Antifertility activity of *Artemisia vulgaris* leaves on female Wistar rats

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[ABSTRACT]

AIM: To evaluate the antifertility activity of *Artemisia vulgaris* leaves on female Wistar rats.

METHOD: The plant extract was tested for its effect on implant formation at two dose levels, 300 and 600 mg·kg$^{-1}$, respectively. The effective methanolic plant extract was further studied for estrogenic potency on ovariectomised immature female Wistar rats.

RESULTS: The data presented in this study demonstrate the antifertility potential of *Artemisia vulgaris* methanolic leaf extract, which shows a strong and significant decrease in implant formation (100%), and a strong estrogenic effect resulting in a significant increase in uterine weight in immature ovariectomised rats. These observations suggest that the methanolic extract of *Artemisia vulgaris* leaves has strong anti-implantation activity and estrogenic activity.

CONCLUSION: The methanolic plant extract of *A. vulgaris* has antifertility activity.

[KEY WORDS] Antifertility activity; Anti-implantation activity; *Artemisia vulgaris*; Estrogenic activity


Introduction

Antifertility agents are those drugs which are capable of inducing the termination of pregnancy. The world population explosion has pointed out the need for new, effective, and safe contraceptive agents, or methods of maximum protection. The side effects of the existing synthetics on the normal human body are much more aggressive and unpredictable on prolonged use. Therefore, the present time is therefore alerting us to think of alternatives in the field of contraception. Hence efforts are made to think back on the available natural products[^1].

*Artemisia* is a large and diverse genus of plants with between 200 to 400 species belonging to the daisy family Asteraceae. It comprises hardy herbs and shrubs known for their volatile oils. They grow in temperate climates of the Northern Hemisphere and Southern Hemisphere, usually in dry or semi-dry habitats. The fern-like leaves of many species are covered with white hairs[^2]. *Artemisia vulgaris* L. (Asteraceae) is commonly known as mugwort, and contains a volatile oil, flavonoids, sesquiterpene lactones, coumarin derivatives, and triterpenes[^3]. Traditionally mugwort has been used to stimulate irregular or suppressed menstruation. It is believed that it stimulates the uterus, and that it is useful for menstrual pain and cramps. Furthermore, it has been used to induce miscarriage, probably due to the herbs ability to interfere with menstruation[^4].

An ethnomedical survey revealed that the alcoholic extract of *A. vulgaris* leaves, taken at toxic doses, is used to interrupt pregnancy in females. However, despite the abortifacient claim of *A. vulgaris* leaf extract in folklore medicine, there is no published scientific evidence that has either substantiated or refuted this claim. Therefore, this research work was carried out to provide scientific evidence to the claimed antifertility potential of the alcoholic extract of *A. vulgaris* leaves in pregnant rats using parameters such as implantation index, estrogenicity, and reproductive hormones.

Materials and Methods

*Artemisia vulgaris* was obtained from local area of Kadapa and authenticated by Sri Madhava Chetty botanist, Tirupati, (A.P). The collected plant material (leaves) of *A. vulgaris* was washed thoroughly in water, and air-dried for two weeks at 35–40 °C. Extraction was carried out with 200 g of *A. vulgaris* leaves in 500 mL of 70% methanol by soxhlation for 18 h by using Soxhlet apparatus. The extracts were concentrated...
under reduced pressure, dried, and stored at 4 °C in air-tight containers for further studies. Healthy adult female Wistar rats weighing 150–200 g and healthy immature female rats weighing about 30 to 40 g were obtained from Raghavendra enterprises (Bangalore). The animals were housed in stainless steel cages at a controlled room temperature of 24 °C, under a 12 h light and 12 h dark cycle. After one week of acclimatization, the animals were used for experimentation. The experimental protocol was approved by the Institutional Animal Ethical Committee of P. Rami Reddy Memorial College of Pharmacy (1423/PO/a/11/CPCSEA).

**Experimental design**

**Assessment of anti-implantation activity**

Healthy adult Wistar strain female rats (150–200 g) of proven fertility and regular estrus cycles were selected and caged with males of proven fertility in the ratio of 3 : 1 in the evening of estrus phase and examined the following day for the evidence of copulation. Those rats showing thick clumps of spermatozoa in their vaginal smears and presence of HCG (human chorionic gonadotrophin) in their urine samples (detected by using urine pregnancy kits) were separated, and that day was designed as day 1 of pregnancy.

Those rats were divided into three groups containing six rats in each. Group I received vehicle only [1% carboxy methyl cellulose (CMC) suspension 10 mL·kg⁻¹] and served as the control. Groups II and III received MEAV (methanolic extract of *Artemisia vulgaris*) at a dose of 300 and 600 mg/kg body weight, respectively. Doses were given from day 1 to 10 of pregnancy by oral feeding needle.

Twenty-four hours after the last dose, the animals were sacrificed under excess ketamine anesthesia using sterile conditions, and the uteri were examined to determine the number of implantation sites [⁶]. The treatment schedule is shown in Table 1.

<table>
<thead>
<tr>
<th>No.</th>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>No. of days of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Vehicle</td>
<td>0.5% CMC 10 mL·kg⁻¹</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>MEAV-I</td>
<td>Methanolic extract of <em>A. vulgaris</em></td>
<td><em>A. vulgaris</em> 300 mg·kg⁻¹</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>MEAV-II</td>
<td>Methanolic extract of <em>A. vulgaris</em></td>
<td><em>A. vulgaris</em> 600 mg·kg⁻¹</td>
<td>10</td>
</tr>
</tbody>
</table>

**Assessment of estrogenic activity**

The results obtained from the anti-implantation testing indicates the potency of the methanolic extract of *A. vulgaris* in pregnant rats which significantly increased the weight of reproductive organs. Therefore, it was subjected to detailed investigation for its possible estrogenic-like activity.

The estrogenic potential of MEAV was evaluated in ovariec tomised immature female rats. This activity was carried out according to the previous reported study [⁶].

Colonies bred, immature, bilaterally ovariectomized female rats (21–23 days) weighing between 30 to 40 g were divided into four groups consisting of six rats. Group I received vehicle only (0.5% CMC suspension 10 mL·kg⁻¹) and served as the control given by oral feeding needle.

The group II rats received synthetic estrogen preparation which serves as standard [ethinylestradiol (0.03 mg) + levonorgestrel (0.15 mg)] in olive oil, administered subcutaneously using a 1 mL syringe. Group III received the methanolic extract of *A. vulgaris* at 300 mg·kg⁻¹ orally, and group IV received the methanolic extract of *A. vulgaris* at 600 mg·kg⁻¹ orally by oral feeding needle. Doses were given for 7 days, and on the 8th day of the experiment, all of the animals were sacrificed under excess ketamine anesthesia. The uteri were dissected out; surrounding tissues were removed, blotted on filter paper, and weighed quickly. The treatment schedule is as shown in the Table 2.

<table>
<thead>
<tr>
<th>No.</th>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>No. of days of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Vehicle</td>
<td>0.5% CMC 10 mL·kg⁻¹</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>MALA-D (tabs) + olive oil</td>
<td>Olive oil + Levonorgestrel (0.15 mg) + Ethinylestradiol (0.03 mg)</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>MEAV-I</td>
<td>Methanolic extract of <em>A. vulgaris</em></td>
<td><em>A. vulgaris</em> (300 mg·kg⁻¹)</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>MEAV-II</td>
<td>Methanolic extract of <em>A. vulgaris</em></td>
<td><em>A. vulgaris</em> (600 mg·kg⁻¹)</td>
<td>7</td>
</tr>
</tbody>
</table>

**Statistical analysis**

All of the data are expressed as mean ± SEM. Statistical significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad.). Statistical significance was taken as *P* < 0.05.

**Results**

**Selection of dose**

The doses were selected according to the acute toxicity
studies conducted earlier [7]. The LD₅₀ of the methanolic leaf extract of plant *A. vulgaris* was found to be more than 3 g·kg⁻¹. Hence one-tenth of this dose i.e., 300 mg·kg⁻¹ body weight was fixed as the low dose and 600 mg·kg⁻¹ was fixed as the high dose.

**Effect of the crude extract on implantation activity**

The effect of MEAV was studied at the doses of 300 and 600 mg·kg⁻¹. The results revealed that the methanolic extract of *A. vulgaris* leaves shows significant (*P* < 0.05), dose-dependent inhibition of implant formation, the extract at the dose of 600 mg·kg⁻¹ showed 100% inhibition of implant formation, while the extract at the dose of 300 mg·kg⁻¹ had shown 50% inhibition of implants, when compared to that of control group animals, as shown in the Table 3.

Table 3  Anti-implantation activity of *Artemisia vulgaris* leaves (mean ± SEM, *n* = 6)

<table>
<thead>
<tr>
<th>No.</th>
<th>Dose mg·kg⁻¹</th>
<th>Treatment (in days)</th>
<th>No. of rats without implants on day 11/no. of rats used</th>
<th>No. of implants on day 11</th>
<th>Antifertility activity/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (CMC-0.2 mL)</td>
<td>10</td>
<td>0/6</td>
<td>6.5 ± 0.56</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>MEAV-I 300 mg</td>
<td>10</td>
<td>3/6</td>
<td>1.5 ± 0.71 *</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>MEAV-II 600 mg</td>
<td>10</td>
<td>6/6</td>
<td>0 ± 0</td>
<td>100</td>
</tr>
</tbody>
</table>

*P* < 0.05 vs control

The results also revealed that in the 10 day treatment with the methanolic extract of *Artemisia vulgaris* at a doses of 300 and 600 mg·kg⁻¹, the respective reproductive organ weights, and the other vital organs weights like liver, kidney, heart, and spleen increased, as compared to the control group rats. However, a very slight difference was observed in their body weights as compared to the respective initial body weights, as shown in the Table 4. Photographic images showing the presence of implants in the uterine horns in each group are shown in the respective Figs. 1–6, and Fig. 7 is the graphical representation of the number of implants in each group.

**Fig. 1**  Photograph showing presence of nine implants in a control group animal

**Fig. 2**  Photograph showing presence of six implants in a control group animal

**Fig. 3**  Photograph showing presence of four implants in MEAV-I treated group animal

**Fig. 4**  Photograph showing absence of implants in MEAV-II treated animals

**Effect of the crude extract on estrogenic activity**

The effect of the MEAV on the immature rat uterus is shown in Table 5. Oral administration of the methanolic *A. vulgaris* leaf extract at the doses of 300 and 600 mg·kg⁻¹ p.o. caused a significant (*P* < 0.05) increase in uterine weight in immature ovariectomised rats. The extract, at the doses of 300 and 600 mg·kg⁻¹ body weight, induced vaginal opening and the smear showed proestrous or estrous. The number of cornified cells in the vaginal smear was considerably higher (+ to ++) than the control, but notably less than that of standard (ethinyl estradiol 0.03 mg + levonorgestrel 0.15 mg).
Discussion

One of the most critical problems facing a developing country like India is its geometrical increase in human population. About 90% of the world’s contraceptive users are women [8]. This gender-based usage has occurred due to the emphasis of family planning programs and contraception research. Condoms, vasectomy, and withdrawal are the only male contraception devices available with less assurance for men. It has, therefore, become necessary to use biologically active botanical substances or fertility-regulating agents of plant origin which are eco-friendly in approach and interfere with the natural patterns of reproduction. Nearly 80% of the world population relies on traditional medicines for primary health care, most of which involve use of plant extracts [9].

Plants that have contraceptive and abortifacient properties may act through rapid expulsion of the fertilized ova from the fallopian tube, inhibition of implantation due to a disturbance in estrogen–progesterone balance, fetal abortion, perhaps due to lack of supply of nutrients to the uterus and the embryo, and also on the male side through affecting sperm count, motility, and viability [10-12]. The present research was carried out to evaluate the anti-implantation and anti-zygotic, anti-blastocytic, as well as the estrogenic properties of the plant extract. The estrogenic activity assessment of the plant extract revealed that oral administration of the methanolic extract of Artemisia vulgaris leaves at a dose of 600 mg·kg⁻¹ body weight showed a significant decrease in the number of implantation sites compared to the control. This may indicate that the crude extract inhibited the process of implantation.

The possible cause of the termination of pregnancy on oral administration of the plant extract may be due to anti-zygotic, anti-blastocytic, as well as the estrogenic properties. Plant extracts can cause endometrial alterations resulting in non-receptive endometrium, and thus cause implantation failure. Earlier work reported that an extract of the leaves of Hibiscus rosa-sinensis caused endometrial alteration and resulted in blastocyst implantation failure in mice [13]. It is known that administration of high levels of exogenous estrogenic substances to mice also causes implantation failure [14]. In mice, rats, and humans, estrogen also plays a pivotal role in implantation because it participates in estrogen/progesterone balance and, therefore, can affect the uterine receptivity to the embryo [15], as reported that administration of low concentrations of compounds with estrogenic activity to many species during early pregnancy resulted in rapid passage of ova through the oviducts and expulsion of the ova from the oviduct. Furthermore, degeneration of the fertilized ova when transported into the uterus too early can also decrease the number of implants and result in decreased fertility [16].

The results of the anti-implantation experiments (Table 3) revealed that oral administration of the methanolic extract of Artemisia vulgaris at a dose of 600 mg·kg⁻¹ body weight showed a significant decrease in the number of implantation sites compared to the control. This may indicate that the crude extract inhibited the process of implantation.

When the dose was decreased to 300 mg·kg⁻¹ body weight there was only a 50% decrease in the implantation sites as compared to the dose at 600 mg·kg⁻¹ body weight. This shows that the effect of the extract on implantation de-

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**Table 4** Body weight (g), organ weight (g) of control and Artemisia vulgaris-treated animals (mean ± SEM, n = 6)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight/g</th>
<th>Vital organ weight/g</th>
<th>Entire reproductive organ weight ovaries, uterine horns, and uterus</th>
<th>Relative reproductive organ weight and final body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.2 mL CMC)</td>
<td>Initial 128.3±2.04 Final 120.3±4.86 Ovaries 0.026±0.01 Uterus 0.083±0.01 Liver 4.06±0.21 Kidney 1.13±0.05 Heart 0.63±0.06 Spleen 0.266±0.02 Weight ovaries, uterine horns, and uterus 0.23±0.02 Weight and final body weight 0.23/120.3=0.0019</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAV (300 mg·kg⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAV (600 mg·kg⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P value not significant

**Table 5** Estrogenic activity of MEAV in ovariectomised female rats (mean ± SEM, n = 6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Uterine weight</th>
<th>Vaginal cornification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.5% CMC)</td>
<td>10 mL·kg⁻¹</td>
<td>0.028 ± 0.003</td>
<td>No cells observed</td>
</tr>
<tr>
<td>Standard (ethinyl estradiol + levonorgestrel)</td>
<td>0.03 mg + 0.15 mg</td>
<td>0.125 ± 0.004**</td>
<td>+++</td>
</tr>
<tr>
<td>MEAV</td>
<td>300 mg·kg⁻¹</td>
<td>0.066 ± 0.004**</td>
<td>+</td>
</tr>
<tr>
<td>MEAV</td>
<td>600 mg·kg⁻¹</td>
<td>0.103 ± 0.004*</td>
<td>++</td>
</tr>
</tbody>
</table>

*** P < 0.001; *P < 0.05 vs control; +: nucleated epithelial cells; ++: nucleated and cornified cells; +++: cornified cells
pends on the dose. This result is in agreement with the finding of Golam Sadik et al., who showed that ethanolic leaf and stem extract of *Pergularia daemia*, when administered orally to mice, inhibited implantation depending on the dose [17]. A similar finding was reported by Montanari et al., who showed that administration of a hydroalcoholic leaf extract of *Maytenus ilicifolia* orally to mice resulted in a reduced rate of implantation of embryos [18]. Gebrie et al. also reported that the methanolic root extract of *Rumex steudelii* when administered orally to pregnant rats resulted in significant decrease in number of litters [19]. It is not always the case that medicinal plants result in anti-implantation effect. For example administering 70% ethanolic extract of the aerial parts of *Ruta graveolens* L. orally to mice did not cause pre-implantation embryonic loss and did not affect implantation [20].

A number of plants have been reported to inhibit implantation by their estrogenic mode of action [21]. The process of implantation of an egg to the uterine wall depends upon the hormonal milieu of the uterus [22]. Anti-implantation agents are effective by virtue of their hormonal properties, namely estrogenic or progestational properties, or by antagonizing the effects of female sex (estrogen and progesterone) hormones. It is well-established that some conventional plants result in anti-implantation effect. For example, administering 70% ethanolic extract of the aerial parts of *Embelia ribes* fruits were reported to alter the level of estrogen and progesterone leading to improper implantation [22]. Therefore in any of the conditions, the secretion of estrogen and/or progesterone governs all the preparatory changes in the uterus for implantation.

In the present investigation, MEAV (methanolic extract of *A. vulgaris*) exhibited either 50% or 100% anti-implantation activity, depending on the dose. In immature female rats the methanol extract exhibited definite estrogenic activity at 600 mg/kg−1 dose as it was observed that the weights of reproductive organs had increased significantly. Hence, the anti-implantation activity of the methanol extract may be due to an imbalance of endogenous estrogen and progesterone levels as evidenced by the significant increase in uterine weight, or may be due to their anti-zygotic, blastocytotoxic activity [23]. The observation of reduced implantation sites and increased uterine weight confirms the antifertility activity of the methanol extract of *A. vulgaris* leaves.

Phytochemical examination revealed the presence of steroids, flavonoids and saponins. Flavonoids have been reported to possess antifertility activity [24]. The presence of flavonoids in MEAV may contribute to its antifertility activity. On the basis of these observations it may be concluded that methanolic extract of *A. vulgaris* owing to its estrogenic nature alters the biochemical milieu of the uterus which leads to a change in the normal status of reproduction in female reproductive tract of rats and thus produces a significant antifertility effect.

In summary, the present study was aimed at the possible anti-implantation and estrogenic effects of the methanolic extract of *A. vulgaris*. It was shown that the plant extract inhibits the process of implantation depending on the dose, further, the extract possess strong dose-dependent estrogenic effect. Hence, the study suggests that the methanolic plant extract of *A. vulgaris* has antifertility activity.

**Acknowledgements**

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**References**