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Antifertility and Anti-Implantation Activity of Methanol Fraction of *Melia azedarach* Linn. Seed Extract in Female Albino Rats

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Abstract: ***To meet the demands of rapidly increasing human population and to prevent the economic loss, it becomes imperative to develop and apply various types of rodent management strategies, amongst which fertility regulating agents of plant origin that can interfere with the natural patterns of reproduction are considered to be the most valuable. Therefore, the present investigation was aimed to study the effect of methanol 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 treatment groups. However, the occurrence of various stages of estrous cycle was not disturbed significantly. Pre-implantation, post-implantation and total prenatal mortality was significantly increased during D1-D7pc and D7-D18pc @24mg/kg body weight as compared to control. The average gestation period was increased where as litter size, live birth index and average life span of young ones decreased considerably. Thus, the results indicate that the methanol 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 implantation and development.***

Keywords: Antifertility, Anti-implantation, Estrous cycle, Female rats, *Melia azedarach*

1. Introduction

Rodents are considered to be the most destructive vertebrate pests of agriculture produce in India as they cause 5-15% damage in different crops [1], [2]. 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 diseases, resistance and environmental contamination. So, a promising alternative for rodent control [3] is the use of inexpensive biologically active fertility regulating botanical substances which are not passed on to the next trophic level and interfere with the natural patterns of reproduction that can meet the needs of National Rodent Control programme [4].

Extracts of many plants are known to possess abortifacient activity in various rodents [5]. *Melia azedarach* (Family: Meliaceae) seed extract [6] and hydroalcoholic root extract [7] have been evaluated for antiimplantation , estrogenic / antiestrogenic and progestational / antiprogestational activity. *Azadirachta indica* , another member from the same family Meliaceae has also been reported to have antifertility and antiimplantation activity from geographically distant areas [8]-[13].

Present author 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 fertility in rats. So, the present study proposes to evaluate the effects of methanol fraction of *Melia* seed extract on the estrous cycle and post-coital efficacy in albino rats.

2. Materials and Methods

(1) 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 methanol fraction was extracted at 35°C [15],[16] which was considered as a polar fraction and the doses were made on per kg body weight basis. The mode of administration was oral.

(2) 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] daily between 9.00AM to 10.00AM. Rats showing at least three regular four day cycles and in diestrous stage of estrous cycle were selected for the study.

2.1 Preliminary screening for antifertility activity

Mature virgin regular cyclic female rats were divided into six groups (7rats/ group).
Group I received olive oil (vehicle)
Group II, III, IV, V, VI were administered with methanol 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.

2.2 Estrous cycle

It was determined as described in experimental design. The rats were divided into two groups (6 rats / group).

Group I was administered olive oil orally for 18 days.
Group II was administered 24 mg /kg body weight / day of methanol fraction of *Melia* seed extract orally for 18 days.

2.3 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 received olive oil (vehicle) from D1-D7pc
Group II received methanol fraction @24mg/kg body weight for D1-D7 pc
The rats (Group I & Group II) were sacrificed on day 11 post-coitum.
Group III received olive oil (vehicle) from D7-D18 pc
Group IV received methanol fraction @24mg/kg body weight for D7-D18 pc
The rats (Group III & Group IV) were sacrificed on day 19 post-coitum.

Determination of effect on gestation period and Live Birth Index:
Group V received olive oil (vehicle) from D7-D18 pc
Group VI received methanol 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 the 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].

3. Results

The fertility index was reduced considerably in female rats administered with methanol fraction of *Melia* seed extract @ 3mg, 6mg, 12mg and 24mg/kg bw for 18 days (Table 1), the reduction being more @ 24mg(66.67%) when compared with the control (100%) that received olive oil. Pre-implantation mortality (19.05%) and total prenatal mortality (19.05%) was highest @ 24mg dose as compared to control where the mortality rate was found to be zero per cent.

Table 1: Effect of administration of methanol fraction of M*elia 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	100.00	9.00	0	0	0
III	3.0	75.00	8.00	0	0	0
IV	6.0	75.00	8.50	0	0	0
V	12.0	75.00	7.67	0	0	0
VI	24.0	66.67	4.25	19.05	0	19.05

The occurrence of various stages of the estrous cycle was not significantly disturbed in the rats administered methanol fraction @ 24mg/kg body weight / day for 18 days (Table 2), yet presence of two or more successive stages was observed.

There was a significant reduction in the average number of embryos in rats administered methanol fraction @ 24mg/ kg body weight (6.50) for D1-D7 pc as compared to control (10.00) (Table 3). Pre-implantation mortality was significantly high during D1-D7 pc treatment (15.15%) and D7-D18 pc treatment (9.38%) as compared to the control (zero per cent). Significant increase in post-implantation mortality was observed during D1-D7 pc treatment (7.14%) as compared to D7-D18 pc treatment (zero per cent) and control (zero per cent). Total prenatal mortality was also found to be significantly high at D1-D7 pc (21.21%) and D7-D18 pc (9.38%) treatments as compared to control (zero percent). So, overall there was a considerable increase in the mortality percentage during D1-D7 pc and D7-D18 pc treatment periods.

Table 2: Effect of administration of methanol fraction of *Melia azedarach* seed extract on the percent occurrence of various stages of estrous cycle in cyclic albino rats

Group	*Diestrous (%)*	*Proestrous (%)*	*Estrous (%)*	*Metestrous (%)*
Control (olive oil)	30.79±0.40	26.32±0.88	22.37±0.44	21.58±1.02
Methanol Fraction @24mg/kg b.wt.	33.39±1.89	25.79±2.07	18.95±2.35	22.37±2.01
CD (P = 0.05)	NS	NS	NS	NS

Values are mean ± SE
NS- Non significant

Table 3: Effect of methanol fraction of *Melia azedarach* seed extract on implantation in female albino rats

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 (24mg/kg b.wt.)	D1-D7	6.50±1.26*	15.15±2.22*****	7.14±1.55*	21.21±0.83*****
III Control	D7-D18	10.00±0.36	0	0	0
IV (24mg/kg b.wt.)	D7-D18	9.67±1.17	9.38±0.12*	0	9.38±0.12*****

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

The average gestation period increased by about 11 days (31 days) in methanol 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 the treatment were observed.

Table 4: Effect of administration of methanol fraction of *Melia azedarach* seed extract for D7-D18 pc on gestation period, litter size, live birth index and life span of young ones in albino rats

Group	*Gestation Period (Days)*	*Average Gestation Period (Days)*	*Litter Size*	*Live Birth Index (%)******	*Average Life Span of Young ones*
V Control	19-21	20.00	10.00	100.00	survived for 3.5-4 years
VI (24mg/kg b.wt)	30-32	31.00	4.00*	93.75*****	9.0 Days

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

4. Discussion

The reduction in fertility index and average number of embryos in adult cyclic rats after 18 days of treatment with methanol 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 [6]. Reduction in the number of viable fetuses was also observed in rats after administration of various plant extracts and reproductive toxicity was found to be the main factor in reducing the number of viable fetuses [19]-[21]. Increase in pre-implantation mortality may be attributed to reduced vasculature permeability which is essential for normal implantation [22].

Non-significant variation in the occurrence of various stages of estrous cycle in rats administered methanol fraction depicts non-estrogenic nature of the fraction. A number of plants possessing anti-estrogenic activity have been reported to interrupt pregnancy [23]-[27]. Presence of two or more successive stages of estrous cycle in the present investigation might be an indicative of hormonal disturbances. 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 [28]-[30] that creates an imbalance in progesterone estrogen ratio [31] 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 [32]. So, the methanol fraction might be interfering with the production of estrogen, thus disturbing progesterone estrogen ratio and rendering uterus unfavorable for implantation. 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 [7],[33] 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. It has already been reported that neem oil and hydroalcoholic extract of *Melia* does not have any estrogenic, antiestrogenic or progestational activity [7], [34]. Yakubu *et al* [35] reported the presence of alkaloids and flavonoids in plant extract of *Cnidoscolous aconitifolius* that 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.

Increase in average gestation period and delay in parturition in the present investigation may be the result of suppressing effects of methanol fraction on prostaglandin (PG) synthesis by pregnant rats [36]. No external malformations or

abnormalities reported 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 [37].

In conclusion, the results of present investigation reveal that methanol 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 implantation.

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