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Effect of *Melia azedarach* on nicotine induced changes on female infertility

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ABSTRACT

Nicotine in cigarette smoke is one of the toxic substances which impair the fertility. In this research the effects of nicotine tartrate on female albino rat's reproductive system was studied. The rats were divided into 4 groups. Group I: control animals received normal saline, Group II: injected with nicotine 4mg/kg, Group III: injected with nicotine4mg/kg and Meliaazedarach 100mg given orally. Group IV: given Meliaazedarach 100 mg/kg orally. The experimental group were injected subcutaneously 4 mg/kg of nicotine tartrate daily for 30 days. Estrous cycle was examined daily by vaginal smear and animals in estrus phase were selected. At the end of the experiment, uterus and ovaries were removed and after weighing were prepared for histometric and histological studies. Results showed that the length of estrous cycle in group II animals was increased significantly. Histological studies showed that there were atretic follicles in 4 mg/kg treated groups. In group III rats these pathological changes were reversed and there is no change in group IV rats.

Keywords: Ovary; Uterus; Nicotine; Estrous cycle; atretic follicle

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INTRODUCTION

Reproduction is an exceptionally complex process being highly vulnerable at many stages. The environmental and life style factor have an adverse effect on fertility. Nicotine in cigarette smoking is thought to affect female fertility via a number of alterations in ovarian function, including irregularities in the estrous cycle, depleted ovarian reserves, impaired ovulation and spontaneous abortion [1].Treatment of rats with nicotine is associated with a decrease in estrogen dependent parameters, including diameter of uterus and thickness of endometrium and myometrium [2].

Meliaazedarach commonly called the "pride of India" Persian lilac in English, Bakain in Hindi, Malaivembu in Tamil is a member of meliaceace is widely grown as an ornamental trees. The plant is traditionally used for the treatment of leprosy, inflammations, uterine illnesses and cardiac disorders. Its fruits extracts possess ovicidal and larvicidal activity [3].The leaf extracts also possess antiviral, antimalarial and anthelmintic activity [4].

MATERIALS AND METHODS

Female rats were divided four groups. Group I control, group II nicotine induced, group III nicotine induced and *Meliaazedarach* treated and group IV only drug *Meliaazedarach* treated. Group II received a subcutaneous injection of nicotine tartrate (4mg/kg bw per day for 30 days). Along with nicotine, *Meliaazedarach* was given at the dosage of 100mg per kg body weight for Group III rats.

The estrous cycle of both control and treated rats was monitored through observation of cell types in the vaginal smear according to Montes and Luque (1988)[5].Vaginal fluid was collected with the help of smooth dropper filled with normal saline and placed on a slide to prepare a smear and allowed to air dry. Unstained material was observed under a light microscope, without the use of the condenser lens, with 10 and 40 x objective lenses.

Micrometric measurements such as diameter of uterus, thickness of endometrium and endometrium were also made from randomly selected 20 sections which appeared round in cross section from each group. Micrometric measurements were made by using stage and ocular micrometer. The ovary and uterus were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned for histology.

RESULTS AND DISCUSSION

It is cleared that the group I & group IV rats exhibited regular 4–5 day estrous cycle. Treatment with 100mg/kg *m.azedarach* and nicotine (Group III) causes moderate decrease in the number of estrous cycle and the duration of proestrus, estrus, metestrus with moderate increase in the diestrus phase. Group II rats were treated with only nicotine (4mg/kg) causes significant cessation of the number estrous cycle and the duration of proestrus, estrus, metestrus with the simultaneous increase in the diestrus phase.

Table 1 Effect of nicotine and *Meliaazedarach* L. on estrous cycle

	No. of cycles	Proestrus Estrus		Metaestrus	Diestrus
Group I	5.63±0.08	5.49±0.041	7.63±0.06	5.8±0.01	11.20±0.04
Group II	3.08±0.31**	2.88±0.042**	4.14±0.02**	2.66±0.041**	20.55±0.11**
Group III	4.32±0.18*	3.84±0.091**	6.23±0.07**	4.5±0.13	15.5±0.05
Group IV	5.32±0.34	5.5±0.062	7.7±0.05	5.6±0.27	12.62±0.07

*Values are Mean ±S.E. ** P < 0.01, * P < 0.05*

Table 2 Effect of nicotine and *Meliaazedarach* on histometric changes in female rats

	Uterus diameter µm	Endometrium thickness µm	Myometrium thickness µm
Group I	213.52±0.56	12.33±0.26	7.49±0.09
Group II	163.71±0.35**	7.44±0.17 **	5.3±0.27 **
Group III	187.29±0.67**	10.5±0.09 *	6.52±0.12*
Group IV	214.03±0.89	12.49±0.427	7.16±0.31

*Values are Mean ±S.E.**= P < 0.01, * = P < 0.05*

Fig (1): Section of ovary of control showing normal histo-architecture with well organized surface epithelium and different follicles viz. primary, secondary, developing and mature graffian follicles. Stromal cells of ovarian follicles and corpus luteum are also well developed (H & E 40X)

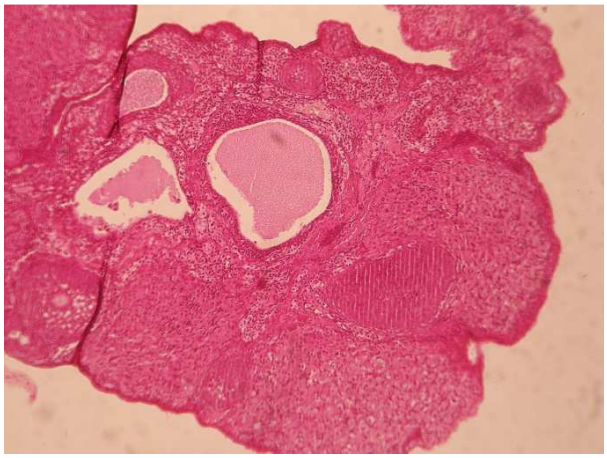


Fig (2) Section of 30 days nicotine treated ovary of rats showing highly atrophic, degenerative, vacuolated changes with less cytoplasmic material, reduced nuclei of follicular cells, granulosa cells, thecal and stromal cells of ovarian follicles (H & E 100X)

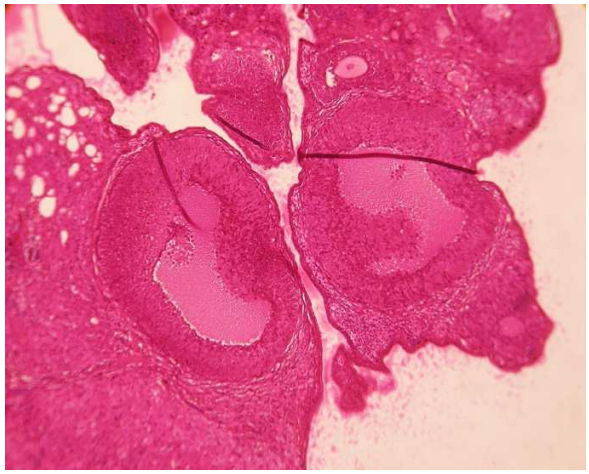


Fig (3): Section of 30 days Nicotine and *Meliaazedarach* treated ovary showing normal histo-architecture of ovary but hypertrophied cells found in corpus luteum. The interstitial tissues are also atrophied in condition and showing areas of vasoconstriction (H & E 10X)

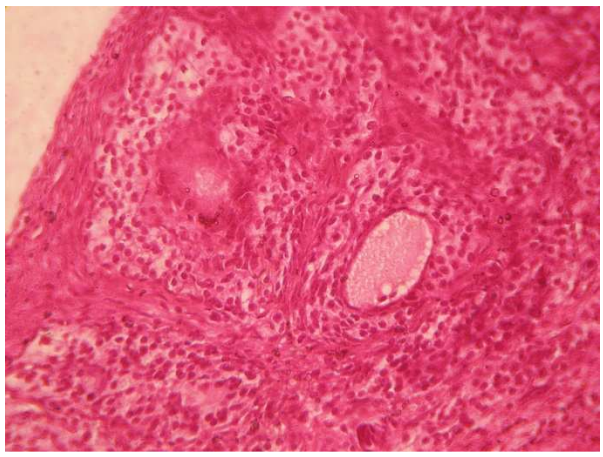


Fig (4): Section of ovary of *Meliaazedarach* treated group showing normal histo-architecture with well-organized surface epithelium and different follicles such as developing and mature graffian follicles. Stromal cells of ovarian follicles and corpus luteum are also well developed (H & E 40X)

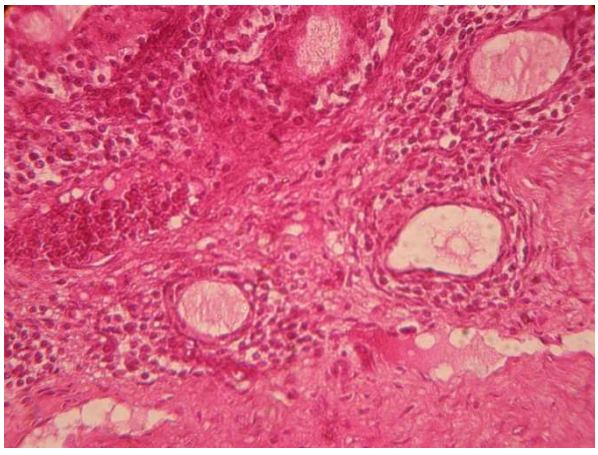


Fig (5): Section of control uterus of Rat showing well organized outer layer of serosa, middle layer of myometrium with muscle layers and inner layer of endometrium with uterine gland. Endometrium is lined by simple columnar epithelium. Lumen is also well developed (H & E 40X). epithelial cells

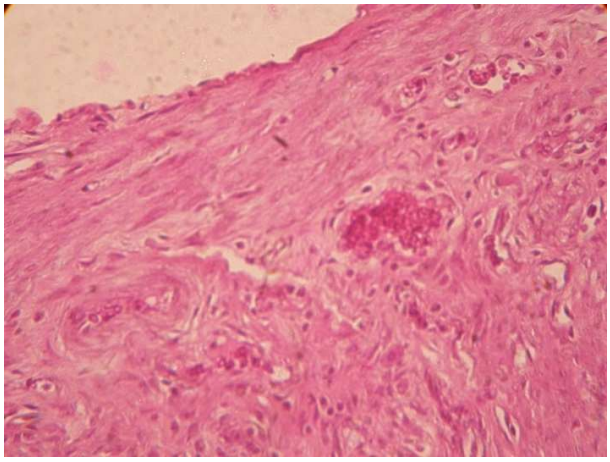
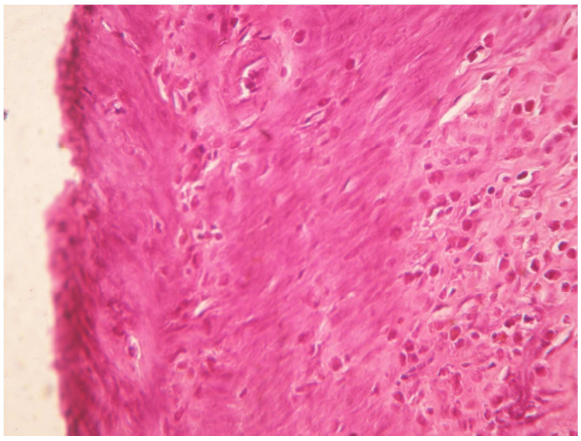


Fig (6): Section of 30 days nicotine treated uterus of Rat showing areas of vasoconstriction, smooth muscle atrophy and degenerative epithelial layer of lumen. Vacuolization, fibrosed, degenerative and atrophic changes found in endometrium, myometrium and serosa (H & E 10X)



In this study the control rats exhibited regular estrous cycle of 4–5days. Cyclic changes of the vaginal smear observed in the frequency of estrous cycle gave a reasonable index of ovarian activity and gonad disruption. Estradiol was responsible for changes in the reproductive tract, mammary glands and for the regulation of gonadotropins. The stages of estrus cycle and their interconversions were mainly governed by the hormones viz., estrogens and progesterone [6]. Any change in these hormones would lead to changes in the cyclicity and impaired fertility. In this study we examined the estrous cycle variables of nicotine exposed female rats as a holistic indicator about the toxic effects of nicotine on the female reproductive system.

The data obtained in the present study revealed that the rats treated with nicotine caused a significant decrease in the number of estrous cycle and duration of proestrus, estrus and metestrus with concomitant significant increase in diestrus phase. This may be due to non-availability of estrogen parameter and reduced steroidogenesis of the ovary as the estrogen was essential for the cornification of vaginal epithelial cell during estrus phase. Similar results have been reported with other organophosphorus pesticides in rats [7]. The alteration in the estrous cycle with prolonged diestrus in nicotine treated rats may be due to the hormonal imbalance in the estrogen: progesterone ratio. Pomerleau et al., [8] have reported effect of chronic nicotine administration on ovaries and sexual cycle. These cyclic changes were reversed in *Meliaazedarach* treated rats along with nicotine .In *Meliaazedarach* alone treated groups the estrus cycle was as that of the control groups.

Fig (7): Section of nicotine and *Meliaazedarach* treated uterus of rat showing areas of vasoconstriction, smooth muscle atrophy, vacuolated endometrium with slightly degenerated

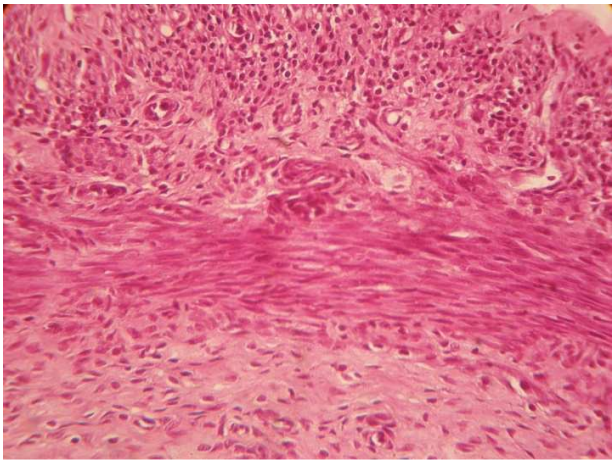
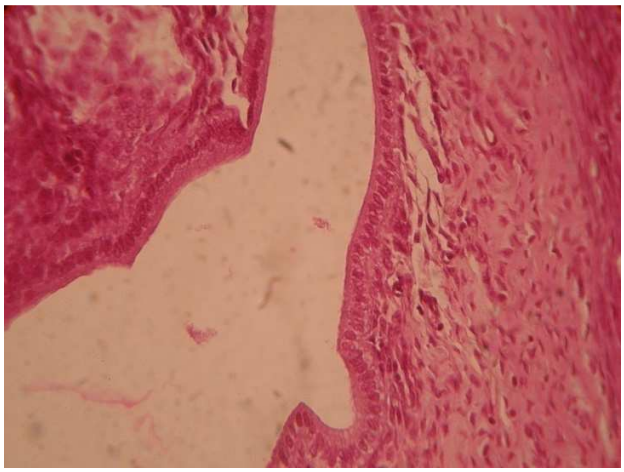


Fig (8): Section of control uterus of Rat showing well organized outer layer of serosa, middle layer of myometrium with muscle layers and inner layer of endometrium with uterine gland



Histometric analysis showed that there was significant reduction in the diameter of uterus and thickness of endometrium and myometrium in nicotine treated groups compared to the control groups. But in the treatment of *Meliaazedarach* along with the nicotine improved the diameter and thickness. In group IV rats there was no change in uterus.

Uterine growth depends upon the ovarian estrogen secretion. Estrogen primarily acts upon the surface epithelium and the glands within endometrium [9].Progesterone acts on estrogen primed uterus and prepares the uterine epithelium from proliferative to secretory state . In the present investigation, reduction in the uterine diameter, reduced thickness of its myometrium and endometrium and reduced secretions from endometrial glands indicate the inhibition of ovarian steroid biosynthesis necessary for growth of the uterus and reproductive cyclicity. In the present investigation decreased uterine diameter and thickness of the endometrium and myometrium were reversed by the treatment with *Meliaazedarach.*

Histopathological studies of ovary of albino rat of group I and VI Showed the normal cellular organization with all kinds of follicles, stroma and vascularity (Fig.1&4). Nicotine exposure caused atrophic changes in the developing follicles and loose stroma with normal vascularity (Fig. 2). Treatment with *Meliaazedarach* after nicotine administration improved the ovary architecture to a great extent. It showed significant improvement in follicles, stroma, vascularity and corpora lutea also seen (Fig. 3). Photomicrograph of the T. S. of Uterus of albino rat of control group showing the normal histoarchitecture of endometrium, uterine lumen, uterine glands, musculature and

vascularity (Fig. 5). Nicotine Exposure caused atrophic changes in the uterine musculature, uterine glands and endometrium (Fig. 6). Treatment with *Meliaazedarach* after nicotine administration improved the uterus architecture to a great extent. It showed significant improvement in uterine musculature, uterine glands, uterine lumen and vascularity (Fig.7)

By the histological studies, it has noted that there is marked decrease in growth of follicles in ovary under the nicotine challenge. Graafian follicles with an oocyte are observed in the ovarian cross section of control group of rats. But no graafian follicles are observed in the ovarian cross section when the animals are exposed in nicotine. This is probably due to the decrement of estrogen level in nicotine treatment. Growth of follicles are increased as well as oocyte appeared with normal form in the ovary after *Meliaazedarach* supplementation which indicates that *Meliaazedarach* restores the normal ovarian function by minimizing the toxic effect of nicotine which in turns increases estrogen levels. According to Soares *et al*. (2007), heavy smoking disrupts the stability of cells in the lining of uterus differently which overall reduces general pregnancy rate. He also reported that a change of endometrium in heavy smokers. In present study noted that endometrium of the uterus of nicotine treated rats becomes thinner than that of control group. *Meliaazedarach* regains the normal structure and architecture of endometrium as a result of which the endometrium becomes thick. Many scientists have already shown that curcumin has a broad range of biological activities which are beneficial for our health [10,11]. Our results are in line with the observations reported earlier.

CONCLUSION

In conclusion, this study indicates that *Meliaazedarach* improves the ovarian functions in nicotine treated rats through alterations in estrogen concentration. Our results also suggest that *Meliaazedarach* may have a significant beneficial effect for infertility.

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