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 metaphasfsis 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 antispermaticgenic and degenerative changes on mice testes (9). In the present study hydroalcoholic extract of Achillea santolina (300mg/kg/day intraperitoneally 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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Effect of Achillea santolina on mice spermatogenesis

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

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

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

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

**RESULTS**

The results showed that injection of Achillea santolina, at the dose of 300mg/kg/day, intraperitoneally, for 20 days, 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 Achillea santolina flower on mouse body and testes weight (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Testis (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>37.98 ±0.73</td>
<td>36.71 ±1.34</td>
<td>293.08 ±10.62</td>
</tr>
<tr>
<td>Control</td>
<td>38.16 ±1</td>
<td>38.22 ±0.76</td>
<td>329.6 ±6.58</td>
</tr>
</tbody>
</table>

* NS : No Significant

DISCUSSION
The results of this investigation confirm that Achillea santolina (300mg/kg intraperitonealy 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 genotoxic activity of Achillia milifolium 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 intraportionally) which was employed in present study.

ACKNOWLEDGEMENT
This research was supported by a grant from the research council of the Golestan University of Medical Sciences. We thank, Department of Anatomy and Pathology and Dr Solimany for their co-operation and Mr. Krimabady for photography.

REFERENCES


