Indian Journal of Experimental Biology
Vol. 45, October 2007, pp. 853-860

Antifertility effect of *Melia azedarach* Linn. (dharek) seed extract in female albino rats

Reshu Mandal & Patwant Kaur Dhaliwal**

*Department of Zoology, Panjab University, Chandigarh 160 014, India
**Department of Zoology and Fisheries, Punjab Agricultural University, Ludhiana 141 004, India

Received 12 September 2006; revised 3 July 2007

In the present study, the effect of oral administration of *Melia azedarach* Linn. (dharek) seed extract on fertility index, uterine weight and various histological and biochemical parameters of uterus were studied in the adult cyclic Wistar rats. Average number of embryos and implantation losses in the pregnant animals treated with dharek seed extract was also studied. The extract was prepared using a flash evaporator at 35°C and dissolved in olive oil to prepare doses on per kg body weight basis. The results indicated a reduction in fertility index and average number of embryos in mated rats treated with the dharek extract. Pre-implantation, post-implantation and total prenatal mortalities were increased in rats treated with dharek seed extract during early (D_1-D_7) and late (D_7-D_{18}) stages of gestation period at doses of 5, 10 and 20 mg kg^{-1} body wt day^{-1}. Histological studies showed a significant reduction in myometrial thickness, uterine gland diameter, luminal diameter of uterine glands and luminal epithelial cell height in rats treated with dharek seed extract at 1mg kg^{-1} body wt day^{-1} for 18 days. Pits and folds in luminal epithelial, mitotic activity in luminal and glandular epithelial cells of uterus were observed to be absent. Biochemically, a significant increase in protein and glycogen contents was observed. Thus, in conclusion, the application of this plant extract in rodent control programme may help to elevate the socioeconomic status of the society.

Keywords: Antifertility, Implantation losses, *Melia azedarach*, Post-natal mortality, Rat, Seed extract, Uterus

Rodents are the most destructive vertebrate pests of agricultural produce. Persistence of rodent problem is attributed to their high rate of reproduction, complex behavior and their ability to adapt under diverse ecological conditions. Almost all the species of Indian rodent pests mature at the age of 2-3 months, exhibit short gestation period (12-47 days), and high rate of annual productivity (10.6 to 53.4 offsprings/ female/year). This high rate of annual productivity results in damage to agricultural produce causing up to 8-15% losses[1,2].

Continuous and heavy usage of rodenticides has created ecological problems like resistance in animals, environmental contamination and death of non-target species. A promising alternative for chemical rodenticides/pesticides is the application of biologically active substances of natural origin, which can meet the needs of the National rodent control programme[3].

Anti-fertility and anti-implantation activity of neem (*Azadirachta indica)* has been reported from different geographically distant areas[4-6]. Implantation was inhibited in rats administered with neem oil during D_1-D_7 of gestation[7] and on the 10th day of gestation[8]. Further, early post-implantation contraceptive effect of a purified (hexane) fraction of neem seeds have also been reported in albino rats[9].

Reduction in fertility was observed due to infiltration of leukocytes in uterus resulting in inhibition of implantation and blockage of pregnancy[10,11]. Secondly, anti-implantation activity has been reported due to histo-pathological changes produced by inhibition of oestrogen induced changes in the uterus[12]. Thirdly, the cause of implantation losses was observed due to steroidogenic depression evidenced by reduced plasma progesterone and LH levels[13] or to imbalance in the progesterone:estrogen ratio[14].

Anti-fertility activity of *Azadirachta indica* (neem) and *Melia azedarach* (dharek) on the ovaries has been reported earlier[15,16]. But there is no evidence of anti-fertility effect of *Melia azedarach* on uterus[16]. Therefore, the present work was undertaken to investigate the effect of dharek (*Melia azedarach* Linn.) on fertility in female Wistar rats.

Phone: 9872011971
e-mail: reshu_mandal@yahoo.co.in

Materials and Methods

Mature female Wistar rats (72) weighing 150 ± 10 g were obtained from small animal colony of the Institute. The animals were provided with standard rat pellet diet and water *ad libitum*. Stage of the oestrous cycle of each animal was determined by taking vaginal smears daily in the morning between 9.00 to 10.00 AM (using 0.9% NaCl, w/v). Rats showing at least three regular four-day cycles and in the diestrous stage of the cycle were used for the present study. For each dose regime, 6 animals were used for the present study. During the present investigations, dharek seed extract was administered to adult female cyclic Wistar rats for 18 days before mating (3 doses of seed extract) for calculating fertility index (total 24 rats), to pregnant female rats during gestation periods (D_1-D_7 and D_7-D_{18} post-coitum, total 36 rats) for studying implantation and prenatal mortalities, and a treatment was given to adult cyclic Wistar rats for 18 days for studying histological and biochemical parameters (total 12 rats). A control was maintained in each experiment.

Preparation of dharek seed extract—Ripe drupes of *M. azedarach* were collected from trees growing in the campus of Punjab Agricultural University, Ludhiana. Seeds of shade-dried matured drupes of *M. azedarach* were powered. Extraction of the bioactive material was done as per the method of Singh[17] and modified Singh[18] using chloroform:methanol (9:1). The filtrate was evaporated at 35°C to a constant weight under vacuum in the flash evaporator and dark brown slurry thus obtained was 6.85 g from 100 g of powder (yield: 6.85%). The dark brown slurry obtained was administered to the female albino rats on per kg body weight basis by dissolving in olive oil (vehicle). Animals administered with olive oil served as control (Group-I).

Experimental design—The fertility was tested by treating female rats with 3 doses of dharek seed extract at 0.5, 1.0 and 5.0 mg kg^{-1} body wt day^{-1} in Group-II (6 rats/dose) and only olive oil to control rats in Group-I (6 rats) for a period of 18 days. The control and treated females were paired with male rats of proven fertility in the ratio of 2:1 for next 7 days. On the 8th day, male rats were isolated. Thirteen days after separation, the female rats of control and treated groups were autopsied and the fertility index was calculated using the following formula:

$$\text{Fertility index} = \frac{\text{Total number of females pregnant}}{\text{Total number of females mated}} \times 100 \quad \ldots (1)$$

Experiment I—Adult female Wistar rats at pro-oestrous phase of oestrous cycle were selected and they were allowed to mate with male rats of proven fertility in a ratio of 2:1. Next morning vaginal smear of each female was examined for the presence of thick clumps of spermatozoa. The day of presence of spermatozoa in vaginal smear was designated as day 1 (D_1) of gestation. After, 24 hr, mated female rats were divided into two groups as control (Group-I) and treated (Group-II). The rats in Group-I and II were further divided into 2 subgroups Group-IA and IB (controls) and Group-IIA and IIB (treated).

The rats in Group-IIA were treated with dharek seed extract at doses of 5 and 10 mg kg^{-1} body wt day^{-1} on days D_1-D_7 post-coitum and rats in Group-IIB were treated with dharek seed extract at doses of 10 and 20 mg kg^{-1} body wt day^{-1} on days D_7-D_{18} post-coitum (6 rats /dose). Olive oil was administered to the control mated rats from D_1-D_7 post-coitum in Group-IA (6 rats) and on D_7-D_{18} post-coitum in Group-IB (6 rats). The animals in Group-IA and Group-IIA were autopsied on 11th day post-coitum and the animals in Group-IB and IIB were sacrificed on 19th day post-coitum.

Number of corpora lutea (CL) was counted in both the ovaries of rats in control Group-IA and IB, and treated Group-IIA and IIB. The total number of embryos (implantations) in both the uterine horns gave an estimate of litter size. Resorbing embryos were counted in both the uterine horns of female rats in Groups-IIA and IIB. An implantation was listed as resorbing if its size was smaller in comparison to that of others in the litter. Then, pre-implantation mortality, post-implantation mortality and total prenatal mortalities were calculated [pre-implantation mortality is the loss of embryos prior to implantation and is measured as the difference between CL and the number of implantation sites (living and resorbing embryos) whereas post-implantation mortality is the mortality of implanted embryos and hence it is the resorbed embryos rate].

The computations were done as follows:

$$\text{Pre-implantation mortality} = \frac{A-B}{A} \times 100 = X\% \quad \ldots (2)$$

Post-implantation mortality (resorbtions) =

$$\frac{B-C}{B}\times 100 = Y\% \quad \ldots (3)$$

Total prenatal mortality = $\frac{A-C}{C}\times 100 = Z\%$ …(4)

where, A – Total number of corpora lutes (CL); B – Total implanted embryos; C – Number of normal embryos.

Experiment II—Dharek seed extract at a dose of 1 mg kg^{-1} body wt day^{-1} was orally administered to six adult female cyclic Wister rats in Group-II (treated) and only olive oil to six female rats in Group-I (Control) for 18 days. The rats of control and treated groups were autopsied on 19th day. Their body weights were recorded. Uteri of both the sides were dissected out and weighed. One uterus of each rat was fixed in Bouin's fluid, dehydrated in graded ethanol, cleared in benzene, embedded in paraffin wax, sectioned at 7 μm and stained with haematoxylin and eosin. The parameters studied were luminal epithelial cell height, uterine gland diameter, myometrium thickness, stroma, eosinophils and mitotic activities in the uterine glands and in epithelium from all the sections of each uterus. Uteri of the contralateral side were processed for biochemical analysis of proteins[19] and glycogen[20]. The experimental data was subjected to statistical analysis using Student's *t* test.

Results

Fertility index—The fertility index in adult cyclic rats after 18 days of treatment of dharek seed extract was reduced with the increase in dose in Group-II as compared to that of control Group-I. Fertility in cyclic rats was 50% at dose of 0.5 mg kg^{-1} body wt day^{-1}, but at 1 and 5 mg kg^{-1} body wt day^{-1} doses of dharek seed extract, the inhibition of pregnancy was observed to be 100% (Table 1).

Table 1—Effect of administration of dharek seed extract (0.5, 1 and 5 mg kg^{-1} body wt day^{-1}) on the Fertility Index in Wistar rats
[Values are mean of 6 observations]

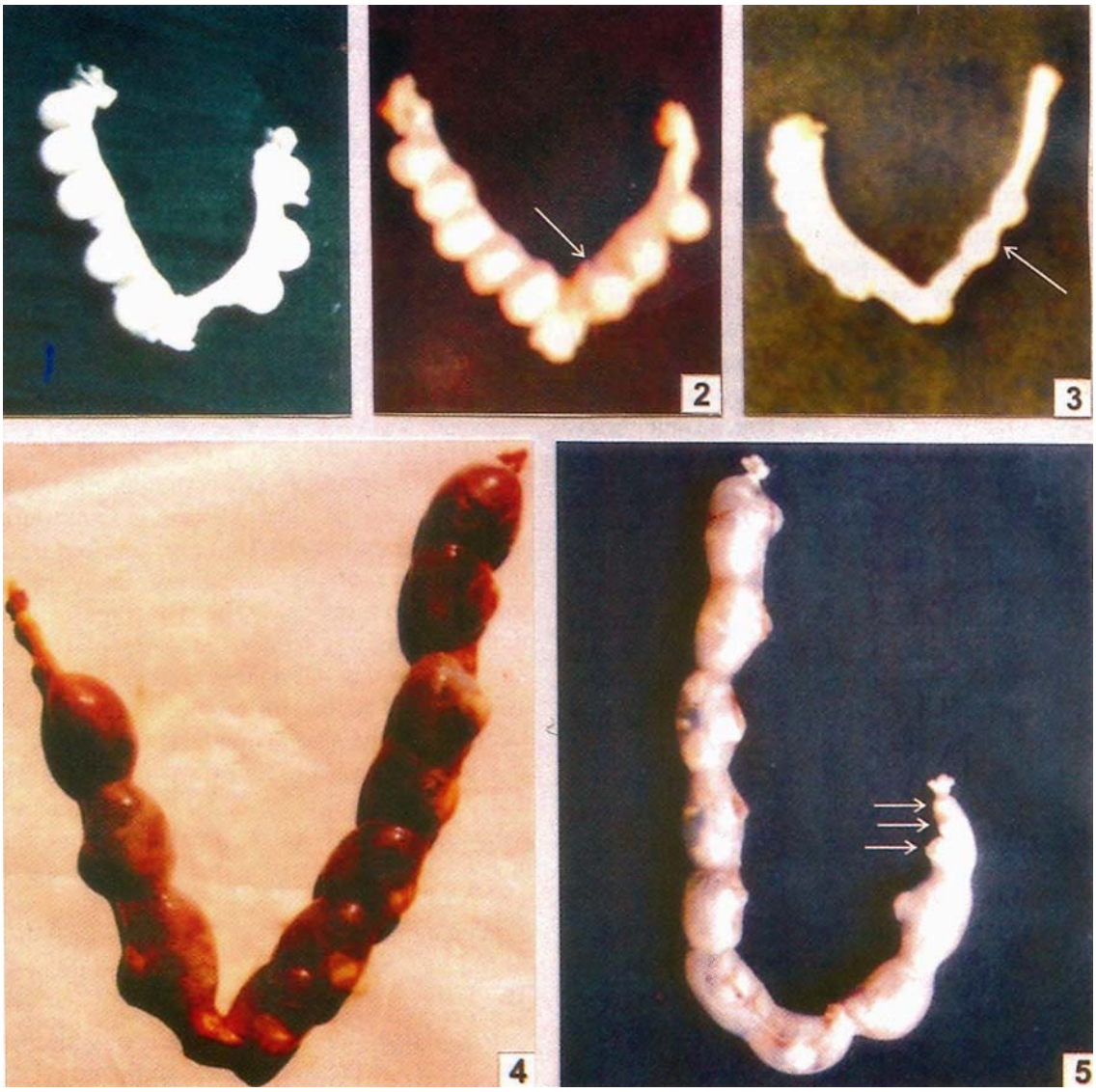
Group	Treatment [a]	Dose[a] (mg kg^{-1} body wt day^{-1})	Fertility index (%)
I	Vehicle (olive oil)	—	100
II	Seed extract of dharek	0.5	50
		1.0	0
		5.0	0

[a] Days 1-18
(Group-II compared with control, Group-I)

Implantation loss during D_1 to D_7 of treatment with dharek seed extract—The data regarding the effect of administration of dharek seed extract during early days of gestation (D_1-D_7) in rats resulted in reduction in number of implantations treated at doses of 5 and 10 mg kg^{-1} body wt day^{-1} in Group-IIA as compared to that in the control Group-IA (Figs 1-3). Pre-implantation mortality increased in both the treatments of dharek seed extract. Similar trend was observed in post-implantation mortality. Total prenatal mortality increased to 50% (49.95 ± 2.72) at 10 mg kg^{-1} body wt day^{-1} dose as compared to that at a dose of 5 mg kg^{-1} body wt day^{-1} of dharek seed extract in Group-IIA (Table 2).

Implantation loss during D_7 to D_{18} of treatment with dharek seed extract—Observations on the effect of administration of dharek seed extract during later stages of gestation (D_7-D_{18}) also resulted in reduction in number of implantations and increase in implantation losses. The average number of implantations was reduced at doses of 10 and 20 mg kg^{-1} body wt day^{-1} of dharek seed extract in Group-IIB as compared to that in control Group-IB (Table 3). Decrease in the size of implants was recorded in rats at dose of 10 mg kg^{-1} body wt day^{-1} as compared to that in the control (Figs 4, 5). Pre-implantation, Post-implantation and total prenatal mortalities were increased in rats treated at dose of 10 mg kg^{-1} body wt day^{-1} of dharek seed extract. But total pre-implantation loss (100%) of embryos was observed at 20 mg kg^{-1} body wt day^{-1} dose in the treated Group-IIB (Table 3).

Histological studies—Uterine weights of Wistar rats were decreased non-significantly after the treatment with dharek seed extract at 1 mg kg^{-1} body wt day^{-1} for 18 days in Group-II as compared to that control, Group-I (Table 4). The uterine lumen in control rats was wide, but in treated rats, it was reduced to S-shape and T-shape (Figs 6-8). The size of uterus did not vary much after treatment with dharek seed extract. Numerous pits and folds in luminal epithelium in control rats were observed, whereas after the administration of dharek seed extract resulted in disappearance of pits and folds (Figs 6-8). Tall columnar cells with nuclei in basal region in cells of luminal epithelium were observed in the control, whereas low columnar cells, with nuclei slightly above their basal parts in luminal epithelium were recorded in treated rats. The height of luminal epithelium was significantly reduced in the treated



Figs 1-5—1: Uterus of rat treated with the vehicle (olive oil) on days 1-7 post-coitum and autopsied on day 11 post-coitum in control (Group-IA) showing the normal size of all the implantations. 2: Uterus of rat treated with the dharek seed extract at a dose of 5 mg kg^{-1} body wt day^{-1} on days 1-7 post-coitum and autopsied on day 11 post-coitum in Group-IIA showing resorbing implantation and the presence of blood clot (arrow). 3: Uterus of rat treated with the dharek seed extract at a dose of 10 mg kg^{-1} body wt day^{-1} on days 1-7 post-coitum and autopsied on day 11 post-coitum in Group-IIA showing resorbing implantation (arrow). 4: Uterus of rat treated with the vehicle (olive oil) on days 7-18 post-coitum and autopsied on day 19 post-coitum in control Group-IB showing normal size of implantations. 5: Uterus of rat treated with the dharek seed extract at a dose of 10 mg kg^{-1} body wt day^{-1} on days 7-18 post-coitum and autopsied on day 19 post-coitum in Group-IIB showing resorbing implantations (arrows).

group as compared to that in the control (Table 4). Mitotic divisions were frequent in luminal epithelial cells of control group, but were totally absent in rats treated with dharek seed extract.

Stroma in control rat uterus was edematous with evenly spaced nuclei and moderate blood vascularity, whereas the medullary region of stroma was observed to become dense after administration of dharek seed extract in Group-II. Eosinophils were present in large numbers in stroma of control rats, whereas they occur only in cortex and were not present in medulla of treated rats in Group-II.

Decrease in the number and size of uterine glands was observed in treated rats as compared to those in

Table 2—Effect of administration of dharek seed extract (5 and 10 mg kg^{-1} body wt day^{-1}) on days 1-7 post coitum on the implantation and prenatal mortalities in Wistar rats
[Values are mean ± S.E.M. of 6 rats)

Group	Treatment[a]	Dose[a] (mg kg^{-1} body wt day^{-1})	Pregnant[b]/ total rats	Corpora[b] lutea	Implantations[b]	Prenatal mortality[b] (%)		
						Pre-implantation	Post-implantation	Total
IA	Vehicle (olive oil)	—	6	9.00 ± 0.36	9.00 ± 0.36	0.00	0.00	0.00
IIA	Seed extract of dharek	5	6	6.50 ± 0.76	5.67 ± 0.67	12.43 ± 2.79	19.20 ± 4.37	28.78 ± 5.97
		10	6	7.83 ± 0.87	5.33 ± 0.56	31.69 ± 1.28	26.43 ± 4.71	49.95 ± 2.72

[a] Days 1-7 post-coitum; [b] Day 11th post-coitum (Group-IIA compared with control, Group-IA)

Table 3—Effect of administration of dharek seed extract (10 and 20 mg kg^{-1} body wt day^{-1}) on days 7-18 post coitum on implantation and prenatal mortalities in Wistar rats
[Values are mean ± SEM of 6 rats]

Group	Treatment[a]	Dose[a] (mg kg^{-1} body wt day^{-1})	Pregnant[b]/ total rats	Corpora[b] lutea	Implantations[b]	Prenatal mortality[b] (%)		
						Pre-implantation	Post-implantation	Total
IB	Vehicle (olive oil)	-	6	10.00 ± 0.58	10.00 ± 0.58	0.00	0.00	0.00
IIB	Seed extract of dharek	10	6	10.00 ± 1.34	8.00 ± 1.12	20.31 ± 1.78	18.75 ± 2.01	34.91 ± 2.17
		20	6	9.33 ± 2.18	0.00	100	100	100

[a] Days 7-18 post-coitum; [b] Day 19th post-coitum (Group-IIB compared with control, Group-IB)

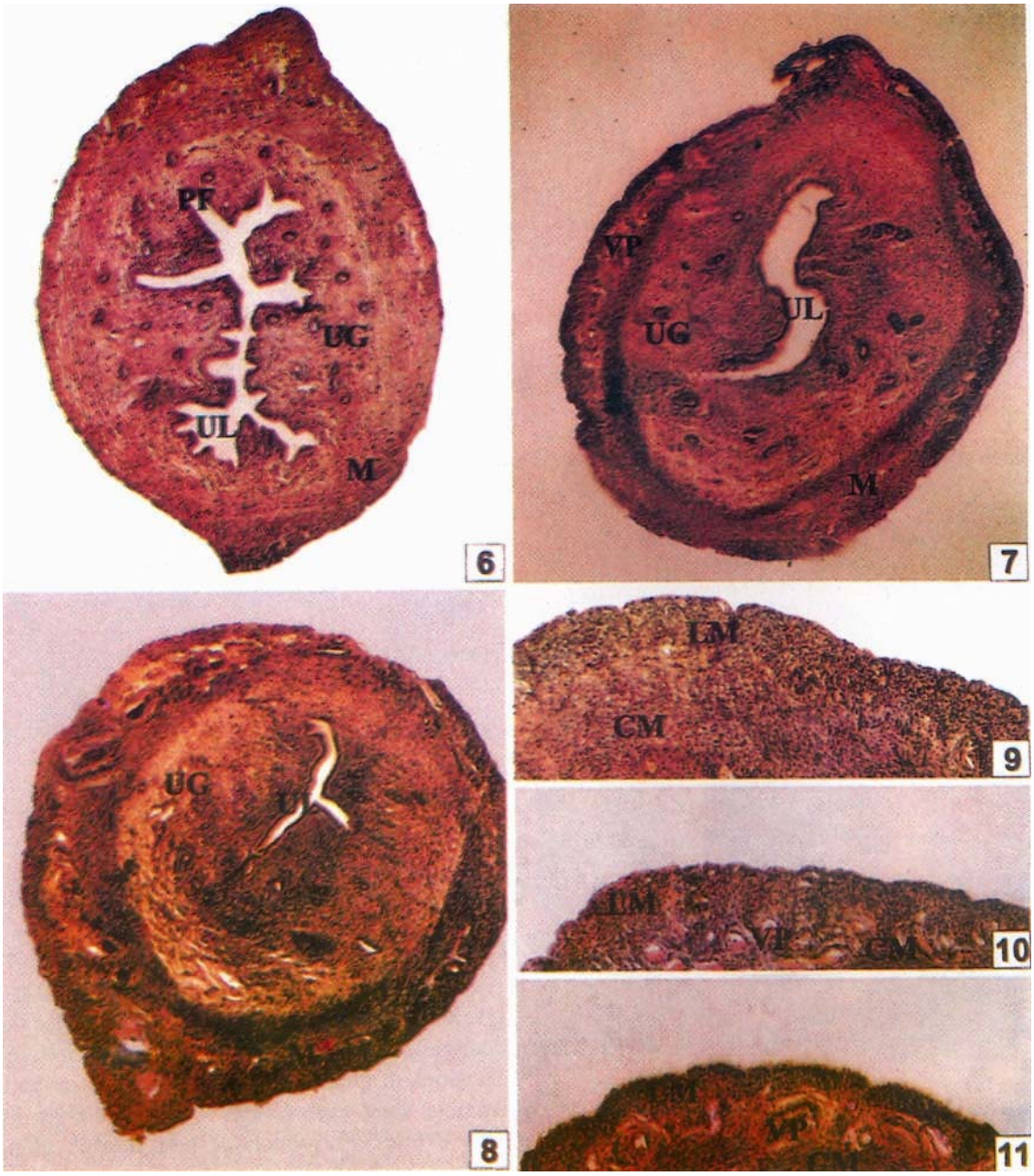
Table 4—Effect of administration of dharek seed extract (1 mg kg^{-1} body wt day^{-1}) on uterine histomorphometry and protein and glycogen contents in Wistar rats.
[Values are mean ± SEM of 6 rats]

Parameters	Group-I (control)	Group-II (treated)
Uterine weight[€]	67.20 ± 2.94	64.55 ± 1.75
Luminal epithelial cell height[$]	32.00 ± 0.89	21.00 ± 0.50*
Uterine gland diameter[$]	142.00 ± 4.83	38.00 ± 0.85*
Luminal diameter of uterine gland[$]	99.00 ± 2.91	22.00 ± 0.86*
Longitudinal muscle layer[$]	91.00 ± 2.80	150.00 ± 2.17*
Circular muscle layer[$]	57.00 ± 2.07	63.00 ± 1.76*
Protein[ℓ]	20.29 ± 0.40	28.73 ± 0.70*
Glycogen[ℓ]	20.46 ± 0.52	22.99 ± 0.94*

Units: [€] mg /100g body weight; [$] µm; [ℓ]mg g^{-1} tissues
P-values: *Significant at $P < 0.05$ (Group-II compared with control, Group-I)
All other relevant comparisons were statistically non-significant

the control group (Figs 6-8). The uterine gland diameter decreased significantly in treated rats in Group-II as compared to control, Group-I (Table 4). The luminal diameter of uterine gland also showed similar trend exhibiting decrease diameter in treated rats compared to control (Table 4). Numerous mitotic divisions in gland cells were observed in control rats, whereas they were absent in rats treated with dharek seed extract. Formation of new uterine glands from luminal epithelium was frequent in control rat uterus, whereas the ability of luminal epithelium to form new glands was decreased in rats treated with dharek seed extract.

The thickness of longitudinal and circular muscle layers decreased significantly in uteri of treated rats (Group-II) than those in control, (Group-I; Table 4). In treated rats, layers were disorganized, cells were smaller and less number of eosinophils were present in circular muscle layer in treated group as compared to control. The vascular plexus between longitudinal and circular muscle layers was observed to be



Figs 6-11—6: Transverse section of uterus of control rats of Group-I showing wide uterine lumen (UL), presence of pits and folds in the uterine epithelium (PF), uterine glands (UG) and myometrium (M) (× 136). 7 and 8: Transverse section of uterus of treated rats in Group-II showing reduced uterine lumen (UL), absence of pits and folds (PF) in the epithelium, reduced number of uterine glands (UG) and decreased myometrial thickness (M) after administration of dharek seed extract at a dose of 1 mg kg^{-1} body wt day^{-1} for 18 days (×136). 9: Transverse section of uterus of control rats in Group-I showing moderate vascular plexus (VP) between the longitudinal muscle layer (LM) and circular muscle layer (CM) of myometrium (M) (× 850). 10 and 11: Transverse section of uterus of treated rats in Group-II showing extensive vascular plexus (VP) between the longitudinal muscle layer (LM) and circular muscle layer (CM) of myometrium after the administration of dharek seed extract at a dose of 1 mg kg^{-1} body wt day^{-1} for 18 days (× 850)

extensive in rats treated with dharek extract as compared to control (Figs 9-11).

Biochemical studies—The total protein and glycogen contents were found to be increased significantly in the uterus of rats in Group-II treated with dharek seed extract at a dose of 1 mg kg^{-1} body wt day^{-1} dose as compared to that in control, (Group-I; Table 4).

Discussion

The data recorded on reduction in fertility index in adult cyclic rats after 18 days of treatment with dharek seed extract during present investigations supported the findings of Sinha *et al*[4] who have observed reduction in fertility after vaginal administration before mating and unilateral administration of neem oil in the uterus of female Wistar rats[11].

The fertility block during pre-implantation period in the present investigations may be due to lowering in EGFR localization in the luminal and glandular epithelium caused by massive leukocytes infiltration into the uterus results in degeneration of the early embryos and by causing the post-implantation embryonic resorptions in the uteri[11]. In a previous study, a number of retrieved unimplanted embryos have been found to have the attached leukocytes to the zona pellusida layer. It is believed that this secretion of leukocytes might be responsible for under development of early embryos or by initiating a cellular immune response in the uterus leading to blocking of implantation[10,11] which is still under investigation.

According to the finding of Hiremath *et al*[14], other reason for resorptions during D_1-D_7 of treatment may be due to an imbalance in the progesterone estrogen ratio. The failure to maintain pregnancy in mice may be attributed to a direct or indirect effect on the corpus luteum resulting in an inhibited synthesis and/or secretion of progesterone. Lal *et al*[21,22] have reported partial resorption of fetuses after oral administration of neem oil from D_1-D_{10} of gestation and other at various doses from 0.05-0.5 ml/rat/day from D_1-D_3 of gestation exhibited 100% blastocidal effect in rats[7].

The anti-estrogenic quality of neem oil also explains its anti-implantation effect. But the post-implantation effect, which caused implanted fetuses to be either resorbed or expelled, also may be due to direct toxicity, fall in progesterone level or interference with the uterine utilization of progesterone[23]. Therefore, pre- and post-implantation losses observed during the present investigations may be attributed to the disturbance of both uterine metabolism and indirect or direct effect on CL resulting in an inhibition of synthesis and/or secretion of progesterone.

Dixit *et al*[24] and Patil *et al*[25] have reported decreased myometrial volume in proportion to uterine weight and marked regression of uterine glands in female gerbils treated intraperitoneally with *Cannabis* extract and decreased thickness of myometrium and height of luminal epithelium in uterus of rats administered nicotine at 2 and 4 mg kg^{-1} body wt for 20 days, respectively. Morphological changes observed in the myometrium of uterus of treated group in the present study might either be result in stimulation[26] or inhibition[27] of uterine contractions in rats.

Further, Shukla *et al* [28] have reported complete obliteration of lumen of uterus in rats treated with neem extract, whereas Kholkute *et al*[29] have observed atrophic uteri and uterine epithelium devoid of mitotic figures in rats after administration of benzene extract of *H. rosa-sinensis* flowers for 30 days. Along with it, subcutaneous administration of neem seed oil has been reported to cause significant damage to luminal epithelium and uterine glands[12,30], which might be the reason in anti-implantation effect of dharek seed extract in the present study.

The increased protein content observed in the present investigation might be due to the extensive vascular plexus between longitudinal and circular muscle layers of myometrium in the treated rats. Increased glycogen content might be due to inhibition of phosphorylase system of the tissue in which phosphorylase changes glycogen to glucose-1-phosphate and rest of the glycogen shorter than one glucose unit. Further, the activity levels of aldolase and G-6-PDH might be inhibited by dharek extract that indicated inhibition of hexose-mono and diphosphate pathways, the operation of which is essential for implantation which needs further study[31,32].

In conclusion, dharek seed extract resulted in reduction in fertility by change in the uterine metabolism. Thus, it is consistent with its use in the folk medicine as an anti-conceptional agent which may help to elevate the socio-economic status of the society.

References

1 Parshad V R, Kaur P & Guraya S S, Reproductive cycles of mammals: Rodents, in *Reproductive cycles of Indian vertebrates,* edited by S K Saidapur (Allied Publishers Limited, New Delhi) 1989, 347.

2 Sheikher C, Rodent problem in Indian Agriculture: An Overview, *J Punjab Acad Sci,* 1 (1999) 229.

3 Dixit V P, Plant products/non-steroidal compounds affecting fertility in the Indian desert gerbil, *Meriones hurrianae* Jerdon in *Rodents in Indian agriculture,* Vol. 1, edited by I Prakash and P K Ghosh (Scientific Publishers, Jodhpur, India), 1992, 595.

4 Sinha K C, Riar S S, Bardhan J, Thomas P, Kain A K & Jain R K, Neem oil as a vaginal contraceptive, *Indian J Med Res,* 79 (1984a) 131.

5 Sinha K C, Riar S S, Tiwari R S, Dhawan A K, Bardhan J, Thomas P, Kain A K & Jain R K, Anti-implantation effect of neem, *Indian J Med Res,* 80 (1984b) 708.

6 Dhaliwal P K, Roop J K & Guraya S S, Effect of neem-seed oil on the quantitative aspects of follicular development in cyclic female rats, *Indian J Eco*, 26 (1999) 162.

7 Tewari R K, Mathur R & Prakash A O, Post-coital antifertility effect of neem oil in female albino rats, *IRCS Med Sci,*14 (1986) 1005.

8 Khare A K, Srivastava N C, Sharma M K & Tewari J P, Antifertility activity of neem oil in rabbits and rats, *Probe,* 23 (1984) 90.

9 Mukherjee S, Garg S & Talwar G P, Early post implantation contraceptive effects of a purified fraction of neem (*Azadirachta indica*) seeds, given orally in rats: Possible mechanisms involved, *J Ethnopharmacol,* 67 (1999) 287.

10 Upadhyay S N, Kaushic C & Talwar G P, Antifertility effects of neem (*Azadirachta indica*) oil by single intrauterine administration: A novel method for contraception, *Proc R Soc Lond Sci B (Biol Sci),* 242 (1990) 175.

11 Juneja S C, Williams R S, Farooq A & Chegini N, Contraception potential of neem oil: Effect on pregnancy success in the mouse, *J Assist Reprod Genet*, 13 (7) (1996) 578.

12 Pakrashi A & Chakrabarty B, Antioestrogenic and anti-implantation effect of aristolic acid from *Aristolochia indica* (Linn), *Indian J Exp Biol,* 16 (1978) 1283.

13 Adhikary P, Chowdhury D, Banerji J & Chatterjee A, Antifertility effect of crude alcoholic extract of *Piper betle* stalk, *Indian J Physiol Allied Sci,* 52 (1998) 22.

14 Hiremath S P & Rao S H, Antifertility efficacy of the plant Striga lutea (Scrophulariacea) on rats, *Contraception,* 42 (1990) 467.

15 Choudhary R R, Plants with possible Antifertility activity, *Special Report* (*Indian Council Med Res*, New Delhi) 1966, 3.

16 Roop J K, Dhaliwal P K & Guraya S S, Extracts of *Azadirachta indica* and *Melia azedarach* seeds inhibit folliculogenesis in albino rats, *Brazil J Med Biol Res,* 38(06) (2005) 943.

17 Singh R P, Comparison of antifeedant efficacy and extract yields from different parts and ecotypes of neem (*Azadirachta indica* A Juss) in *Natural pesticides from neem tree and other tropical plants,* edited by N S Randhawa and B S Parmar (PSI, New Delhi) 1987, 185.

18 Singh K, *Bioactivity of Melia azedarach L. against diamondback moth, Plutella xylostella* L. M.Sc. Thesis, Punjab Agric Univ, Ludhiana, India, (1994) 87.

19 Lowry O H, Rosenbrough N J, Farr A L & Randall R, Protein measurement with folin phenol reagent, *J Biol Chem,* 193 (1951) 265

20 Kemp A & Kits Van Heigningen A J M, A colorimetric micro-method for the determination of glycogen in tissues, *Biochem J,* 56 (1954) 646.

21 Lal R, Gandhi M, Shankaranyana A, Mathur V S & Sharma P L, Antifertility effect of *Azadirachta indica* oil administered *per se* to female albino rats on selected days of pregnancy, *Fitoterapia,* 63 (1986a) 239.

22 Lal R, Shankaranarayana A, Mathur V S & Sharma P L, Antifertility effect of neem oil on female albino rats by the intravaginal and oral routes, *Indian J Med Res,* 83 (1986b) 89.

23 Riar S S, Bardhan J, Thomas P, Kain A K & Parshad R, Mechanism of antifertility action of neem oil, *Indian J Med Res,* 88 (1988) 339.

24 Dixit V P, Arya M & Lohiya N K, Mechanism of action of chronically administered *Cannabis* extract on the female genital tract of gerbils, *Indian J Physiol Pharmacol,* 20 (1976) 38.

25 Patil R S, Patil S, Bhaktaraj B & Patil S B, Effect of graded doses of nicotine on ovarian and uterine activities in albino rats, *Indian J Exp Biol,* 37 (2) (1999) 184.

26 Veale D J H, Havlik I, Oliver D W & Dekker T G, Pharmacological effects of *Agapanthus africans* on the isolated rat uterus, *J Ethnopharmacol,* 66 (3) (1999) 257.

27 Jayme V, Rosacortes A & Aoki K, Effect on rat uterus contractility of *Tagets lucida* cav leaf extracts, *Phyton* (Buenos Airses), 62 (1-2) (1998) 161.

28 Shukla S, Mishra A & Prakash A O, Effect of extract of *Azadirachta indica A.* Juss (seeds) on the vital and reproductive organs of cyclic rats, *Int Conf Recent Advances in Medical, Aromatic and Spice Crops,* (Indian Council Medical Research, New Delhi, India) 1989, 10.

29 Kholkute S D, Chatterjee S & Udupa K N, Effect of *Hibiscus rosa-sinensis* Linn. on oestrous cycle and reproductive organs in rats, *Indian J Exp Biol,* 14 (1976) 703.

30 Tewari R K, Pathak R S S H & Prakash A O, Biochemical and histological studies of reproductive organs in cyclic and ovariectomized rats supporting a non-hormonal action of neem oil, *J Ethnopharmacol,* 25 (3) (1989) 281.

31 Piyachaturawat P, Ercharuporn S & Subramran A, Uterotrophic effect of *Curcuma comosa* in rats, *Int J Pharmacogn,* 33 (4) (1995) 334.

32 Chattopadhyay R R, Effect of *Azadirachta indica* leaf extract on oestrous cycle of rat, *Environ Ecol*, 11 (1993) 958.