EFFECT OF *NIGELLA SATIVA* ON THE ESTROUS CYCLE AND OVARIAN ACTIVITY IN ALBINO RATS

Sudha Yadav and Dr.Meera Agarwal

Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur-302004.

Corresponding Author: Dr. Meera Agarwal*
Assistant Professor ,Centre for Advanced Studies, Department of Zoology,University of Rajasthan, Jaipur-302004.
E-mail: ysudha90@gmail.com
Phone No.: +91-9460346801

Summary

Present study is aimed to investigate effect of *Nigella sativa* (NSSAq) on reproduction in female rats. For this study rats were divided in control (vehicle treated) and treatment group (NSSAq 200 mg/kg b.wt/day for 40 days). Administration of NSSAq brought about significant decline in weight of ovaries, uterus and vagina. Protein and glycogen level of reproductive tissue were also declined. Significant increase in cholesterol level of reproductive tissues was observed. Diestrous phase of estrous cycle was prolonged. Ovarian follicle showed degenerative changes. Haematological parameters were unaltered. Thus administration of NSSAq in female rats had shown anti-estrogenic nature without altering general physiology.
KEYWORDS: *Nigevlla sativa*, Thecal cells, Haematology, Granulosa cells.

The status of herbal medicine has been fast gaining ground all over the world during the last few decades. The World Health Organization has set up a Task Force on Plant Research for Fertility Regulation with an objective to find new orally active non-steroidal compounds having anti-implantation property (1). A wide variety of synthetic contraceptive agents are available but these are not without side effects. Some plants like *Prangos ferulacia* has been reported to have abortificient effect on the pregnant rats (2). Antifertility effect has been studied in the plant *Woodfordia fructicosa* (3). *Croton roxburghii* and *Zizyphus jujuba* antisteroidogenic activity has been observed in mice (4).

Nigella sativa is one of the most used as herbal medicine all over the world and it is a genus of about 14 species of annual plants in the family Ranunculaceae, native to southern Europe, North Africa and Southwest Asia. *Nigella sativa*, known as kalonji, black cumin is used as a spice in Indian and Middle Eastern cuisine, it is an amazing herb with a rich historical and religious background (5). The plant grows to 20-90 cm tall, with finely divided leaves, the leaf segments narrowly linear to threadlike and its flowers are white, yellow, pink, pale blue or pale purple, with 5-10 petals. The fruit is a capsule composed of several united follicles, each containing numerous seeds (6).When the fruit capsule is matured, it opens up and the seeds contained within are exposed to the air, becoming black in color (7).

Seeds of *Nigella sativa* are source of the active ingredients of this plant. It is the black seed referred by the prophet Mohammed as having healing powers (8). Four dolabellane-type diterpene alkaloids have been isolated from the seeds of Nigella sativa (9,10). *Nigella* sativa seeds contain other ingredients, including nutritional components such as carbohydrates, fats, vitamins, mineral elements, and proteins, including eight of the nine essential amino acids (11, 12). It has hypoglycemic effect (13) and used as immunopotentiating, immunomodulating and interferon like activities (14,15).

The antifertility potential of this plant in females has not been explored in detail and hence the present study was undertaken. The present work was conducted to monitor the antifertility effect of *Nigella sativa* seed extract (NSSAq) on reproductive organs and fertility in adult female albino rats.

Material and Method

Test substances used:
The seeds of *Nigella sativa* were purchased from the local market and authenticated by the Department of Botany, University of Rajasthan, Jaipur. All the chemicals used in the experimentation were of analytical grade.

Preparation of the *aqueous* extract:
Aqueous extract of the seeds was prepared according to the method of the National Institute of Health & Family Welfare, New Delhi, India. The seeds were grinded with a electrical grinder into powder and the aqueous extracts was prepared by soaking 100mg of powdered seeds with 500ml of Double-distilled water and kept for 12 hrs .The resulting extract was filtered using What man No. 1 filter paper and was allowed to dry at reduced pressure and finally lyophilized. The drug yield was calculated to be 10.02% i.e. 10.02 gm extract was formed from 100 gm powder of seeds of *Nigella sativa*. The crude extract of the seeds (NSSAq) was dissolved in distilled water to the required concentration.

Animal Model Used:

20 Adult female albino rats, weighing 200-210 g were used for the present study. The females showing regular estrous cycle were selected by observing vaginal smear for two consecutive cycles. They were maintained at standard laboratory conditions. Animals were housed individually in the polypropylene cages and maintained under standard conditions (12-h light/dark cycle; 25±3°C temperature; 35–60 relative humidity), and were fed on standard rat feed procured from Aashirwad Food Ltd., Chandigarh (India). The water was given *ad libitum.*

Treatment Protocol:

To study the effect of aqueous extract of *Nigella sativa* on the estrous cycle, Body and Organ weight, Tissue Biochemistry of reproductive organs and Fertility study were done. The animals were divided into two groups; group 1 served as control (Vehicle treated) and group 2 received aqueous extract at dose of NSSAq 200 mg/kg b.wt for 40 days. During this period the vaginal smear of the rats were examined daily early in the morning (6.00-7.00 am) as per the method described by Zarrow et al (16) and reviewed by Cooper et al (17). The duration of the estrous cycle together with that of various phases was determined as described by Makonnen et al (18). On the day 41st the rats were sacrificed under light ether anesthesia. The ovaries, uterus and vagina were dissected out, weighed and half of the tissues were kept at -20°C for the biochemical estimations. Ovary was fixed in Bouin's solution for histopathology. Ovarian tissues were dehydrated in an ascending grade of alcohol ,cleared in xylene and embedded in paraffin wax after the method of Drury and Wallington (19). Serial sections of 5microns thick were obtained using microtome. The deparaffinized sections were stained routinely with haematoxylin and eosin. Photomicrographs of the desired results were obtained using light microscope.

Blood Analysis:

Total erythrocyte count and Haematocrit were measured by Microhaematocrit method as described by Schalm et al (20). Total leucocyte count was estimated as described by Lynch et al (21) and the Haemoglobin level was estimated by Cynomethanoglobin method as described by Makrem A (22).

Biochemical Estimation:

Protein and glycogen level were estimated according to the method of Lowry et al (23) and Montgomery (24). The cholesterol content was measured using the method of Oser (25).

Ethical Aspects:

The study was approved by the ethical committee, Center for Advance Studies, Department of Zoology, University of Rajasthan, Jaipur (India). The Indian National Sciences Academy, New Delhi (INSA, 2000), (26) guidelines were followed for maintenance and use of experimental animals.

Statistical analysis:
Statistical significance between the control and experimental data were subjected to one way analysis of variance (ANOVA).

Results

Effects on body and organs weight:
The treatment of rats with the *Nigella sativa* seeds extract caused almost significant increase in the body weight of treated animals; whereas, a highly significant decrease in weight of Ovaries, Uterus and Vagina in the treated group were observed (Table: 1).

Table 1. Effect of *Nigella sativa* seeds extract on Body and Organ weight of female albino rats

TREATMENT GROUPS	BODY WEIGHT (g)		REPRODUCTIVE ORGAN WEIGHT(mg/100gm b. Wt.)		
	Initial	Final	Ovaries	Uterus	Vagina
GROUP 1: Control	202±1.48	205±1.67	44±1.52	238±2.12	219±1.31
GROUP 2 : N. sativa treated	208±1.71 P<0.028	212±1.31* P<0.010	26±1.05*** P<0.000	217±2.49*** P<0.000	175±1.00*** P<0

*** Highly significant (P<0), ** Significant (P<0.05), * Almost significant (P<0.01-0.04), ns Non-significant (P<0.1) compare to control.

Effect on estrous cycle of rats:
Effect of *Nigella sativa* on the different phase of estrous cycle was found to be a highly significant decrease in the Proestrous and Metaestrous phases. Whereas a highly significant increase in the duration of Estrous and Diestrous phases in experimental animals was observed when compared to their respective control.

Table 2.Effect of *Nigella sativa* seeds extract on Estrous Cycle of female albino rats

TREATMENT GROUPS	Proestrous phase	Estrous phase	Metaestrous phase	Diestrous phase
GROUP 1: Control	1.33±0.01	0.82±0.01	0.94±0.01	1.92±0.01
GROUP 2 : N.sativa treated	0.42±0.01*** P<0.000	0.89±0.01*** P<0.004	0.76±0.01*** P<0.000	2.98±0.01*** P<0

***Highly significant (P<0), ** Significant (P<0.05), * Almost significant (P<0.01-0.04), ns Non-significant (P<0.1) compare to control.

Effect on tissue Biochemistry:

The total protein and glycogen content decreased highly significantly in the Ovaries, Uterus and Vagina with comparison to the respective control. However, there was a highly significant increase in the Cholesterol content of ovaries, uterus and vagina of the experimental animals (Table: 3,4,5).

TABLE 3. Effect of *Nigella sativa* seeds extract on Protein Content of female albino rats

***Highly significant (P<0), ** Significant (P<0.05), * Almost significant (P<0.01-0.04), ns Non-significant (P<0.1) compare to control.

TREATMENTGROUPS	Protein content(mg/gm)		
	OVARY	UTERUS	VAGINA
GROUP 1: Control	180±0.70	178±0.89	142±1.38
GROUP 2 : N. sativa treated	134±0.83*** P<0	138±1.26*** P<0	118±1.58*** P<0

Table 4. Effect of N*igella sativa* seeds extract on Glycogen Content of female albino rats

TREATMENT GROUPS	Glycogen content(mg/gm)		
	OVARY	UTERUS	VAGINA
GROUP 1: Control	8.02±0.05	16.28±0.13	8.13±0.06
GROUP 2 : N. sativa treated	4.60±0.49*** P<0.0001	10.02±0.12*** P<0	4.12±0.11*** P<0

***Highly significant (P<0), ** Significant (P<0.05), * Almost significant (P<0.01-0.04), ns Non-significant (P<0.1) compare to control.

Table 5. Effect of *Nigella sativa* seeds extract on Cholesterol Content of female albino rats.

TREATMENT GROUPS	Cholesterol content(mg/gm)		
	OVARY	UTERUS	VAGINA
GROUP 1: Control	8.18±0.17	3.98±0.08	5.10±0.07
GROUP 2 : N. sativa treated	12.02±0.42*** P<0	8.11±0.10*** P<0	8.50±0.22*** P<0

***Highly significant (P<0), ** Significant (P<0.05), * Almost significant (P<0.01-0.04), ns Non-significant (P<0.1) compare to control.

Effect on Histopathology of rats:

The ovary of the control group showed normal histological features, illustrating a well defined zonal granulosa surrounding the nucleated oocyte and compact theca folliculi and the presence of some primordial follicles. The ovaries of the control rat exhibiting mature and healthy follicles with distinct Thecal layers. Follicles of all sizes are present from nest of oocyte to large Graffian follicle (Figure7a). The ovaries of the *Nigella sativa* treated group showed some cellular hypertrophy of the theca folliculi, complete distortion/destruction of the basement membrane separating the theca folliculi from the zona granulosa. Degenerative and atrophic changes were observed in the developing oocyte. Zona pellucid surrounding the oocyte was not visible in the treated ovary. Graffian follicles showed pyknosis of the nuclei of granulose and Thecal cells (Figure7b). Extensive degenerative changes of nuclei of oocyte and granulose cells is similar to that caused by gossypol acetate (27, 28).

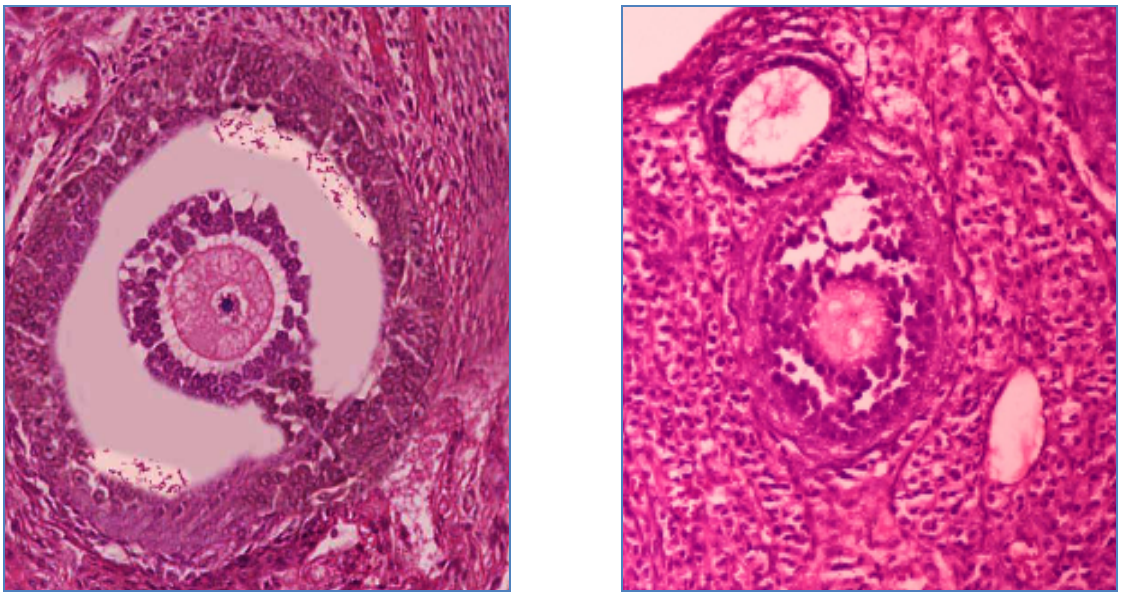

Figure: 7 . (a) Micrographs of ovary of control –Haematoxylin and Eosin stain and (X200) and (b) Treatment group–Haematoxylin and Eosin stain (X200).

Effect on Haematological studies:

There were non-significant changes in the haematological parameters in both the group.

Table 6.Effect of *Nigella sativa* seeds extract on Heamatological parameters of female albino rats

TREATMENT GROUPS	RBC COUNT million/mm3	WBC COUNT Per. Cu.mm.	HEAMOGLOBIN gm %	HEAMATOCRIT %
GROUP 1: control	3.68±0.01	7.2±0.13	14.62±0.01	48.24±0.01
GROUP 2 : N.sativa treated	3.72±0.02^{ns} P<0.169	7.4±0.16 ^{ns} P<0.346	14.40±0.72 ^{ns} P<0.760	47.84±0.39 ^{ns} P<0.335

***Highly significant (P<0), ** Significant (P<0.05), * Almost significant (P<0.01-0.04), ^{ns} Non-significant (P<0.1) compare to control.

Discussion

Many crude extracts and active principles derived from medicinal plants were evaluated for their antifertility effects in animal models (29, 30). Plant products as contraceptive will be more acceptable for economic reasons and less side effects than chemical agents.The present study revealed a significant increase in the level of cholesterol which may be the probable cause of increase in the body weights as cholesterol is the forms of fat present in the body (31). The present work reports that the oral administration of *Nigella sativa* seed extract to the female rats lead to declined female rat fertility as it caused estrogen inhibition due to its antiestrogenic nature. The antiestrogenic nature of *Nigella sativa* was shown by the decrease in the weight of ovaries and uterus since antiestrogenic substance decreases the net weight of the uterus (32). Sharma and Bhinda (33) also noticed similar effects on the reproductive organs weight following the oral administration of steroidal extract of *Trigonella foenum-graceum* seeds in female rats.The change in estrous cycle may be due to the hormonal imbalance and decrease in the level of gonadal steroids which are essential for normal functioning of the gonads (34).

Cholesterol derived from the different sources is the precursor for the steroidogenesis of ovarian endocrine tissue (35). Highly significant increase in the cholesterol level of the group receiving extract indicates that cholesterol was not used for steroidogenesis hence accumulated in the ovary (36). The highly significant decrease of the glycogen content of uterus treated with *Nigella sativa* confirms the antiestrogenic nature of the drug (37). Also, the highly significant reduction in protein content of the female genital tract of *Nigella sativa* treated rats suggests an inhibition of estrogen production (38).

It is well established that tests of blood parameters form the very front-line investigations on which diagnosis of various diseases is based. Unaltered haematological parameters of the treatment group , suggests that NSSAq did not cause any adverse effects on general health of the animals.

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Rats were kept for two weeks before the start of the experiment for acclimatization. The animals were then housed 5/cage and received normal basal diet and tap water ad libitum at a room temperature of about 28 Â± 2Â°C, a room humidity of 60 Â± 5%, and a 12 h light and 12 h dark cycle. 2.5. Experiment Design. The animals were divided into 4 groups, each consisting of 10 rats. Effect of Nigella. sativa of the estrous cycle and ovarian activity in. albino rats. Pharmacologyonline.Â On the contrary, rats pretreated with MTZ and HSP showed significant decrease in NO, MDA levels, and MPO activity, while, activities of SOD and GPx were increased ($P<0.001$). Oxidative stress induced by CP in the rat ovary causes infertility in the female rats. HSP and MTZ could reverse this effect and provide protection of fertility against CP-induced toxicity.