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Effect of *Melia azedarach* Linn. Seed Extract on Estrous Cycle and Reproductive Performance in Female Rats

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Abstract: The present investigation was aimed to study the effect of hexane fraction of seed extract of *Melia azedarach* Linn. (Family: Meliaceae) on estrous cycle, fertility and implantation in female albino rats @1mg, 3mg, 6mg, 12mg, 24mg/kg body weight. Fertility index and average number of embryos were considerably reduced in the adult cycling rats after 18 days of treatment @6mg, 12mg and 24mg/kg b.wt. However, the occurrence of various stages of estrous cycle was not disturbed significantly in the treated rats. Pre-implantation, post-implantation and total prenatal mortality was significantly increased in the treatment group (24mg/kg b.wt.) as compared to control during D1-D7pc and D7-D18pc treatments. The average gestation period was increased by about 9.5 days, where as litter size, live birth index and average life span of young ones @24mg/kg b.wt. decreased considerably. Thus, the results indicate that the hexane fraction of *M. azedarach* can be used as a fertility regulating agent as it possess antifertility, anti-implantation / abortive activity that may be attributed to its antiestrogenic property resulting in hormonal imbalance creating an unfavorable uterine environment for nidation.
Keywords: Estrous cycle, Antifertility, *Melia azedarach*, Anti-implantation, Female rats.

Introduction

Rodents because of their high rate of reproduction impose great loss to the agricultural produce in India[1] by inflicting 5-10% damage in different crops. Therefore they are considered to be the most destructive vertebrate pests. Moreover, population all over the world has increased during the past years. To meet the demands of ever increasing human population and to prevent the spread of various diseases and economic loss, it becomes imperative to develop and apply various types of rodent management strategies. The indiscriminate use of persistent and toxic rodenticides has created serious problems like rodenticide resistance and environmental pollution. So, a promising alternative for rodent control[2] is the use of biologically active fertility regulating botanical substances which are considered ecologically and environmentally safe and interfere with the natural patterns of reproduction and can meet the needs of National Rodent Control[3].

Extracts of many plants are known to possess abortifacient activity in various rodents[3-5]. Hydroalcoholioc extract of *Melia azedarach* roots[6] and *Melia azedarach* seed extract[7] have been evaluated for antiimplantation , estrogenic / antiestrogenic and progestational / antiprogestational activity. *Azadirachta indica* , a member from the same family Meliaceae as *M. azedarach* has been reported to have antifertility and antiimplantation activity from geographically distant areas[8-13].

Present authors also investigated the effect of *A. indica* and *M. azedarach* seed extracts on folliculogenesis in albino rats[14]. But there is no evidence of the effects of fractions of *Melia* seed extract on the

estrous cycle and post-coital efficacy in rats. So, the present study proposes to evaluate the effects of hexane fraction of *Melia* (Family: Meliaceae) seed extract on the estrous cycle and post-coital efficacy in albino rats.

Materials and Methods

Preparation of Plant Extract

Ripe drupes of *M. azedarach* (dharek) were collected from trees growing at Punjab agricultural university, Ludhiana in the months of Feb-March. Seeds of shade dried matured drupes were powered (100gm) and hexane fraction was extracted at 35°C [15,16] which was considered as non-polar fraction. This fraction was then dissolved in olive oil to prepare doses on per kg body weight basis. The mode of administration was oral.

Experimental Design

Mature cyclic female albino rats weighing 135±10 gm bred in the Small Animal Colony of Punjab Agricultural University, Ludhiana were used for the present investigation. The animals were provided standard diet (Hindustan Lever Pvt. Ltd.) and water *ad libitum.* All the rats were caged in standard laboratory conditions (temperature 22±3°C and 14 hour light / 10 hour dark cycle). The stage of estrous cycle of each rat was determined by taking vaginal smears[17] (using 0.9%NaCl, w/v) daily between 9.00am to 10.00 am. Rats showing at least three regular four day cycles and in diestrous phase of estrous cycle were selected for the study.

(A) Preliminary screening for antifertility activity

Mature regular cyclic female rats were divided into six groups (7rats/ group) .
Group I: Olive oil (vehicle)
Group II, III, IV, V, VI: Hexane fraction @1mg, 3mg, 6mg, 12mg, 24mg/kg body weight /day, respectively.
The different doses of extract and vehicle were orally administered for 18 days.

The treated female rats were then paired with the male rats of proven fertility in the ratio 2:1 (2 females: 1 male) for 7days. On the 8th day, male rats were separated. Thirteen days after the separation, the rats in all the groups were autopsied for determination of fertility index, average number of embryos, pre-/post- implantation mortality and total prenatal mortality, using the formulas:

Fertility Index =

(Total number of females pregnant/ Total number of females mated) x100
Pre-implantation Mortality = (A – B/A) x 100 = x%
Post-implantation Mortality (resorptions) = (B – C/ B) x 100 = y%
Total Prenatal Mortality= (A – C/ A) x 100 = Z%
Where, A = Total number of corpora lutea
B = Total implanted embryos
C = Number of normal embryos

Depending upon the results of antifertility activity the dose was selected to determine its effect on the estrous cycle and post-coital efficacy.

(B) Estrous cycle

It was determined as per described in experimental design.
The rats were divided into two groups (6 rats / group).
Group I: Olive oil orally administered for 18 days.
Group II: 24 mg /kg body weight / day of hexane fraction of *Melia* seed extract orally administered for 18 days.

(C) Anti-implantation and abortifacient effects

Adult cyclic female albino rats at proestrous phase of estrous cycle were selected and allowed to mate with the male rats of proven fertility in the ratio 2:1(2females:1male). Next morning presence of spermatozoa in the vaginal smear provided evidence of copulation. That day was designated as day 1 post-coitum (D1 pc).The pregnant rats were then divided into six groups (7 rats/ group).

Group I: Olive oil (vehicle) from D1-D7pc

Group II: Hexane fraction @24mg/kg body weight for D1-D7 pc
The rats (Group I & Group II) were sacrificed on day 11 post-coitum.
Group III: Olive oil (vehicle) from D7-D18 pc
Group IV: Hexane fraction @24mg/kg body weight for D7-D18 pc
The rats (Group III & Group IV) were sacrificed on day 19 post-coitum.

(D) Determination of effect on gestation period and Live Birth Index:

Group V: Olive oil (vehicle) from D7-D18 pc
Group VI: Hexane fraction @24mg/kg body weight for D7-D18 pc
Thereafter, the animals were daily observed for the day of parturition, live and dead fetuses born and abnormalities in young ones. The conceptus was classified as live if fetal movements could be elicited.
The data were analysed as per standard procedures of completely randomized block design[18].

Results

Female rats administered different doses of hexane fraction of *Melia* seed extract for 18 days (Table 1) exhibited decrease in the fertility index, the decrease being more (50%) at doses 6mg, 12mg and 24mg when compared with the control (100%) that received olive oil as vehicle. Pre-implantation mortality (37.50%) and total prenatal mortality (43.75%) was highest @ 12mg dose and post-implantation mortality was highest (16.67%) @ 24mg dose as compared to control where the mortality rate was found to be zero per cent.

Table 1: Effect of Administration of Hexane Fraction of *Melia Azedarach* Seed Extract on Fertility Index in Cyclic Albino Rats

Group	Treatment (mg/kgb.wt.)	Fertility Index (%)	Average number of embryos	Pre-implantation Mortality (%)	Post-implantation Mortality (%)	Total prenatal Mortality (%)
I Control	-		10.0	0	0	0
II	1.0	100	10.0	0	0	0
III	3.0	100	7.50	0	0	0
IV	6.0	50	8.50	0	0	0
V	12.0	50	4.50	37.50	10.00	43.75
VI	24.0	50	5.00	10.00	16.67	25.00

The occurrence of various stages of the estrous cycle was not significantly disturbed in the rats administered hexane fraction @ 24mg/kg body weight / day for 18 days (Table 2) , yet overlapping of two or more successive stages i.e., mixed type of smear was observed. Moreover, vaginal smear showed distorted epithelial cells in the proestrous phase and debris along with pale yellow fluid in proestrous, metestrous and diestrous phases.

Table 2: Effect of Administration of Hexane Fraction of *Melia Azedarach* Seed Extract on the Percent Occurrence of Various Pahses of Estrous Cycle

Group	Diestrous	Proestrous	Estrous	Metestrous
Control (olive oil)	30.79±0.40	26.32±0.88	22.37±0.44	21.58±1.02
Hexane Fraction @24mg/kg b.wt.	29.74±1.31	25.26±1.67	25.66±2.11	19.34±1.24
CD (P = 0.05)	NS	NS	NS	NS

Values are mean±SE NS- Non significant

There was a significant reduction in the average number of embryos in rats administered with hexane fraction @ 24mg/ kg body weight (5.80) for D1-D7 pc as compared to control (10.00) (Table 3). Pre-implantation mortality was significantly high during D1-D7 pc treatment (32.56%) as compared to D7-D18 pc

treatment (zero per cent) and control (zero per cent). Significant increase in post-implantation mortality was observed during D7-D18 pc treatment (14.29%) as compared to D1-D7 pc treatment (zero per cent) and control (zero per cent).Total prenatal mortality was also found to be significantly high at D1-D7 pc treatment (32.56%) as compared to D7-D18 pc treatment (14.29%). So, it can be said that hexane fraction considerably increases the mortality percentage during D1-D7 pc and D7-D18 pc treatment periods.

The average gestation period increased by about 9.5 days (29.50 days) in hexane fraction treated rats as compared to control where it was about 20 days (Table 4). The litter size, live birth index and average life span of young ones decreased to a great extent in the treatment group as compared to control.

No malformations or developmental variations indicative of a treatment were observed.

Table 3: Effect of Hexane Fraction of *Melia Azedarach* Seed Extract on Implantation in Female Rats When Administered From D1-D7 And D7-718 Post-Coitum @24mg/kg b.wt..

Group	Duration of Treatment (pc)	Average number of embryos	Pre-implantation Mortality (%)	Post-implantation Mortality (%)	Total prenatal Mortality (%)
I Control	D1-D7	10.00±0.36	0	0	0
II	D1-D7	5.80±0.71	32.56±2.12*	0	32.56±1.40*
III Control	D7-D18	10.00±0.36	0	0	0
IV	D7-D18	6.00±0.17	0	14.29±1.29*	14.29±2.00*

Values are mean±SE
Level of significance *p< 0.05 when compared to control

Table 4: Effect of Administration of Hexane Fraction of *Melia Azedarach* Seed Extract (D7-D18 Pc) on Gestation Period, Litter Size, Live Birth Index and Life Span of Young ones in Albino Rats

Group	Average Gestation Period (Days)	Litter Size	Live Birth Index (%)*	Average Life Span of Young ones
V Control	20.00	10.00	100.00	survived for 3.5-4 years
VI (24 mg/kg b.wt)	29.50	3.25	76.92*	3.0 Days

*Live birth index= (number of live fetuses born/total number of fetuses born) x100
*P < 0.05 when compared to control

Discussion

The data recorded on reduction in fertility index and average number of embryos in adult cyclic rats after 18 days of treatment with hexane fraction of *Melia* seed extract during the present investigation supported the findings that *Melia* seed extract administered @1.0mg and 5.0mg/kg body weight/day for 18 days resulted in 100% fertility reduction[7]. Vaginal administration of neem oil before mating[9] and unilateral administration of neem oil in the uterus of female rats[19] resulted in reduced fertility. Reduction in the number of viable fetuses was also observed in rats after administration of various plant extracts[20-22]. Reproductive toxicity was found to be the main factor in reducing the number of viable fetuses[22]. Increase in the implantation mortality when the duration of treatment was 18 days in adult cyclic rats in the present investigation may also be attributed to the immune cellular response in the uterus leading to the blockage of implantation[7, 19]. Pre-implantation mortality may also be due to the reduced vasculature permeability which is essential for normal implantation[23].

Non-significant variation in the occurrence of various stages of estrous cycle in the rats administered hexane fraction for 18 days depicts the non-estrogenic nature of the fraction. Results of study on neem (neem seed oil @ 2.0mg, 3.3mg and 4.6mg/kg body weight/day for 18 days) another member of Family Meliaceae,

also resulted in non-significant variation in estrous phase of estrous cycle [24] , indicating that neem seed oil is not typically estrogenic. A number of plants possessing anti estrogenic activity have been reported to interrupt pregnancy[25-30]. Presence of overlapping of two or more successive phases of estrous cycle in the present investigation might be an indicative of hormonal disturbances. Yakubu *et al* [31] reported that the presence of alkaloids and flavonoids in plant extract of *Cnidoscolous aconitifolius* reduced the concentration of luteinizing hormone, estradiol and follicle stimulating hormone which are necessary for follicle growth and ovulation and may impair fertility and conception in female rats.

The high rate of implantation losses (D1-D7 pc) may be due to direct / indirect effect on corpus luteum resulting in an inhibited synthesis and/or secretion of progesterone[32-34] that creates an imbalance in progesterone estrogen ratio[35] necessary for implantation. It has been reported that estrogen is an indispensable hormone for nidation and there is a surge for estrogen on day 4-day 5 after fertilization, which is essential for sensitization of the uterus for induction of decidualization[36]. So, the hexane fraction might be interfering with the production of estrogen, thus disturbing progesterone estrogen ratio and rendering uterus unfavorable for implantation. It has already been reported that neem oil does not have any estrogenic, antiestrogenic or progestational activity[37]. So, other causes for implantation loss may be the disruption of events which are pre-requisite for fertilization and an impairment in the production of cytokines[38,39], growth factors and various types of adhesion molecules either by the developing blastocyst or by the uterine epithelium around the site of implantation,[40-42] increase in the phagocyte activity and antigen presenting ability of macrophages that induce the production of interferon-gamma and leukocyte accumulation leading to embryonic damage and finally fertility [39,43].

Marked increase in mortality during D7-D18 pc treatment may be attributed to direct toxicity, fall in progesterone level or interference with the uterine utilization of progesterone[6, 44] i.e., disturbance of both uterine metabolism and direct / indirect effect on corpus luteum resulting in an inhibited synthesis and / or secretion of progesterone that might lead to abortions.

Increase in average gestation period and delay in parturition in the present study may be the result of inhibitory effects of hexane fraction on prostaglandin (PG) synthesis by pregnant rats[45]. No external malformations or abnormalities in the present study might be due to decrease in PG synthesis as increase in PG production of post implanted embryos may be associated with retardation and abnormalities during the gestation period[46].

In conclusion, the present study reveals that hexane fraction of *Melia azedarach* Linn can be used as a fertility regulating agent as it possess antifertility, anti-implantation and abortive activity that might probably be due to its antiestrogenic property resulting in hormonal imbalance creating an unfavorable uterine environment for nidation.

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