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EFFECT OF IVNT ON OVARIAN WEIGHT, FOLLICLE NUMBER, ESTRUS FREQUENCY AND REVERSIBILITY OF REPRODUCTIVE CYCLE IN ALBINO RAT

Hare Ram Singh*[1], Raja RamSingh [2], Ajit KumarDubey [3], Santosh Kumar Jha [1]

Department of Biotechnology, Birla Institute of Technology [1], Mesra, Ranchi-835215, Jharkhand, India
Department of Zoology, V.K.S. University [2], Ara, Bihar, India
G.N.M. College [3], Parsathuan, Rohtas, Bihar, India

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Correspondence to Author:

Dr. H.R. Singh

Assistant Professor, Department of Biotechnology, Birla Institute of Technology, Mesra, Ranchi-835215, India

E-mail: hrsingh@bitmesra.ac.in

ABSTRACT: The cyclic albino rats of body weight group (120-130 gm.) were intravaginally treated with single dose (0.1 ml) neem oil at various durations to study its anti-fertility efficiency. It was observed that a significant increase in ovarian weight in weekly three months treated rat group to control and a significant decrease in follicle numbers in single ovary in weekly and fortnightly one and three months treated rat group in comparison to control. The disturbed reproductive cycle showed a highly significant increase estrus frequency from control in case of weekly and fortnightly one and three month treated rat group. But in rat group treated for monthly one and three months showed a significant increase. The reversibility showed a highly significant decrease in fortnightly one month in comparison to weekly one month treated rat group whereas monthly one month treatment showed a significant decrease from fortnightly one month treated group. The fortnightly three months treatment showed a significant increase in the reversibility with weekly and monthly three months treated group. All such findings might be an indication of antifertility efficacy of neem oil through intravaginal routs of treatment which affects hypothalemo-pituitary-ovarian axis and through cell mediated immune reaction and monthly three months treatment showed a better reversibility therefore this may through light on the way and duration of IVNT is highly applicable.

INTRODUCTION: Various plants screened for their antifertility potential but none of them are found up to mark. Recently neem *Azadirchata indica A juss* is widely prevalent and highly esteem wonder tree of Indian sub-continent [1]. Earlier findings of various investigators have reported that neem leaves, bark, seeds and oils possesses antifertility properties [2, 3, 4, 5, 6].

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The tree is regarded as "village dispensary" in India as reported in a book called "Neem - a tree for solving global problems" [7]. Its non-wood product is neem oil which contains a number of chemical compounds. Azadirachtin is one of them having a mixture of seven structurally related isomers of tetranortriterpenoid which may use as spermicidal and contraceptive [4, 8]. Neem oil proved to be an ideal contraceptive as it affect the fertility in female albino rats, whether administered Intravaginal or oral routes [9]. *In vitro* and *in vivo* it has spermicidal and anti-implantational action [10, 11]. In this study it was evaluated the antifertility potential of Neem oil through intravaginal treatment.

MATERIALS AND METHODS: For the study of estrous cycle after intravaginal neem oil treatment, intact healthy and cyclic female albino rats of Charl's foster strains of 120-130 gm body weight were employed in this investigation. Rats were divided into seven groups each consists of 12 rats. Their regular estrous cycle was studied by vaginal smear technique of Long and Evans [12] (1922). The rats showing their regular estrous cycle were selected for experimentation. One group of 12 rats was considered as control group and other groups were considered as experimental groups. All the experimental as well as control rats were maintained at uniform animal husbandry condition of light, temperature, water and food.

TABLE 1: DETAILS OF MATERIAL EMPLOYED DURING EXPERIMENTATION.

Sl. No	Status of rats	Group No.	Body weight of rats (gm)	Amount of neem oil (in ml)	Frequency of neem oil	Total duration of experimentation
1	Cont.	I	120– 130	-	-	-
2	Exp.	II	”	0.1	Weekly	1 month
3	Exp.	III	”	0.1	Fortnightly	1month
4	Exp.	IV	”	0.1	Monthly	1 month
5	Exp.	V	”	0.1	Weekly	3months
6	Exp.	VI	”	0.1	Fortnightly	3 months
7	Exp.	VII	”	0.1	Monthly	3 months

Neem oil was procured from khadi and village industries commission, Patna. Different experimental group on the basis of duration of treatment were divided into six groups as shown in table no 1.For treatment of neem oil, experimental rats were held in the head down position. A fine polyethylene tube fitted to syringe with the help of needle was passed gently into vagina as deep as possible. The animals were allowed to stay in the same position for one to two minutes for proper dispersion of oil into vagina. In the similar way control rats were treated with distilled water by following the same time, frequency and duration.

The vaginal smear of control as well as treated rats were studied by the accepted procedure involving identification of the cell types and their relative quantities present in the slide preparation, obtained from the vagina either scarping or swabbing the vaginal wall.

The vaginal smear studied daily at the fixed time every early in the morning throughout experimental period. The frequency of estrus in the reproductive cycle /estrous cycle (in days) was calculated and results were analyzed by student t-test statistically. 6 rats of each experimental group left for the reversibility of normal estrous cyclicity after last treatment. After two days of last treatment, the group of 6 rats out of 12 were sacrificed and ovary were taken out for taking weight and count the follicle numbers in one ovary and other ovary was employed for study of other biochemical parameters.

RESULTS AND DISCUSSION: As indicated in table 2, it was observed that a significant increase in ovarian weight in weekly three months treated rat group to control and a significant ($p<0.05$) decrease in follicle numbers in single ovary in weekly and fortnightly one month treated rat groups and it was up to the level of ($p<0.01$) decrease in weekly and fortnightly three months treated rat groups in comparison to control.

The disturbed reproductive cycle showed a highly significant ($p<0.001$) increase from control in case of weekly one month treated rat group. In case rat group which was treated for fortnightly one month, weekly and fortnightly three months duration showed a highly significant ($p <0.01$)increase of estrus frequency. But in rat group treated for monthly one and three months showed a significant ($p<0.02$) increase.

The reversibility showed a highly significant ($p <0.01$) decrease in fortnightly one month in comparison to weekly one month treated rat group whereas monthly one month treatment showed a significant ($p <0.05$)decrease from fortnightly one month treated group. The fortnightly three months treatment showed a significant ($p <0.05$) increase in the reversibility with weekly and monthly three months treated group.

Physiology of reproduction in mammals is subjected to strict regimen of cyclical changes. Albino rats are polyestrous non seasonal rodent and their estrous cycle has been divided into four stages namely-estrus, metestrus, diestrus and proestrus having 4-7 days length depending upon the genetic and environmental factors [13]. Different stages of estrous cycle and their interconversions are governed by hypothalamo-pituitary-ovarian axis [14, 15].

Genetically determined endogenous programme, perhaps modulated by exogenous factors can also be effective in regulation of reproductive cycle. During metestrus and diestrus there is low level of estrogen in the blood that stimulates the release of FSH which together with LH induces the production of estrogen in the developing follicle. In the proestrus stage there is sharp increase in the concentration of LH in the blood brought about by stimulating effect of relatively high concentration of estrogen in the blood. The proestrus anabolic phase of estrous cycle during which active growth is in progress in female genital tract for implantation while metestrus is a catabolic stage of degenerative changes. The diestrus is a quiescence phase of slow growth.

Neem oil when administered intravaginally observed by a mucosal surface of vagina, reached in circulation and caused an increase in polymorphonuclear leukocyte and a variety of cytokines in various reasons of genital tract and primary sex organs.

The cytokines produced in the ovary caused inhibitory effect on progesterone synthesis and secretion and ultimately making estrogen dominance [5, 16, 17]. This cytokines may be a factor together with endocrinological factor affecting ovarian physiology. Pala et al. (1999)[18] reports indicated that estradiol and progesterone varied during different phases of estrous cycle. During the initial development, the ovarian follicular cells produced estrogen and during the later stage under the influence of LH they produced progesterone together with some estrogen [19].

It might be possible that disturbed endogenous gonadal hormones affect the follicular growth and development and caused increased ovarian weight and decreased follicle number and increase estrous frequency after intravaginal neem oil treatment in different duration treated rats. Shaik *et al* (2009) [20] also reported that higher level of FSH and lower level of LH, estradiol and progesterone level after oral administration of neem oil indicated inhibitory effect on follicles.

In our present finding a significant marked decreased in reversibility days in fortnightly one month to weekly one month treated rat group might be possible due to the immune-modulatory effect of neem oil in different duration treated rats as earlier finding of Riar et.al,1990[8] & 1991[2] and Sai Ram et.al, 1997[21] suggested that volatile fraction of neem oil called Nim-76 had immunomodulatory effect in a dose dependent manner and it may be a causative factor in the resumption of normal estrous cycle.

TABLE 2: EFFECT OF IVNT ON OVARIAN WEIGHT, FOLLICLE NUMBER, ESTRUS FREQUENCY AND REVERSIBILITY OF REPRODUCTIVE CYCLE IN ALBINO RAT

Sl. No.	Group No.	Ovarian weight of single ovary (in mg)	No. of ovarian follicles in single ovary	Estrus frequency (in days)	Reversibility of reproductive cycle
1	I	19.88+1.12	12.00+1.10	0.20+0.03	-
2	II	19.48±0.95	10.00±1.10	0.67±0.04	16.17±0.98
3	III	19.46±1.34	9.60±0.94	0.43±0.03	10.40±0.98
4	IV	19.17±1.28	12.50±0.73	0.38±0.04	06.50±0.92
5	V	21.46±1.76	8.00±0.17	0.40±0.02	07.13±0.97
6	VI	20.29±1.27	09±1.46	0.36±0.01	13.83±2.01
7	VII	20.24±0.79	11.00±1.10	0.31±0.01	05.50±0.92

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