* flowers in particular with high dose, cause a significant decrease in fertility parameters in male rats.

The animal model used in this work has been previously with minor changes used by several researchers to assess the effect of various extracts obtained from medical plants on reproductive functions in male(6, 14).

The previous studies showed that *Achillea millefolium* (200mg/kg/day ntraperitoneally for 20 days) and different variety of *Achillea santolina* (300mg/kg intrapertonealy for 20 days) have an antispermatogenic and degenerative changes on mice testes (21, 22).

Table 1: Effects of alcoholic extract on body and organs weight and motility and viability

Group	Body weight(g)	GSI(1000 × g)	Epedidymis weight (1000 × g)	Sperm motility(%)	Sperm viability (%)
Control	87/5±6/21	7/52 ±0/19	62/22 ±2/42	81/33±4/24	82/5 ±2/3
A	94/25±4/35	7/37 ±0/27	53/33 ±2/63*	77/67±3/6	74/17 ±1/64
B	82/17±7/96	6/57 ±0/3*	49/87 2/22**	74/83±3/48	77/83 ±3/11

*: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, compared with the control

Table 2: Effects of alcoholic extract on ESR, DSP, testosterone and fertility

Group	ESR(million)	DSP(million)	Testosterone concentration(ng/dl)	Fertility(%)
Control	242/17±10/29	25/15±1/16	569/67±18/23	77/5±3/64
A	234/17±11/11	20/4±1/06*	501/33±13/12*	74/83±3/05
B	197/67±8/11*	16/43±1/15***	445/5±18/43***	63/33±2/76*

*: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, compared with the control

The decrease in GSI (testes weight/body weight) and epididymis weight may be attributed to the increase level of damage on the testes tissue of experimental rates that showed in previous studies(21,22). In addition, in this study, oral administration of alcoholic extract of *Achillea millefolium* (200 and 400 mg/kg), for 55 days, reduced the hormone level of testosterone. The reduction in testosterone might be due to this extract, which altered androgen hormones synthesis of Leydig cells. Reduction in ESR and DSP parameters may be also due to altered androgenic synthesis and spermatogenesis.

Decrease in pregnancy in untreated females rats which were mated with treated males

may be due to failure of fertilization as indicated by reduction in ESR, DSP and testosterone concentration parameters in this study and antispermatogenic effects and testes degenerative changes in previous studies(21,22).

The exact mechanism of these effects is not clear and might be due to substances present in *Achillia miliflolim* flowers extract, which leads to its antifertility effects, but it may be due to presence of chemical composition of *Achillia miliflolim*

In its chemical composition, literature describes the presence of essential oil with terpens (cineol, borneol, pinens, camphor, azulen), terpenics and sesquiterpenics derivatives, tannins, coumarins, resins, saponins, steroids, fatty acid, alkaloids and principles of bitter taste (15,28,29). It is reported the presence of flavonoids, apigenin, luteolin and its glycosides, artemetin and rutin in the flowers and leaves (30,31).

Studies showed that apigenin(a flavonoid) was an effective inhibitor of aromatase (human estrogen synthetase) and 17b-hydroxysteroid dehydrogenase activities in human placental microsomes(32). In addition, apigenin *Induce Apoptosis* human promyelocytic leukemia(33). suggesting that chemical composition as flavonoids may be effective in androgenic synthesis.

In conclusion, alcoholic extract of *Achilea mellefolium* flowers has antifertility effect, but its mechanism is not clear.

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