Effects of Artemisia lanata Extract on Reproductive Parameters of Female Rats

Nava Ainehchi¹*, Afshin Zahedi²

Abstract
Objective: Until date, there is no report on safety of Artemisia lanata. This study aimed to determine the possible undesirable effects of A. lanata on reproduction of female rats.

Materials and Methods: The pregnant rats were treated (i.p.) with vehicle or 200 and 400 mg/kg of A. lanata hydroalcoholic extract from the 2-8 day of pregnancy. Then, number and weight of neonates, duration of pregnancy, and percent of dead fetuses were determined. Furthermore, cytotoxicity of this plant was tested using fibroblast (L929) and Chinese hamster ovary (Cho) cell lines.

Results: The A. lanata had no significant effect on duration of pregnancy, average number of neonates, and weight of neonates. However, administration of 200 and 400 mg/kg of the extract led to 30 and 44% abortion in animals, respectively. The extract at concentrations ≥ 200 µg/ml significantly (P < 0.001) inhibited the proliferation of L929 fibroblast cells. Regarding the Cho cells, the extract induced toxicity only at concentration of 800 µg/ml (P < 0.010).

Conclusion: Our results showed that continuous consumption of A. lanata in pregnancy may increase the risk of abortion and also may have toxic effect on some cells.

Keywords: Artemisia lanata, Female Rats, Reproductive

Introduction
Until date, there is no report on safety of Artemisia kopetdaghensis. This study aimed to determine the possible undesirable effects of A. kopetdaghensis on reproduction of female rats. The pregnant rats were treated (i.p.) with vehicle or 200 and 400 mg/kg of A. kopetdaghensis hydroalcoholic extract from the 2-8 day of pregnancy. Then, number and weight of neonates, duration of pregnancy, and percent of dead fetuses were determined. Furthermore, cytotoxicity of this plant was tested using fibroblast (L929) and Chinese hamster ovary (Cho) cell lines. The A. kopetdaghensis had no significant effect on duration of pregnancy, average number of neonates, and weight of neonates. However, administration of 200 and 400 mg/kg of the extract led to 30 and 44% abortion in animals, respectively. The extract at concentrations ≥ 200 µg/ml significantly (P < 0.001) inhibited the proliferation of L929 fibroblast cells. Regarding the Cho cells, the extract induced toxicity only at concentration of 800 µg/ml (P < 0.010). Our results showed that continuous consumption of A. kopetdaghensis in pregnancy may increase the risk of abortion and also may have toxic effect on some cells.

Today, medicinal plants are widely used around the world as an alternative to pharmaceutical drugs. Although herbal products are considered to have fewer adverse effects compared with synthetic drugs, they are not completely free from side effects or toxicity (1). Adverse effects of medicinal plants may result from contamination of herbs with toxic metals, adulteration with active synthetic compounds, improperly prepared herbal products, misidentification of herbal ingredients, and inherent toxicity of certain herbs (2). Therefore, the potential side effects of any medicinal plant need to be

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Materials and Methods

High glucose Dulbecco's modified eagles medium (DMEM) and fetal bovine serum (FBS) were purchased from gibco. Penicillin, streptomycin, and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were obtained from sigma. Dimethyl sulfoxide (DMSO) was purchased from Fluka. Tween 80 was purchased from Merck.

The fresh A. lanata was collected from native stores and identified by the Pharmacognosia Department of Tabriz Research Institute, Iran. The aerial parts of plant were cleaned and grounded to fine powder with a blender. Then, macerated extract was prepared as described previously (6,7), briefly by suspension of 200 g of the powder in 500 mL of 50% ethanol and incubation for 72 h at 37 °C. The hydroalcoholic extract was then dried on a water bath and the yield dissolved in distilled water containing 1% DMSO.

Male and female Wistar rats (200-250 g) and female mice (26-32 g) were obtained from Laboratory Animals Research Center and housed in cages with controlled lighting (12 h dark, 12 h light) and temperature (22 ± 2 °C). The animals were given standard pellets diet and water ad libitum. All animal procedures were in accordance with Ethical Guidelines approved by the Animal Care Use Committee of Tabriz Research Institute, Iran.

Prior to the mating, the female rats were isolated for 30 days to rule out preexisting pregnancy. Then, they were caged overnight with a male rat of proven fertility in the ratio of 1:1. Rats exhibiting vaginal plug on the following morning were separated, and that day was considered as the 1st day of pregnancy. The pregnant rats were randomized into three groups: (1) control group receiving 1% Tween 80 as vehicle (n = 8), (2) experimental rats treated with 200 mg/kg of A. lanata extract (n = 10), and (3) experimental rats receiving 400 mg/kg of the plant extract (n = 9). The extract was injected intraperitoneally from the 2nd to the 8th day of pregnancy (early period of organogenesis). The animals were kept individually in cages until parturition. Then, number and weight of neonates, duration of pregnancy, and percent of dead fetuses were determined.

Acute toxicity of A. lanata extract was evaluated by the method of Akhila et al. (8). Five groups of two mice received vehicle (1% Tween 80) or 400, 800, 1600, and 3200 mg/kg of the plant extract intraperitoneally. The treated animals were monitored for 24 h and also 1 week for mortality. The lowest dose which led to death of animals and the highest dose which did not kill any mice were recorded.

The L929 (mouse fibroblast) and Cho cells were seeded in 96-well plates and cultured for 24 h in DMEM supplemented with 10% FBS, penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37 °C and 5% CO2. Then, the medium was changed to fresh one containing vehicle (1% DMSO) or 50-800 μg/ml of A. lanata extract. The cells were further incubated for 24 h at 37 °C and 5% CO2. At the end of the treatment, the cell proliferation was measured using MTT assay as previously described (9-11). The assay was carried out using 2 culture plates, 4 wells for each concentration (n = 8).

The values were compared using the one-way analysis of variance, followed by Tukey's post-hoc test for multiple comparisons. The P < 0.050 were considered to be statistically significant. All results are presented as mean ± standard error of mean.

Results

Effect of A. lanata on reproduction

As shown in table 1, the A. lanata extract at concentrations of 200 and 400 mg/kg had no significant effect on duration of pregnancy. However, administration of 200 and 400 mg/kg of the extract led to 30 and 44% abortion in animals, respectively. The percent of dead neonates was 6% in control group and 7% and 0% in experimental groups treated with 200 and 400 mg/kg, respectively. The average number of the neonates in the animals receiving vehicle during pregnancy was 8.13 ± 1.5 (Figure 1). None of the A. lanata doses could cause a significant change in the neonate number. Likewise, the extract had virtually no significant effect on weight of neonates (Figure 2).

Acute toxicity of A. lanata

Different groups of mice (n = 2) were treated with 400, 800, 1600, and 3200 mg/kg of A. lanata hydroalcoholic extract. After 24 h, it was found that 1600 and 3200 mg/kg are the highest dose which did not kill any mice and the lowest dose which led to death of both mice, respectively. The treated animals were further monitored until 1 week and no mortality or any sign of toxicity was observed at doses ≤ 1600 mg/kg.
Table 1. Effect of A. lanata on reproduction of female rats. The pregnant rats were treated (i.p.) with vehicle or A. lanata hydroalcoholic extract from the 2-8th day of pregnancy. Values are mean ± SEM (n = 8-10)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of pregnancy (day)</th>
<th>Abortion (%)</th>
<th>Percent of dead fetuses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22 ± 0.3</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>A. lanata (200 mg/kg)</td>
<td>23 ± 0.3</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>A. lanata (400 mg/kg)</td>
<td>22 ± 0.4</td>
<td>44</td>
<td>0</td>
</tr>
</tbody>
</table>

A. lanata: Artemisia lanata; SEM: Standard error of mean

Figure 1. Effect of Artemisia lanata hydroalcoholic extract on number of neonates
The pregnant rats were treated (i.p.) with vehicle or 200 and 400 mg/kg of A. lanata hydroalcoholic extract from the 2-8th day of pregnancy. Values are mean ± standard error of mean (n = 8-10)

Figure 2. Effect of Artemisia lanata hydroalcoholic extract on weight of neonates
The pregnant rats were treated (i.p.) with vehicle or 200 and 400 mg/kg of A. lanata hydroalcoholic extract from the 2-8th day of pregnancy. Values are mean ± standard error of mean (n = 8-10)

Cytotoxicity of A. lanata
Figures 3 and 4 show the effect of A. lanata hydroalcoholic extract on proliferation of L929 and Cho cells. Following incubation of L929 fibroblast cells with 50, 100, 200, 400, and 800 μg/ml of the extract, approximately 15, 17, 45, 72, and 77 percent inhibition in cell growth, was observed, respectively, as compared with untreated cells. The cytotoxic effect of A. lanata was statistically significant at concentrations ≥ 200 μg/ml (P < 0.001). On the other hand, the extract induced toxicity on Cho cells only at concentration of 800 μg/ml (P < 0.010). In the presence of 50, 100, 200, 400 and 800 μg/ml of A. lanata, surviving of Cho cells was 103 ± 1.3, 100 ± 3.2, 102 ± 2, 102 ± 2 and 85 ± 2%, respectively, as compared to untreated cells (100 ± 5).

Discussion
Many medicinal plants are used by pregnant women for their therapeutic effects. For example, it has been shown that about 36% of pregnant women in Norway use herbs (8,12). However, these plants are consumed mostly based on personal experience or traditional knowledge and in most cases it is unclear how safe the use of them is during pregnancy. Previous studies highlighted that some of plants have different antifertility activities (13). The present study aimed to examine the possible toxic effects of A. lanata.
on reproduction of female rats. Our data demonstrated that the plant extract has no effect on duration of pregnancy and number or weight of neonates. However, it can induce abortifacient effect when consumed at early period of pregnancy. This antifertility effect of *A. lanata* has been also reported for some other plants of the Asteraceae family such as *Achillea millefolium* and *Aspilia africana* which showed antispermatogenic and antiovulatory activities, respectively (14,15). On the other hand, we observed that administration of *A. lanata* extract (400 mg/kg) did not lead to stillbirth. The exact cause of this discrepancy should be explored in the future experiments. However, it may be attributed to high rate of abortion in animals receiving high concentration of the extract.

According to the previously published work, amphene, camphor, davanone, eucalyptol, eugenol, and geranial are of major components of *A. lanata*. Camphor accounted for about 1.5 g/100 g of this plant (16). Rabl et al. have reported that camphor crosses the placenta and may lead to abortion (17). In another study, Linjawi reported that camphor induces significant structural changes on uterus of pregnant rats (18). Therefore, it is rational to assume that camphor is involved in the abortifacient effect of *A. lanata*.

Cytotoxicity evaluation of *A. lanata* showed that its hydroalcoholic extract decreases proliferation of fibroblast cells. This finding may describe the camphor induced degeneration of luminal epithelium and decrease of endometrium thickness in uterus of pregnant animals (18).

![Figure 3. Effect of Artemisia lanata hydroalcoholic extract on proliferation of fibroblast L929 cell lines](image1)

*The cells were cultured for 24 h in the medium containing vehicle (1% dimethyl sulfoxide) or 50-800 µg/ml of *A. lanata* extract; Values are mean ± standard error of mean (n = 8)*

![Figure 4. Effect of Artemisia lanata hydroalcoholic extract on proliferation of Chinese hamster ovary cell lines](image2)

*The cells were cultured for 24 h in the medium containing vehicle (1% dimethyl sulfoxide) or 50-800 µg/ml of *A. lanata* extract; Values are mean ± standard error of mean (n = 8)*
In conclusion, our results showed that continuous consumption of A. lanata in pregnancy may increase the risk of abortion and also may have toxic effect on some cells of body. Therefore, its continuous use is not recommended in pregnancy.

Ethical issues
The local ethics committee approved the study.

Conflict of interests
We declare that we have no conflict of interests.

Acknowledgments
We would like to thank Women’s Reproductive Health Research Center that helped us in this study.

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