



ISSN: 2250 – 2688

Received: 20/02/2016

Revised: 09/03/2016

Accepted: 16/03/2016

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## Anti-fertility Effect of Stem bark of *Ailanthus excelsa* in Wistar Albino Rats

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### ABSTRACT

*Ailanthus excelsa* (Family: Simaroubaceae) is commonly known as Mahanimba and Arlu. In this study stem bark of *Ailanthus excelsa* were evaluated by anti-implantation and abortifacient method at three doses i.e. 100mg/Kg, 150mg/Kg and 200mg/Kg of the body weight of the rat. It was observed that treatment with methanolic extract of *Ailanthus excelsa* at the dose of 200 mg/kg was shown significant anti-fertility effect. *Ailanthus excelsa* possess anti-fertility activity by inhibiting implantation and abortifacient action.

**Keywords:** Antifertility, abortifacient, *Ailanthus excelsa*, implantation

### 1. INTRODUCTION

*Ailanthus excelsa* (Simaroubaceae) is commonly known as “Mahanimba” due to its resemblance with neem tree (*Azadirachta indica*). The term Ailanthus is from ailanto which means Tree of heaven and is the name for one of the species in the Moluccas, while in Latin excelsa means tall. It is a fast growing tree extensively cultivated in many parts of India towards the vicinity of villages. The tree is indigenous to central and southern India and is distributed in Madhya Pradesh, Gujarat, some costal districts of Andhra Pradesh, Ganjam and Puri districts of Orissa.<sup>1</sup>

Nowadays population explosion is a major obstacle in economic growth and all-round human development in developing countries. Current pandemic population explosion demands an immediate betterment of new potential contraceptives<sup>2</sup>. Different studies of many years have highlighted the unmet demand for safe, inexpensive, and acceptable contraceptives to avoid unwanted pregnancies and resultant abortions. The quest for the oral contraceptive agent that can control human fertility is as old as recorded history. Although different synthetic contraceptive agents<sup>3,4</sup> are available, these cannot be used continuously due to their severe side effects<sup>5,6</sup>

In Chinese system of medicine bark of *Ailanthus excelsa* is used to treat diarrhoea and dysentery, especially when there is a blood in stool. The bark has been used in Asian and Australian medicine to counteract worms, excessive vaginal discharge, malaria and asthma. It has marked antispasmodic and cardiac depressant properties. The root bark is used to cure epilepsy and heart troubles. In Africa the plant is used to treat cramps, gonorrhoea, epilepsy, tapeworm infestation and high blood pressure. Traditionally the mats made of leaves are used as bed for children suffering from fever. In Bombay the bark and leaves are of great repute as a tonic especially in debility after child birth. They are used in dyspepsia, bronchitis and asthma.

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The plant is used as natural anti-fertility agent by the rural women in Mavanahalla region of the Nilgiri district in Tamilnadu. The fresh juice of stem bark mixed with either honey or sugar is given to pregnant women during evening for three consecutive days to induce permanent sterility. It is also used in gout and rheumatism. Fruits are used in diarrhoea, polyurea, piles and fever.<sup>7</sup>

Anti-fertility agents prevent reproduction by interfering with various normal reproductive mechanisms in both males and females. An ideal contraceptive agent is one which possess 100% efficacy, reversibility of action, which is free from side-effects and is easy to use. Ancient literature has mentioned the use of a number of plants and preparations for regulation of fertility in the form of emmenagogues, ecboolics, abortifacients and local contraceptives. Women have used herbs since time immemorial to control their fertility. Most of these plant's activities and their mechanism of action were not scientifically studied.<sup>8</sup>

It needs to develop and use herbal drugs as anti-fertility agents which are safe and interfere with the natural patterns of reproduction. Therefore in this present study stem bark of *Ailanthus excelsa* have been evaluated for antifertility activity by anti-implantation and abortifacient methods in wistar rat model.

## 2. MATERIALS AND METHODS

### 2.1 Collection of plant materials

The stem bark *Ailanthus excelsa* (Simarubaceae) was collected from Bareilly (U.P). Specimen voucher was identified and authenticated by Dr. G. C. Joshi, Scientist, Regional research institute of Ayurveda, Tarikhet (Ranikhet), India. A voucher specimen of the collected sample was deposited in the departmental herbarium for future reference.



Figure1: stem bark and leaves of *Ailanthus excelsa*

### 2.2 Chemicals used

The chemicals used were of the analytical grade.

### 2.3 Preparation of plant extract

The plant material were air-dried under shade, pulverized by a mechanical grinder, passed through 20 mesh and then stored in airtight containers. The stem barks of *Ailanthus excelsa* were powdered and successive extracted using soxhlet apparatus. These extracts were concentrated to dryness under reduced pressure and controlled temperature to yield solid masses.<sup>9</sup> Methanolic extract was used for the present study.<sup>9</sup>

### 2.4 Animal used

Wister rats between 150-200 g and 25 days old were used to evaluate the anti-fertility activity. They were kept in animal house in well cross ventilated room at  $27 \pm 2$  °C, and relative humidity 55-65%, light and dark cycles of 10- 14 h for 1 week before and during the experiments. Animals were provided with standard rodent diet. Water was given ad libitum. Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC), CPCSEA, India (Reg. No. 837/AC/04/CPCSA with protocol no., 02/PhD/2009). During experiment guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) were followed.

### 2.5 Experimental methods

#### 2.5.1 Acute oral toxicity study and selection of doses

The acute toxicity study of methanolic extract was determined as per the Organization of Economic Co-Operation and Development (OECD) guideline no. 423 (Acute toxic class method). It was observed that *Ailanthus excelsa* were safe up to 2000 mg/kg/oral not produced any toxic symptoms. Hence (100 and 150 and 200 mg /kg) was selected for this study.<sup>10</sup>

#### 2.5.2 Anti-implantation method

Female Wistar rats were divided in four groups and containing 6 rats in each groups, weighing between 150 and 200 g were selected for study and left overnight with male of proven fertile in the ratio of 3:1. The extract was administered to separated group rats at the dose level of 100, 150 and 200 mg/kg from day 1 to day 7 of pregnancy. Control animal received the vehicle (distilled water). The animals on day 10 of the pregnancy were laparotomised under ether anaesthesia and uteri were examined to determine the number of implantation sites.<sup>11</sup>

### 2.5.3 Abortifacient method

The extracts were evaluated in female albino rats for abortifacient activity described earlier by Pare *et al.*, and Khanna *et al.*, The vaginal smears of caged female rats of known fertility were monitored daily. Under a light microscope the unstained material was observed. The proportion among the cells used for the determination of the estrous cycle phases. Female rats were caged with males of proven fertility in the ratio of 3:1, in the evening of proestrus and examined the following day for the evidence of copulation. Rats exhibiting thick clumps of spermatozoa in their vaginal smears were separated and that day was designated as day one of pregnancy. These rats were randomly distributed into four groups, a control group and three experimental groups of six animals each. Group I received vehicle only and served as control. Groups II, III, and IV received methanolic extracts. On the 10th day of pregnancy the animals were laparotomized under light ether anesthesia using sterile conditions. The two horns of uteri were examined to determine the implantation sites. There after the abdominal incision was sutured surgically. Post operational care was taken to avoid any infection. The extract to be tested were then fed to operated pregnant rats, at the dose of 100 mg / kg, 150 mg/kg and 200 mg/kg body weight specified by an intragastric soft rubber catheter from day 8 to day 15 of pregnancy. On 21st day, animals were anaesthetized under diethyl ether anaesthesia and observed for number of litters and percentage of resorption. The animals were allowed to go to full term. After delivery the pups were counted and the anti-fertility activity of extract was evaluated and Litters were examined.<sup>11, 12</sup>

### 2.6 Statistical analysis

Results are expressed as Mean  $\pm$  SEM. One-way analysis of variance (ANOVA), was carried out to determine the significant differences. Mean of control group and test drug treated groups shows statistically significant difference at  $P < 0.0001$ ,  $P < 0.01$  The analysis of the results was carried out using GRAPH PAD Prism 5.0 package software.

## 3. RESULTS AND DISCUSSION

### 3.1 Toxicity study

*Ailanthus excelsa* treated animals did not show any changes in their behavioural pattern in acute oral toxicity study. There was no any significant difference body weight, food consumption as compared to vehicle treated group. Also, no gross pathological changes were seen. Thus, it was concluded that *Ailanthus excelsa* safe up to 2000 mg/kg so dose of 100, 150 and 200 mg/kg were selected for study.

### 3.2 The effect of *Ailanthus excelsa* on implantation

The stem bark of methanolic extract of *Ailanthus excelsa* was administered at the dose of 100, 150, 200 mg/kg for day 1<sup>st</sup> to day 7<sup>th</sup> of pregnancy and the animal were laprotomised on the day tenth of the pregnancy and number implantation sites were determined. Results indicate (Table:1) that there was significant ( $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.0001$ ) decrease in number of implants at the dose of 100, 150 and 200 mg/kg respectively was observed. However at the dose of 200 mg/kg there was 86.78 % anti-implantation effect was observed.

### 3.3 Effect on abortification

The stem bark of methanolic extract of *Ailanthus excelsa* was administered at the dose of 100, 150, 200 mg/kg for day 8<sup>th</sup> to day 14<sup>th</sup> of pregnancy and during the experiments animals were observed for vaginal bleeding on 21<sup>st</sup> day, the animal were laprotomised and observed for numbers of litters and percentage of resorption was calculated. Results indicate (Table: 2) that there was significant ( $**P < 0.01$ ,  $***P < 0.0001$ ) decrease in resorption at the dose of 150 and 200 mg/kg respectively was observed. However at the dose of 100 mg/kg there was no any significant decrease in abortification.

Ravichandran *et al* studied that hydroalcoholic extract of *Ailanthus excelsa* possess antifertility effect by showing anti-implantation (72%) and abortifacient action (56%) at the dose of (200 and 400mg/kg, p.o.). In our study we found that methanolic extract of *Ailanthus excelsa* stem bark shown anti-implantation and abortifacient action at the dose of level of 100,150,200 mg/kg/p.o.<sup>15</sup> Dhanasekaran *et al.*, also found that that Alcoholic extract of leaf and stem bark of *Ailanthus excelsa* at a dose of 250 mg equivalent of plant material/kg body weight, exhibited remarkably high anti-implantation and early abortifacient activities. Our results are also in agreement with this result.<sup>16</sup> Sanaula *et al.*, studied that leaf extract of *Ailanthus excelsa* shown anti-implantation activity.<sup>17</sup>

## 4. CONCLUSION

Plant and their products are integral part of medicine they are broad sources of modern synthetic drugs.<sup>13</sup> From survey of literature *Ailanthus excelsa* is a well-known traditional medicinal plant, possesses diverse biological activities and pharmacological activities reported by different researchers. However, its pharmacological and chemical bases are not well understood.<sup>14</sup>

Table 1: Effect of methanolic extract of *Ailanthus excelsa* stem bark in pregnant female wistar rats using anti-implantation method

Sl. No	Groups	Dose mg/kg	Post coital antifertility method			
			No of animal	No. of implants	No of litters	% anti-implantation
1.	Control	-	6	9.167±0.307	8.833±0.307	0
2.	AE-ME	100	6	6.500±0.341*	6.333±0.210*	28.30
3.	AE-ME	150	6	2.667±0.210**	2.333±0.210**	73.58
4.	AE-ME	200	6	1.333±0.210***	1.167±0.166***	86.78

Results are expressed as Mean ± S.E.M. (n=6), Evaluated by using one way ANOVA.  
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\* $P < 0.05$  when compared with corresponding value of normal control

\*\* $P < 0.01$  when compared with corresponding value of normal control.

\*\*\* $P < 0.0001$  when compared with corresponding value of normal control.

Table 2: Effect of methanolic extract of *Ailanthus excelsa* stem bark in pregnant female wistar rats using abortifacient method

Sl. No	Groups	Dose mg/kg	Abortifacient method			
			No of animal	No of implants	No. of Litters	% resorption
1.	Control	-	6	8.500±0.428	8.333±0.494	0
2.	AE-ME	100	6	6.167±0.307	5.500±0.341	33.99
3.	AE-ME	150	6	4.333±0.421**	3.833±0.401**	54.00
4.	AE-ME	200	6	2.500±0.428***	2.167±0.307***	73.99

Results are expressed as Mean ± S.E.M. (n=6), Evaluated by using one way ANOVA.

\*\* $P < 0.01$  when compared with corresponding value of normal control.

\*\*\* $P < 0.0001$  when compared with corresponding value of normal control.

In the present study we have evaluated for evaluation for anti-fertility activity of methanolic extract of bark of *Ailanthus excelsa*. In this study we found that treatment of rats with bark extract decreased the mean number of litters (Table 1 and 2) suggesting the antifertility effect of the extract. The number of litters appeared to decrease more with higher dose of treatment, which may suggest dose dependant antifertility effect. Results of the study (Table 1 and 2) indicate that in anti-implantation method number of implants significantly decreased. It was also clear from the result that the extract is effective in inhibition of post-coital contraception. It was also indicated that it plant stem bark extract was shown dose dependent anti-fertility action. In another study it was also clearly reveal stem bark extract shown significant abortification action.

Results of the above study indicate that different plant parts and different extracts of *Ailanthus excelsa* possesses anti-fertility action. From this study it can be concluded that methanolic extracts of *Ailanthus excelsa* stem bark extract has anti-fertility effect in wistar rat model. This was mediated via abortification and anti-implantation. Thus, lending support to its folkloric use in the pregnancy for safe treatment.

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