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## Research Article

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### THE EFFECT OF *ARGEMONE MEXICANA* ROOT ON FEMALE REPRODUCTIVE SYSTEM IN WISTAR RATS

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#### ABSTRACT

**OBJECTIVES:** The current study was planned to evaluate the effect of fresh extract of root of *Argemone mexicana* on female reproductive system.

**METHODS :** The fresh extract was prepared by using 0.2 kg of the *Argemone mexicana* roots, which were collected, washed thoroughly and cut into small pieces, grounded by adding 50 ml of water, 5 ml of cooking oil and 0.5 g pepper. Finally the extraction was filtered and the filtrate was administered orally. On the day of sacrifice animals were anesthetized with ether anesthesia and carefully dissected for the uterine horns, immediately weighed and fixed with 10% buffered formalin for 48 h, followed by automatic tissue processing which was carried on the Yorco automatic tissue processor (YSI-3) and processed for embedding by standard histological techniques. Statistical analysis was done by using Sigma Plot 10 statistical software.

**RESULTS:** In the present study body and uterine weight showed slight increase in treated group. The uterus microscopic structure showed unaltered histo-architecture with respect to myometrial and perimetrial thickness in treated and control groups while the endometrial thickness was found to be more in the treated group.

**CONCLUSIONS:** We can conclude from the present study that the treated group exerted a profertility effect in the female Wistar rats this is indicated by the increased thickness of endometrium. Further studies are required with *Argemone mexicana* root to determine the mechanism of action that leads to endometrial thickness increase and thereby exerts profertility effect.

**Keywords:** *Argemone mexicana*, root, uterine horn histology, body weight.

#### INTRODUCTION

In India the role of medicinal plants in folklore medicine is a well established and known fact from ancient time. Herbal medicines involving use of fresh or dried plant parts are overtaken modern synthetic drugs by having minimal or no side effects<sup>1</sup>. The World Health Organization survey estimated that around 80% of the world population of developing nations relies on traditional medication in primary healthcare sectors<sup>2</sup>.

Among various health problems, infertility is a crucial situation that bothers the modern society. Infertility is a worldwide health complication with one in six couples suffering from this condition and with a primary economic burden on the global healthcare industry. Infertility is considered to be a painful and heart breaking conditions for couples and of course for the family members<sup>3</sup>. Failure of a couple to become pregnant after one year of regular, unprotected sexual intercourse is called infertility. In both

women and men the process of fertility is complex. In the regular circumstances the feasibility that a woman will get pregnant during a single menstrual cycle is only about 30% and when conception occur only 50-60% of pregnancies advance beyond the 20<sup>th</sup> week<sup>4</sup>. Infertility varies over regions of the world and is estimated that about 8 to 12% of couples worldwide suffering from infertility<sup>5, 6</sup>.

To overcome the infertility problem the traditional practitioners are using various plants and their products. One such plant is the *Argemone mexicana* (root), which is used to treat female infertility. The *Argemone mexicana* Linn is an indigenous prickly annual herb belonging to the family Papaveraceae, common plant found everywhere road sides, as a weed in the waste and agricultural lands in India, universally distributed throughout the subtropical and tropical regions of the world. It grows up to 1.2 m in height and acclimatized throughout India up an altitude of 1,500 m. The common names are Mexican prickly poppy and Satyanashi<sup>7</sup>.

It is broadly used as traditional medicine for the treatment of various diseases. Different parts of the plant were largely used in Ayurveda, Unani, Homeopathy and Siddha medicinal systems. It is reported to have various pharmacological activities such as antioxidant, antidiabetic, hepatoprotective activity, antibacterial, antimicrobial, antimalarial, allelopathic potential, anticancer, wound healing property, larvicidal activity, molluscicidal, neuropharmacological and anti-HIV<sup>8,9</sup>. The previous phytochemical study of ethanolic extract of root of *Argemone mexicana* showed the presence of phenolics, amino acids, alkaloids and fatty acids. Chloroform extract showed the presence of tannins, alkaloids, phytosterols and flavonoids<sup>10</sup>.

Even though scientific literature is available on the phytochemical constituent, toxicity and medicinal activities of *Argemone mexicana*, the role of *Argemone mexicana* root on female infertility is completely lacking in spite its extensive usage in traditional practice of medicine for treating infertility. So the present study is planned to evaluate the effect of *Argemone mexicana* root fresh extract on female reproductive system.

## MATERIALS AND METHODS

**Plant material:** The root of *Argemone mexicana* is used for the present study. In the month of June and July the plant

material was collected from Hyderabad-17° 22' 31" North, 78° 28' 28" East, Telangana, India, Asia, authenticated by Head of Botany Department, Osmania University, Hyderabad, Telangana, India. The plant specimen was identified as *Argemone mexicana* L., it belongs to family Papaveraceae. The voucher No.023 specimen sample of the plant was deposited in the Department Herbarium for future reference.

**Fresh extraction:** After identification of the plant, the roots were carefully separated from the plant and thoroughly washed with tap water. For the study around 0.2 kg of the roots were cut into small pieces and grounded by adding 50 ml of water, 5 ml of cooking oil and 0.5 g pepper. Finally the extraction was filtered and the filtrate was used for the study.

**Animals:** Twenty five adult nulliparous and non-pregnant female Wistar rats of age around six months and weighing 185-195 g were used for the experimental study. The rats were procured from Teena Biolabs Pvt. Ltd, Hyderabad. The rats were acclimatized to the laboratory conditions for a week before starting the experiment. The rats were maintained as per the Institutional ethical committee (IAEC) norms, with 12 h dark and 12 h light cycle with food and water at ad libitum. The study procedures involving the handling and treatment of animals were approved by the Institutional ethical committee Teena Biolabs Pvt. Ltd. Reg. No. 177/PO/cb/99/CPCSEA. Project No: TBLSTPRJ0032014. Prior to experimentation all the rats were tested for the normal regular estrous cycle. The rats with normal regular estrous cycle were selected for the study. The estrous cycle of rats was determined by vaginal smear. Vaginal smears samples were collected by swab smear (cotton buds) technique.

**Experimental design:** The twenty five rats (estrus phase) were randomly separated into five groups, each group consisting of five rats. The control group (C), the sham control B one day (SCB1) and four to six days (SCB4), *Argemone mexicana* root fresh extract treated for one day (AF1) and for four to six days (AF4) group (Table 1).

**Treatment:** The oral administration was done by utilizing a curved ball-tipped intubation needle affixed to a 2 ml syringe.

**Vehicle** - Mixture of 50 ml of water, 5 ml of cooking oil and 0.5 g pepper

**C** - The control group was given the normal standard diet.

**SCB1**- The sham control B treated with 2.5 ml/kg body weight of vehicle, twice a day for 1 day on the estrus phase of the estrous cycle.

**SCB 4**- The sham control B treated with 2.5 ml/kg body weight of vehicle, twice a day for 4 to 6 days on estrus phase of the estrous cycle to the estrus phase of the next estrous cycle.

**AF 1**- Experimental rats were treated orally 2.5 ml/kg body weight of Argemone mexicana root fresh extract, twice a day for 1 day on the estrus phase of the estrous cycle.

**AF 4**- Experimental rats were treated orally 2.5 ml/kg body weight of Argemone mexicana root fresh extract, twice a day for 4 to 6 days on estrus phase of the estrous cycle to the estrus phase of the next estrous cycle.

**Sacrifice**: All the animals were anesthetized with ether anesthesia on the day of sacrifice.

**Body weight**: On the day of sacrifice the body weight of control, sham control and treated group rats were weighed using Wensar top table balance model no TTB 3.

**Uterine horn weight**: The control, sham control and treated group rats were euthanized and carefully dissected for the uterine horns, cleaned for connective tissue and fat, examined macroscopically and immediately weighed by using Shimadzu AX-200 analytical balance.

**Histological Study**: After collection and weighing the uterine horns were labelled for identification and transferred to cassettes, fixed with 10% buffered formalin for around 48 h, followed by automatic tissue processing which was carried out using the Yorco automatic tissue processor (YSI-3). The automatic tissue processing includes dehydration in graded isopropyl alcohol, clearing in xylene I & II, impregnation in Fisher Scientific paraffin wax I & II. Finally tissue paraffin blocks were prepared by using Thermo Shandon paraffin dispenser and L modes. After embedding, the Leica RM2125RTS Rotationsmikrotom was used for cutting 5 micron thick sections. The sections were fixed to the slide by heat technique followed by staining with the Harris's Haematoxylin and Eosin yellow stain solution - 2%W/V, Nice Chemical Pvt. Ltd<sup>11</sup>. The stained uterine sections were analyzed in Labomed Vision 2000 binocular microscope

using a low power objective lens 4X, 10X and a high power objective lens 40X. The photomicrographs of stained sections were taken with Digi Eye digital microscope camera. The photographs of uterine horns were analyzed for uterine diameter, thickness of wall, endometrial thickness, myometrial thickness and epithelial thickness by using image analysis software Digimizer version 4.5.2.

#### Statistical data analysis

All the data was tabulated by using Microsoft excel worksheet. The mean (x), standard deviation (SD), standard error (SE) and t-test was performed by using Sigma Plot 10 statistical software to find out the significance level. The P value <0.05 is considered as statistically significant. All the values were shown as  $x \pm SE$ .

#### RESULTS

**Body weight**: The body weight of root of Argemone mexicana treated rats is slightly increased, but the increase was not statistically significant when compared with the control and sham control groups (Table-2). The mean of body weight of AF4 group showed 1923 mg (1889 to 1962 mg) which more than the control and sham control groups but statistically insignificant.

**Uterine weight**: The uterine weight of root of Argemone mexicana treated rats is slightly increased, but not statistically significant when compared with the control and sham control groups (Table-3).

#### HISTOLOGICAL PARAMETERS

**Uterine horn**: The root of Argemone mexicana treated rat uterine horn wall showed the normal architecture. The endometrial epithelium showed normal tall columnar epithelium, stroma showed presence of uterine glands. The myometrium showed normal smooth muscle composition.

**Wall thickness**: The control and sham control rats wall thickness were similar, while the treated group rats showed a slight increase in thickness but it was statistically insignificant when compared with control and sham control groups (Table 3).

**Myometrial and perimetrial thickness**: The thickness of myometrium and perimetrium of control and sham control rats was same while the thickness in the treated group exhibited a moderate increase when compared with control and sham control rats (Table 3).

**Endometrial thickness**: The control group endometrial thickness was more than the sham control group but it was statistically

**Table 1: Experimental Design**

| Groups  | Treatment   | Duration                    |
|---------|---|-----------------------------|
| Control | Normal standard diet  |                             |
| SCB1    | 2.5 ml/kg body weight of vehicle  | Twice a day for 1 day       |
| SCB4    | 2.5 ml/kg body weight of vehicle  | Twice a day for 4 to 6 days |
| AF1     | 2.5 ml/kg body weight of Argemone mexicana root, fresh extract with vehicle | Twice a day for 1 day       |
| AF4     | 2.5 ml/kg body weight of Argemone mexicana root, fresh extract with vehicle | Twice a day for 4 to 6 days |

**Table 2: Effect of Argemone mexicana root fresh extract on body and uterine weight in rats**

| Group   | Weight (mg)       |                   |                 |
|---|-------------------|-------------------|-----------------|
|   | Initial body      | Final body        | Uterine         |
| Control (C)                                       | 1897.2 $\pm$ 17.0 | 1901.6 $\pm$ 17.0 | 297.2 $\pm$ 2.7 |
| Sham control B 1 day (SCB1)                       | 1896.6 $\pm$ 12.1 | 1902.2 $\pm$ 11.8 | 296.7 $\pm$ 1.7 |
| Sham control B 4 days (SCB4)                      | 1895.6 $\pm$ 16.2 | 1902.6 $\pm$ 16.3 | 297.6 $\pm$ 3.8 |
| Argemone mexicana root fresh extract 1 day (AF1)  | 1895.8 $\pm$ 9.1  | 1901.2 $\pm$ 8.7  | 296.8 $\pm$ 5.2 |
| Argemone mexicana root fresh extract 4 days (AF4) | 1908.2 $\pm$ 14.5 | 1923.4 $\pm$ 13.6 | 302.0 $\pm$ 5.1 |

All the data were expressed as  $\bar{x} \pm SE$ ,  $n = 5$  in each group,  $\bar{x}$  = mean, SE = standard error.

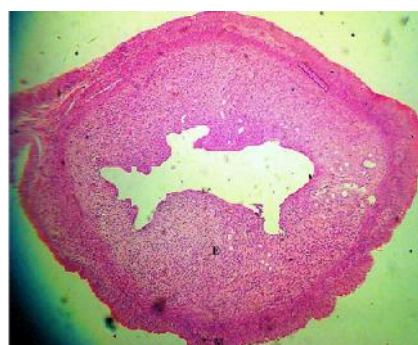
**Table 3: Effect of Argemone mexicana root on wall thickness, myometrial and perimetrial thickness of uterine horns ( $\mu$ m) in rats**

| Group  | WT ( $\bar{x} \pm SE$ ) | MT & PT ( $\bar{x} \pm SE$ ) |
|--|-------------------------|------------------------------|
| Control (C)  | 338.1 $\pm$ 10.9        | 144.6 $\pm$ 8.2              |
| Sham control B 1 day (SCB1)                            | 327.5 $\pm$ 22.6        | 133.5 $\pm$ 11.3             |
| Sham control B 4 to 6 days (SCB4)                      | 344.1 $\pm$ 7.4         | 150.0 $\pm$ 4.0              |
| Argemone mexicana root fresh extract 1 day (AF1)       | 382.5 $\pm$ 20.0        | 153.4 $\pm$ 10.3             |
| Argemone mexicana root fresh extract 4 to 6 days (AF4) | 421.6 $\pm$ 27.4* #     | 173.3 $\pm$ 12.8             |

All the data were expressed as  $\bar{x} \pm SE$ ,  $n = 5$  in each group,  $\bar{x}$  = mean, SE = standard error.

\* Indicates significance level between control and treated. # Indicates significance level between sham controls and treated.

$P < 0.05$ . WT-Wall thickness, MT-Myometrial thickness, PT-Perimetrial thickness.

**Fig. 1a** Control uterus**Fig. 1b** sham control uterus**Fig. 1c** Argemone mexicana fresh extract treated uterus**Figure 1: Photomicrograph of uterus**

EP – Epithelium, E – Endometrium and M – Myometrium

**Table 4: Effect of the Argemone mexicana root on endometrial and epithelial thickness of uterine horns ( $\mu\text{m}$ ) in rats**

| Group  | ET ( $\bar{x} \pm \text{SE}$ ) | EPT ( $\bar{x} \pm \text{SE}$ ) |
|--|--------------------------------|---------------------------------|
| Control (C)  | 188.5 $\pm$ 9.0                | 18.7 $\pm$ 1.1                  |
| Sham control B 1 day (SCB1)                            | 186.0 $\pm$ 10.6               | 19.3 $\pm$ 1.2                  |
| Sham control B 4 days (SCB4)                           | 195.0 $\pm$ 4.6                | 19.4 $\pm$ 0.2                  |
| Argemone mexicana root fresh extract 1 day (AF1)       | 230.2 $\pm$ 12.3* #            | 23.3 $\pm$ 1.5*                 |
| Argemone mexicana root fresh extract 4 to 6 days (AF4) | 247.1 $\pm$ 20.6* #            | 22.1 $\pm$ 1.5                  |

All the data were expressed as  $\bar{x} \pm \text{SE}$ ,  $n = 5$  in each group.

$\bar{x}$  = mean, SE = standard error.

\* Indicates significance level between control and treated.

# Indicates significance level between sham controls and treated.

$P < 0.05$ .

ET- Endometrial thickness, EPT- Epithelial thickness.

insignificant. Moreover treated group exhibited an increased endometrial thickness which is statistically significant ( $P < 0.05$ ) when compared with control and sham control group rats (Table 4 & Fig 1).

**Epithelial thickness:** The epithelial thickness of the treated group was more than the control and sham control group rats. The increased thickness observed was statistically insignificant in AF4 rats while the AF1 treated rats showed statistically significant ( $P < 0.05$ ) increase when compared with control and sham control groups (Table 4).

## DISCUSSION

The present study results showed statistically insignificant increase in body weight and uterus weight. The histoarchitecture showed no cytotoxicity however indicated a profertility activity by exhibiting the increased morphometrical parameters. The increased endometrial thickness was statistically significant among the morphometric parameters. Many studies on antifertility plants have been reported in the electronic system of traditional medicine<sup>12-15</sup>, whereas only few studies reported on pro-fertility effect of plants<sup>16, 17</sup>. One such plant is Argemone mexicana, the root of it showed pro-fertility effect in the present study. There were no previous reports on the effect of root of argemone mexicana in female reproduction.

To maintain the good health of an individual the body weight is necessary factor. The decline in the body weight is generally the sign of the onset of an adverse effect. The rise in bodyweight in present study results which is although statistically insignificant reveal that it is not resulting in any

adverse effect but relatively suggests positive effect. In the literature it is described that the weight and composition of body is well associated with the sexual maturation<sup>18</sup>. It is clear that from the present study that the Argemone mexicana root fresh extract is bringing about a profertility response by increasing bodyweight. The increased weight of the uterine horns in the rats treated with extract for longer duration is indicative of the profertility effect and also prolonged use of the extract may probably bring about more positive effect.

The histomorphometric results of uterus showed mean thickness of uterine wall of treated groups was increased when compared with control and sham control groups this may be due to the increased thickness of endometrial, myometrial and perimetrial thickness. The earlier investigations mentioned that the increased endometrial thickness provides a site and nourishment for an implanting embryo until placenta develops<sup>19</sup>, in another study it is mentioned that the endometrial thickness determines the pregnancy outcome as well as the higher pregnancy rates are significantly linked with the increased thickness of endometrium<sup>20</sup>. Further, studies also have proved a greater chance of pregnancy once the endometrium reaches a threshold thickness<sup>21-23</sup>. In the present study endometrial thickness was increased in fresh extract treated group and the increase was statistically significant ( $P < 0.05$ ) when compared with control and sham control groups. So the fresh extract of root of Argemone mexicana may provide a site, nourishment for an implanting embryo, increase the

pregnancy rate and outcome. The endometrial thickness increase can be due to the phytochemicals present in the root of *Argemone mexicana* which in turn may probably exert the profertility effect by their antioxidant property.

We conclude from the present study that the *Argemone mexicana* root fresh extract is having a profertility effect on the female reproductive system. Further, future studies are required on *Argemone mexicana* root fresh and various extract to evaluate the exact mechanism of action on the female reproductive system.

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