EFFECT OF ACHILLEA SANTOLINA ON MICE SPERMATOGENESIS

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ABSTRACT

Achillea santolina, a common variety of *Achillea* in Golestan province of Iran has been used in traditional medicine for its anti-inflammatory properties.

The effect of hydroalcoholic extract (300 mg/kg/day Intraperitoneally, for 20 days) of *Achillea santolina* on the spermatogenesis of mice was studied by the evaluation of morphologic characteristics by light microscope. The alterations observed were disorganized germ epithelium, exfoliation of immature germ cells, germ cell necrosis and increased number of metaphasfis in germinal epithelium of seminiferous tubules. We concluded that hydroalcoholic extract of *Achillea santolina* 300mg/kg/day intraperitoneally for 20 days as a different variety of *Achillea* has antispermatogenic effect similar to *Achillea millefolium* on mice.

Key Words: *Achillea santolina,* Spermatogenesis, Mice

INTRODUCTION

Achillea is a medicinal herb that has been used in popular medicine for its antihemorrhagic, healing and analgesic properties (1,2). It is native to Europe, North America and Northern Asia (3), and in some parts of Iran. *Achillea santolina* as a variety of achillea has some traditional uses: anti-inflammatory, antidiuretic and antimicrobial effects (4,5, 6).

Achillea millefolium is a variety of *Achillea,* which was used by native people from Northern Europe and North America as a contraceptive, abortifacient, and emmenagogue (7,8).

The previous study showed that *Achillea millefolium* (200mg/kg/day intraperitoneally for 20 days) has an antispermatogenic and degenerative changes on mice testes (9).

In the present study hydroalcoholic extract of *Achillea santolina* (300mg/kg/day intraperitonelly for 20 days), as another variety of *Achillea* which was obtained in the province of Golestan in Iran (south – east of Caspian sea border) was tested on male mice to verify its effect on spermatogenesis.

MATERIALS AND METHODS

Vegetal material and plant extracts

Flowers of *Achillea santolina* were collected freshly from Golestan province. The plant was identified and authenticated by the faculty of pharmacy, Mazandran University of Medical Sciences (vouches specimen Number 721).

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

Adult male NMRI mice were obtained from the Pastur institute (Tehran, Iran) and kept in the animal house in Gorgan faculty of medicine, at 22-25°C, under a natural photo-period (with a 12 h dark and 12 h light cycle).

Treatment

The hydroalcoholic extract was administered at the dose of 300mg/kg/day, intraperitoneally, for 20 days in six treated animals. Control animals (n=5) received the same amounts of vehicle. The mice were weighed at the beginning and at the end of the experiments. The mice were killed by cervical dislocation 24h after the last dose.

The reproductive organs were dissected, the testes were weighed and the weights were expressed in terms of 100g of body weight.

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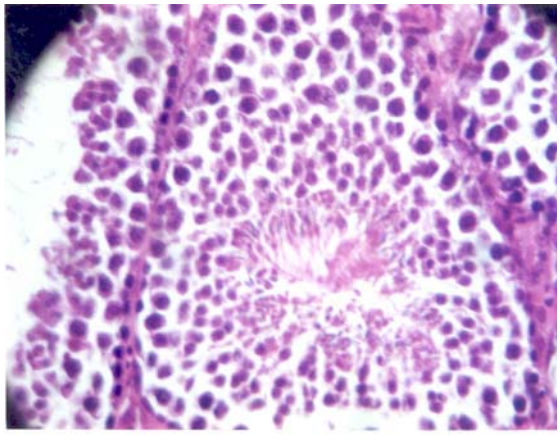

Fig 1: Cross – section of the testis of control animals showing a normal seminiferous tubule. (Hematoxylin and eosin × 100)

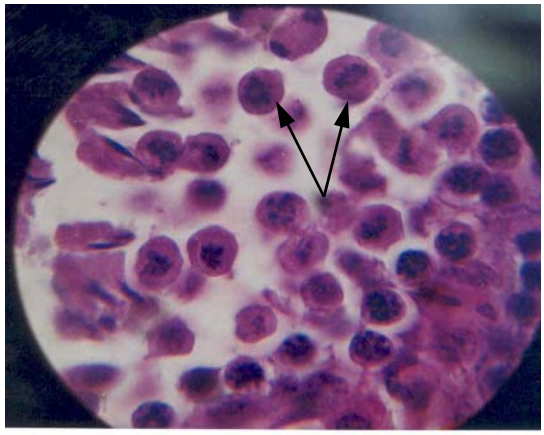

Fig 3. Seminiferous tubule of treated animals, with hydroalcoholic of *Achillea santolina* (300 mg/kg/day intrapertonally for 20 days) with an unusually high number of metaphases (arrow) (hematoxylin and eosin × 1000)

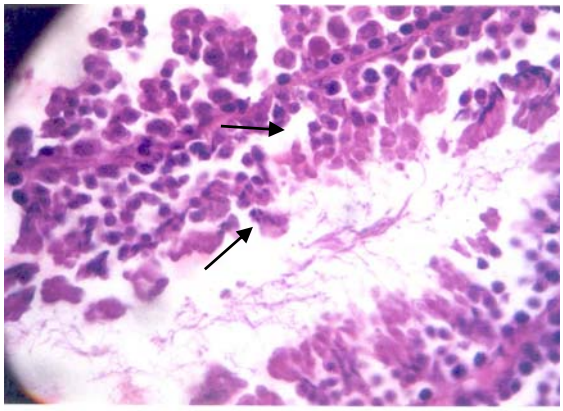

Fig 2 : Cross – section of seminiferous tubule of treated animals, with hydroalcoholic of *Achillea santolina* (300 mg/kg/day intrapertonally for 20days) in which architectural disturbance, degenerative changes (arrow) and dis-organization of germinal epithelium were visualized. (hematoxylin and eosin × 100)

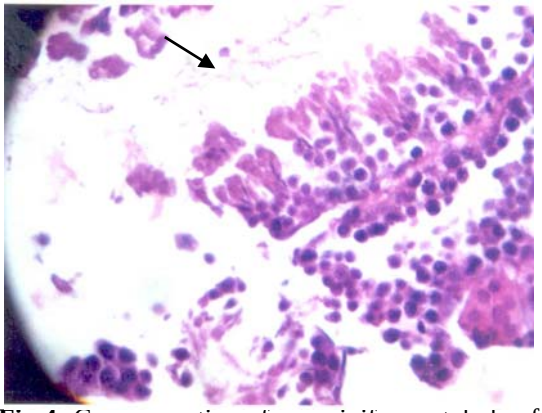

Fig 4: Cross – section of a seminiferous tubule of treated animals, with hydroalcoholic of *Achillea santolina* (300 mg/kg/day intrapertonally for 20days) in which necrotic exfoliated cells (arrow) are observed. (hematoxylin and eosin × 400)

Histological studies

The testes and epididimides were immediately fixed in formaldehyde solution (10%), and after tissue processing were embedded in paraffin wax and sectioned at 5 µm. They were stained with hematoxylin and eosin.

Morphological studies were evaluated by Olympus microscope at 40×, 100× , 400× and 1000× magnifications.

Statistical analysis

The data of the gaining body b and testes weight were analyzed by student t-test and all results were expressed as means±standard deviation. Difference between groups was considered to be significant at $P<0.05$.

RESULTS

The results showed that injection of *Achillea santolina*, at the dose of 300mg/kg/day, intraperitoneally, for 20days, caused no significant reduction of body weight compared with control group (table 1).

In addition, a significant decrease in testes weight were observed between two groups ($p<0.001$).

Histological findings

The seminiferous tubules of control animals appeared normal (Figure 1).

In treated animals (300mg/kg/day, intraperitoneally, for 20 days), there were seen disorganized germ epithelium in the most of seminiferous tubules. In addition, degenerated and

necrotic cells in some of seminiferus tubules were observed (figure 2).
Figure 3 is a section of seminiferous tubules showing the large number of metaphasic cells in germ epithelium.

Table 1: Effect of 300 mg/kg/day (20 days, intraperitonealy) hydroalcoholic extract of Achilliea santolina flower on mouse body and testes weight (mean ± SD)

Group	Initial body weight (g)	Final body weight (g)	Testis (mg/100g)
Treated	37.98 ±0.73	36.71 ±1.34	293.08 ±10.62
Control	38.16 ±1	38.22 ±0.76	329.6 ±6.58
	NS*	NS	P≤0.0001

* NS : No Significant

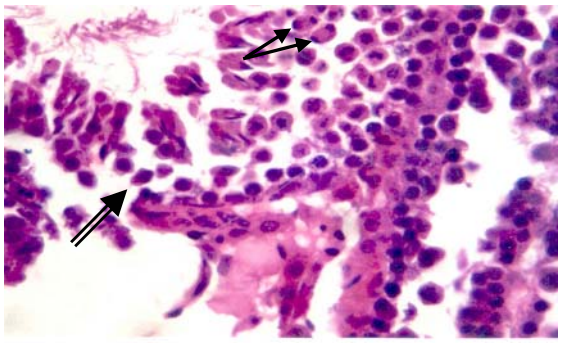

Fig 5 : Cross – section of a seminiferous tubule of treated animals, with hydroalcoholic of *Achillea santolina* (300 mg/kg/day intrapertonally for 20days) with an unusually high number of metaphases (arrow ⟶) , and exfoliation (arrow ⟹) are observed (hematoxylin and eosin × 400).

Also exfoliated germ cells were visualized in the lumina of few seminiferous tubules in treated animals (figure 4). In addition, many round cells, probably exfoliated germ cells together with spermatozoa were visualized in the lumen of few seminiferous tubules (figure 5).
No spermatozoa were observed in the lumina of many seminiferous tubules.
The thinning of epithelial layers (as a characteristic of atrophy in germ epithelium) also were observed in a few seminiferous tubules. The alterations observed in all treated animals, but the above changes were not uniform throughout the testis. There were variations between the seminiferous tubules of a single testis and between the testes of different mice.

DISCUSSION

The results of this investigation confirm that *Achillea santolina* (300mg/kg intrapertonealy for 20 days) has an inhibitory effect on mice spermatogenesis.
The result of this study are similar to those of Montanari et al (9).
It was found that weight of testes in treated animals significantly decreased and this effect was not seen in previous study (9). Also there was found an alteration in the spermatogenesis process, such as disorganized germ epithelium, degenerated and necrotic cells and reduction of germ epithelium. These alterations were also reported with *Achillea millefolium* (9), gossypol (10) and trypterygium wilfordii (11), which are considered to be antispermatogenic agents.
A large number of metaphasic cells were observed in the germ epithelium of treated animals. that might be caused by cell cycle blockage or by a cell proliferation stimulus. It has been reported that vinblastine sulfate causes similar alterations in rat testes (12).
In the present study, no changes of vacuolization in seminiferous tubules, which was previously reported (9) were not observed
The exact mechanism of these effects is not clear and might be due to substances present in *Achillea* extract, which leads to its antispermatogenic effect. This suggestion is confirmed by the results of the previous study (13) in which was shown that components of *Achillea millefolium* are active against mouse leukemia's cells in vivo. In addition, weak genotoixc activity of *Achillia miliflolim* in the somatic cells of drosophila had been reported (14).
Some differences in results of this study and previous one (9) might be either for the use of different variety of *Achillea* or due to higher concentration (300mg/kg/day intra pritonally) which was employed in present study.

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