ANTIFERTILITY ACTIVITY OF *MELIA AZEDARACH* LINN (MELIACEAE) IN FEMALE WISTAR RATS

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Summary

Rapid rise in population has caused serious problem in economic growth and human development. Family planning has been promoted through several methods of contraception, but due to serious adverse effects, such as hormonal imbalance, hypertension, and increased risk of cancer and weight gain, the search for new antifertility molecule with minimum side effects continues. Hydroalcoholic extract of *Melia azedarach* Linn roots were evaluated for antiimplantation, estrogenic /antiestrogenic and progestational/antiprogestational activity. It was found that the extract has very significant antiimplantation and antiprogestational activity and devoid of estrogenic/antiestrogenic activity.

Key words: *Melia azedarach* Linn, antifertility, antiimplantation, estrogenic, progestational.

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Introduction

Global search for antifertility agents is continued to tackle the problem of population explosion that may lead to economic and health impact on the family in particular and the society in general especially in developing countries like India where the population growth is very high. Although contraceptives containing estrogen and progesterone are effective and popular, the risks associated to the drugs have triggered the need to develop suitable product search from indigenous medicinal plants that could effectively be used in the place of pills[1,2].

Melia azedarach Linn, commonly known as mahanimba belongs to family Meliaceae. It is large evergreen tree found throughout India. In folk medicine, a decoction of leaves is regarded as astringent and stomachic; a poultice of flowers is applied to eruptive skin diseases, and for killing lice; an infuson of bark is given in ascariasis; also as a blood-purifying aid[3,4,5]. The pharmacological studies carried out by several workers reported the analgesic[6], immunomodulatory[7], antibacterial[8], antiviral[9], insecticidal[10] and cytotoxic activity [11].

Several workers reported the antifertility activity of differents parts of this plant. In one study, ethanolic leaf extract of *Melia azedarach* were investigated for antifertility activity on male rats in oral dose of 100mg/kg daily for 21 days. There was abolition of libido in 100% males [12]. Another worker reported that there was significant decrease in the number of normal follicles in ovaries of rat, if polar and nonpolar fractions, of *Melia azedarach* Linn. Seed extract was administered at 24mg/kg for 18 days [13]. Ethanolic extract of *Melia azedarach* intercepted pregnancy in 60% and 75% adult female rats[14]. The motility of rat and mice spermatozoa was inhibited with various concentrations of petroleum ether fractions of *Melia azedarach* seed at different time intervals ranging from 20 seconds to 240 seconds as compared to

control. The effect was dose dependent and complete spermatozoa immobilization was seen with 10 and 25 mg concentrations tested for 240-20 seconds, respectively [15]. So the aim of present study is to evaluate the antifertility activity of *Melia azedarach* roots with the mechanism of antifertility activity.

Methods

Plant collection and preparation of extract:

Fresh roots of the *Melia azedarach* were collected from forest research farm, Banki-Sisarama, District-Udaipur, (Rajasthan) in the month of Feb. The plant was authenticated by Dr. S. S. Katewa, Dept. of Botany, College of Science, MLSU, Udaipur. Roots were dried in shade, moderately grinded and macerated with hydro alcoholic solvent (70:30) for 7 days with intermittent shaking. On 8th day, the macerate was filtered through muslin cloth; solvent was evaporated at room temperature and lyophilized in lyophilizer (Step, origin electric, Lonavala) then freeze-dried (Freeze dryer, Allied Frost) to provide dry hydro alcoholic extract of *Melia azedarach* roots (HEMAR). The yield was found to be 10%.

***Animals*:**

Female Wistar rats were used for the study. Mature females, weighing between 150-200 g were used to evaluate antiimplantation activity and progestational/antiprogestational activity. Immature female (20-23 days old), weighing between 50-60 g were used to evaluate estrogenic activity. Institution animal ethic committee approved all experimental procedures. All the animals were maintained under standard husbandry conditions with food (Chakan mill, Sangali, Maharashtra) and water ad libitum.

***Acute oral toxicity*:**
It was determined using OECD/OCDE guideline 425 [16], main test was performed and LD50 was found to be 1030mg/kg.

***Antiimplantation activity*:**
For antiimplantation activity, we selected $1/10^{th}$ and $1/20^{th}$ dose of LD_{50}. Vaginal smear from each rat were monitored daily, those rats showing regular estrus cycle were included in study. They were left overnight with proven fertile male in the ratio of 2:1 (female: male), in proestrous phase and examined the following morning for evidence of copulation. Those rats, which showed thick clumps of spermatozoa in their vaginal smear were separated for the experiment and that day was designated as day 1 of pregnancy and they were divided into three groups containing six rats in each group. The first group was control group received vehicle (1% Carboxy Methyl Cellulose), second and third groups were drug treated, received HEMAR at the dose of 50mg/kg and 100mg/kg respectively. All the treatment were given orally from 1 to 7 days of pregnancy, then laparotomy under light ether anesthesia and semi sterile condition was performed on day 10^{th} of pegnanacy [17,18].

The uteri were examined for implantation sites (I) and total number of corpora lutea (CL) on both the ovaries was counted. The abdominal wound was sutured and animals were allowed to go to term. After delivery, the litters (L) were counted. Percent Preimplantation loss (CL-I/CL×100), percent postimplantation loss (I-L/I×100) and percent antiimplantation activity (CL-L/CL×100) were calculated [19].

Estrogenic activity/ antiestrogenic activity:

Immature female albino wistar rats were ovariectimised [20] under light ether anaethesia and semi sterile conditions. They were divided into six groups, containing six rats in each.

Group I	received 1% CMC served as control
Group II	received Estradiol Valerate 0.1 μg/rat/day s.c. served as standard
Group III	received HEMAR 50 mg/kg bw
Group IV	received HEMAR 100 mg/kg bw
Group V	received Estradiol Valerate 0.1 μg/rat/day, s.c. + HEMAR 50 mg/kg bw
Group VI	received Estradiol Valerate 0.1 μg/rat/day, s.c. + HEMAR 100 mg/kg bw

All the treatment was given for seven days. On eighth day, premature opening of vagina and vaginal cornification was observed. All the rats were sacrificed by decapitation; the uteri dissected out, surrounding tissues were removed and weight of uterus (with fluid) was taken immediately, then uteri were slightly pressed between the folds of filter paper and weight (without fluid) was taken [21,22]. One uterine horn was stored in deep freeze for glycogen estimation [23,24,25] and another horn was fixed in Bouin's fluid and embedded in paraffin wax for histological observations.

The diameter of uterus, thickness of myometrium, endometrium and height of epithelial cells were measured using trinocular optical microscope (Labomed, Ambala).

Progestational / Antiprogestational Activity:

Female wistar rats with regular oestrous cycle were selected in study. They were inseminated by placing them with male rats overnight in the ratio of 2:1 (female: male). On 8th day of pregnancy females were ovariectomized, if found pregnant upon examination of the uterus. All the animals were divided in six group containing six rats in each.

Group I	received Estradiol Valerate 0.1 µg/rat/day s.c.+ 1% Carboxy Methyl Cellulose served as control
Group II	received Estradiol Valerate 0.1µg/rat/day s.c. + Progesterone 3 mg/kg/day, s.c. served as reference standard.
Group III	received Estradiol Valerate 0.1µg/rat/day + HEMAR 50 mg/kg bw
Group IV	received Estradiol Valerate 0.1µg/rat/day + HEMAR 100 mg/kg bw
Group V	received Estradiol Valerate 0.1µg/rat/day + Progesterone 3 mg/kg/day + HEMAR 50 mg/kg bw
Group VI	received Estradiol Valerate 0.1µg/rat/day + Progesterone 3 mg/kg/day + HEMAR 100 mg/kg bw

All the drugs were administered once daily, immediately after ovariectomy from eighth to 20th day of pregnancy. On 21st day, the animals were autopsied and the presence or absence of live embryos was recorded [20].

***Statistical Analysis*:**

The data was analysed by using one-way ANOVA followed by Tukey multiple comparision test. A p value <0.05was considered to be significant.

Results

***Antiimplantation activity*:**

In the present study, hydro alcoholic extract of *Melia azedarach* roots were tested for antiimplantation activity. At the dose of 50mg/kg, extract exhibited more postimplantation loss than pre implantation loss and 51.93 % antiimplantation activity was observed. At 100mg/kg, extract exhibited highly

significant ($p<0.001$) 83.33 % antiimplantation activity when compared with the control group and also significant ($p<0.05$) when compared to the low dose of the extract (Table 1) The post implantation loss was found to be more as compared to the pre implantation loss and significant when compared with the control group ($p<0.05$).

Table 1: Anti-implantation activity of hydroalcoholic extract of roots of *Melia azedarach*

Group	**Treatment**	**Percent Pre implantation loss**	**Percent Post implantation loss**	**Percent Anti-implantation activity**
I (Control)	Vehicle (1 % CMC) p.o.	6.75±3.70	4.69±2.10	11.06±4.20
II (Drug treated)	HEMAR (50mg/kg) p.o.	24.57±3.11	36.75±3.62	51.93±4.19**
III (Drug treated)	HEMAR (100mg/kg) p.o.	39.17±19.24	48.48±18.28*	83.33±10.54***$^{+}$

Data are analyzed using one-way ANOVA followed by Tukey multiple comparison test, a $p<0.05$ and $P<0.001$ is considered to be significant. Values are Mean± SEM. N=6. *$p<0.05$, when compared with control group **$p<0.001$ when compared with control group *** $p<0.001$, when compared with control group, $^{+}p<0.05$, when compared with the group 2nd

Estrogenic/ antiestrogenic activity:

Estrogen treated group showed highly significant ($p<0.001$) increase in uterine weight and glycogen content of uterus. All the animals showed premature opening of vagina and cornified cells in vaginal smear.

Extract treated animals showed insignificant change in uterine weight and glycogen content as compared to control group. Premature opening of vagina and cornification of vaginal cells were also absent (Table 2). Co- administration of estradiol valerate and extract did not show any significant changes in uterine weight and glycogen content as compared to estradiol valerate treated group. Vaginal opening and cornification were also not reduced as compared to estradiol valerate treated group.

Histological studies:

Estrogen treated group, showed significant increase in uterine diameter, thickness of myometrium, endometrium and height of epithelial cells as compared with control and drug treated groups. In drug treated groups there was insignificant changes in uterus. When estradiol was co- administered with extract (group V & VI) then no significant changes was observed in diameter of uterus, thickness of myometrium and endometrium as compared to estradiol treated group (Table 3).

Estradiol treated uterus showed very wide uterine lumen with numerous pits and folds in luminal epithelium. There was secretory stroma with extensive vascular plexus and well defined endometrium, myometrium with circular and longitudinal muscle layer. Drug treated groups and Co-administration of extract with standard estrogen, did not affect the histoarchitecture of uterus as compared to control group and estradiol valerate treated group respectively. This showed that extract have neither estrogenic nor antiestrogenic activity.

Table 2: Estrogenic/ Antiestrogenic activity of hydroalcoholic extract of roots of *Melia azedarach*

Group	Treatment	Uterine weight (with fluid) (mg/100g bw)	Uterine weight, (without fluid) (mg/100g bw)	Premature opening of vagina	Vaginal cornification	Glycogen content (µg/100mg uterus)
I	Vehicle (1%CMC) p.o.	41.43±1.73	38.96±1.92	0/6	-	42.40±6.15
II	EV (0.1µg/rat/day) s.c.	125.20±8.67***	98.96±6.62***	6/6	Cornified cells	74.39±4.82***
III	HEMAR (50mg/kg) p.o.	48.94±3.53	41.67±2.94	0/6	-	48.26±1.99
IV	HEMAR (100mg/kg) p.o.	53.98±3.87	48.45±4.08	0/6	-	40.26±2.45
V	EV + HEMAR (50mg/kg) p.o.	124.11±2.75	99.28±3.18	6/6	Cornified cells	72.30±2.79
VI	EV + HEMAR (100mg/kg) p.o	127.18±2.21	103.53±3.08	6/6	Cornified cells	67.65±2.67

EV-Estradiol Valerate; Data are analyzed using one-way ANOVA followed by Tukey multiple comparison test, a $P<0.001$ is considered to be significant. Values are Mean± SEM. N=6. ***$p<0.001$, when compared to control group.

.Table 3: Effect of of hydroalcoholic extract of roots of Melia azedarach on histology of rat uterus

Group	Treatment	Diameter of uterus (µm)	Thickness of myometrium (µm)	Thickness of endometrium (µm)	Height of endometrial epithelial cells (µm)
I	Vehicle (1%CMC) p.o.	228.38±32.32	41.86±6.78	52.21±11.30	3.41±0.32
II	EV (0.1µg/rat/day) s.c.	430.97±31.8***	80.29±5.74***	162.35±25.18***	10.62±0.77***
III	HEMAR (50mg/kg) p.o.	190.36±16.75	34.60±4.24	46.94±1.19	6.31±1.42
IV	HEMAR (100mg/kg) p.o.	231.64±36.35	33.59±5.95	74.39±15.12	4.34±0.65
V	EV + HEMAR (50mg/kg) p.o.	407.33±10.40	77.43±3.65	150.66±3.30	11.16±1.19
VI	EV + HEMAR (100mg/kg) p.o	426.16±11.25	84.99±2.92	164.83±2.49	11.73±0.79

EV-Estradiol Valerate

Data are analyzed using one-way ANOVA followed by Tukey multiple comparison test. Values are Mean± SEM. N=6 *** $p<0.001$, when compared with control group

Progestational/ Antiprogestational Activity:

No viable fetus were observed in vehicle treated group on 21st day of pregnancy means the animals had complete abortion and pregnancy was not maintained, but conjoint administration of progesterone with estradiol (reference standard group) showed viable fetus (9.5±0.42) when compared to control group and the net success index of pregnancy maintenance was highly significant ($p<0.001$).

when extract and estradiol was administered without any standard progesterone (group III & IV), there was complete abortion and no viable fetus were observed but extract along with standard progesterone and estradiol (group V & VI) showed significant decrease in mean number of viable fetus as compared to reference standard group in dose dependent manner. Net success index of pregnancy was also reduced (Table 4).

Discussion

In rat, blastocyst formation takes place within 5 days and by the end of 5th day of pregnancy, implantation is completed after that there is growth and development of the embryo[26]. In majority of animals implantation takes place at a fixed interval of time after ovulation when the corpus luteum is fully formed the hormonal control ensure maximum endometrial sensitivity when the mature blastocyst is present in uterus. A reaction takes place between the trophoblast and the uterine epithelium so that the endometrium is stimulated to undergo a chain of reactions leading to the formation of the placenta. The response is dependent on progesterone and if the supply of this hormone is cut off the reaction stops and regression takes place, so inhibition of progesterone synthesis or a blockade of receptor binding will result in the failure of blastocyst implantation and interruption of early pregnancy and the maintenance of pregnancy in all phases [27,28].

Table 4: Progestational/ Antiprogestational activity of hydroalcoholic extract of roots of *Melia azedarach*

Group	**Treatment**	**Mean viable fetus**	**Percent net success index**
I	Vehicle (1%CMC) p.o.+ EV(0.1µg/rat/day) s.c.	0	0
II	P(3mg/kg/day) + EV(0.1µg/rat/day) s.c.	9.5+0.42***	95 %
III	HEMAR (50mg/kg) p.o. + EV(0.1µg/rat/day) s.c.	0	0
IV	HEMAR(100mg/kg) p.o. + EV(0.1µg/rat/day) s.c.	0	0
V	HEMAR (50mg/kg) p.o.+ EV(0.1µg/rat/day) s.c.+ P(3mg/kg/day)	$5.83+0.47^{+++}$	58.3%
VI	HEMAR(100mg/kg) p.o. EV(0.1µg/rat/day) s.c.+ P(3mg/kg/day)	$4.16+0.42^{+++\#\#}$	41.6%

EV-Estradiol Valerate, P-progesterone

Data are analyzed using one-way ANOVA followed by Tukey multiple comparison test, a $p<0.05$ and $P<0.001$ is considered to be significant. Values are Mean± SEM. N=6. ***$p<0.001$, when compared with control group, $^{+++}p<0.001$ when compared with group 2nd, $^{\#\#}p<0.01$, when compared with the group V.

Thus the rat, priming with progesterone 24 h before the nidatory ostrogen surge, is compatible with implantation and subsequent embryonic development. Normally, estrogen and progesterone act synergistically upon the uterine endometrium to prepare it for nidation. Under physiological conditions, progesterone is usually secreted after a period of estrogen secretion, so that it can normally act on an estrogen-primed uterus.
It is therefore assumed that a substance, which can impair the synthesis, secretion and function of ovarian steroids, may block the implantation process by hindering the development of oocycte, graffian follicle as well as the endometrial epithelium [29].

In our study we administered the extract for 1-7 days of postcoitum, results showed that post implantation loss was more than pre implantation loss; means the drug mainly affect the development and growth of the uterus or the period of organogenesis. In rat ovarectomy performed during the first half of pregnancy terminates gestation, but the operation performed during the second half of pregnancy does not always result in abortion, because of the capacity of placenta to produce progestin and estrogen. In the pregnancy maintenance test, extract showed reduction in viable fetus when conjointly administered with progesterone as compared to reference standard, hence showed antiprogestational activity which may be acting either as inhibitor in the biosynthesis of progesterone or as blocker in the receptor binding, finally interfere with the maintenance of pregnancy. These findings strongly indicate the late abortifacient activity of the extract. Although, extract also affects the early stages of implantation by affecting the uterine walls for nidation[30,31].

In present study, extract at 100mg/kg body wt., shows highly potent antiimplantation activity (83.33%) and it might be due to antiprogestational activity.

References

1. Gebrie E, Makonnen E, Debella A, Zerihun L. Phytochemical screening and pharmacological evaluations for the antfertility effect of the methanolic root extract of *Rumex steudelii*. J Ethnopharmacol 2005; 96(1-2): 139-143.
2. Ghosh K, Bhattacharya TK. Preliminary study on the antiimplantation activity of compounds from the extracts of seeds of *Thespesia populnea*. Indian J Pharmacol 2004; 36: 288-291.
3. Kapoor LD. Handbook of Ayurvedic Medicinal plants. Ist ed. London: CRC Press; 2005: p.225-226.
4. Khare CP. Encyclopedia of Indian Medicinal Plants. Ist ed. Germany: Springr; 2004: p.305-306
5. Nadkarni KM, Nadkarni AK. Indian Materia Medica. 3rd ed. vol. I, Bombay: Popular Prakashan; 1954: p.784-785.
6. Vohra SB, Dandia PC. Herbal analgesic drugs. Fitoterapia 1992; 63(3):195-207.
7. Benencia F, Courreges MC, Coto C, Coulombie FC. Immunomodulatory activities of *Melia azedarach* L. leaf extracts on human monocytes. J Herb Spices Med Plants 1997; 5(3): 7-13.
8. Sallem R, Ahmed SI, Shamim SM, Faizi S, Siddiqui BS. Antibacterial effect of *Melia azedarach* flowers on rabbits. Phytotherapy Res 2002; 16(8): 762-764.
9. Pifarre MP, Berra A, Coto CE, Alche LE. Therapeutic action of meliacine, a plant derived antiviral, on HSV-induced ocular disease in mice. Exp Eye Res 2002; 75: 327-334.
10. Rani M, Suhag P, Kumar R, Singh R, Kalidhar SB. Chemical component and biological efficacy of *Melia azedarach* stems. J Med Aromatic Plant Sci 1999; 21: 1043-1047.
11. Itokawa H, Qiao ZS. Cytotoic limonoids and tetranortriterpinoids from *Melia azedarach*. Chem Pharm Bull 1995; 43(7): 1171-1175.

12. Choudhary DN, Singh JN, Verma SK, Singh BP. antifertility effects of
leaf extracts of some plants in male rats. Indian J Exp Biol 1990; 28: 714-716.
13. Roop JK. Extracts of Azadirachta indica and *Melia azedarach* seeds inhibit
follculogenesis in albino rats. Braz J Med Biol Res 2005; 38(6): 943-947.
14. Keshri G, Lakshmi V, Singh MM. Pregnancy interceptive activity of *Melia azedarach* Linn. In adult female Sprague-Dawley rats. Contaception 2003; 68: 303- 306 .
15. Sharanabasappa A, Saraswati BP. Spermicidal activity of Melia azedarach: In vitro and in vivo studies. Chem Biol Interface: Synergistic New Frontiers 2004; 25(2): 21-26.
16. OECD guideline for testing of chemicals, 425. adopted: 17th December 2001
17. Khanna U, Chaudhary RR. Antifertility screening of plants. Part I. Investigation of Butea monosperma (Lam) Kutze. Indian J Med Res 1968; 56: 1575-1578.
18. Koneri R, Saraswati CD, Balaraman R, Aheesha EA, Antiimplantation activity of the ethanolic root extract of Momordica cymbalaria Fenzl in rats. Indian J Pharmacol 2007; 39: 90-96.
19. Agrawal SS, Arvinda S. Anti-implantation activity of some H2 receptor blockers. Indian J Pharmacol 1995; 27: 40-42.
20. Vogel HG, Vogel WH. Drug Discovery and Evaluation Pharmacological Assays. 2nd ed. Berlin: Heidelberg Publications Springer Verlag; 1997: p. 1167.
21. Nwafor PA, Okwuasaba FK, Onoruvwe OO. Contraceptive and Non Estrogenic effects of methanolic extract of *Asparagus pubescens* root in experimental animals. J Ethnopharmacol 1998; 62: 117-122.
22. Vasudeva N, Sharma SK. Post-coital antifertility activity of *Achyranthes aspera* Linn. Root. J Ethnopharmacol 2006; 107: 179-181.
23. Wallas O. Effect of oestrogen on the glycogen content of the rat terus. Acta Endocrinol 1952; 10: 175-192.
24. Kemp A, Adrienne JM. A colorimetric micromethod for determination of glycogen in tissue. Biochem J 1954; 56: 646-648.

25. Tewari PV, Mapa HC, Chaturvedi C. Experimental study on estrogenic activity of certain indigenous drugs. J Res Ind Med Yoga & Homeo 1976: 11(4): 7-12.
26. Kulkarni TR, Kothekar MA, Mateenuddin M. Study of anti-fertility effect
of lemon seeds (*Citrus limonum*) in female albino mice. Indian J Physiol Pharmacol 2005; 49(3): 305-312.
27. Finn Ca, Martin L. The control of implantation. J Reprd Fert 1974; 39: 195-206.
28. Shivalingappa H. Sataynarayan ND, Purohit MG. Antiimplantation and pregnancy interruption efficacy of Rivea hypocrateriformis in the rat. J Ethnopharmacol 2001; 74: 245-249.
29. Dickmann Z. Implantation in the rat: progesterone priming requirements. J. Endocrinol 1972; 53: 327-328.
30. Sarma HN, Mahanta HC. Effects of composite root extract on histological structures of graffian follicle and endometrial epithelium in albino rat. Contaception 2000; 61: 335-339.
31. Dhanwad R, Purohit MG, Patil S, Patil SB, Satyanarayan ND, Antiimplantation activity of ethanolic extract of *Cardiospermum helicacabum* linn.(Sapindaceae) in albino rats. Indian Drugs 2005; 42(11): 726-730.