

## EFFECT OF NIGELLA SATIVA ON NUMBER OF CYSTIC FOLLICLES IN LETROZOLE INDUCED POLYCYSTIC OVARIES IN MICE

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### ABSTRACT

**Objective:** To observe the protective effect of Nigella sativa on number of cystic follicles in Letrozole induced polycystic ovaries in mice.

**Study Design:** Laboratory based randomized control trial.

**Place and Duration of Study:** Department of Anatomy, Army Medical College in collaboration with National Institute of Health from Nov 2014 to Nov 2015.

**Material and Methods:** Forty female BALB/c mice were selected and divided in four groups, each having 10 animals. Group A served as control and was given normal diet. Group B was given Letrozole at a dose of 1milligram/kilogram body weight. Group C was treated with Letrozole for eight weeks at a dose of 1milligram/kilogram body weight and Nigella sativa seeds powder at a dose of 10grams/kilogram body weight once daily starting at 22 day and continued up to eight weeks. Group D was treated with Letrozole for eight weeks at a dose of 1milligram/kilogram body weight and Nigella sativa oil at a dose of 4milliliter/kilogram body weight once daily starting at 22 day and continued up to eight weeks. Animals were dissected a day after last dose. Size, shape, color and consistency of ovary was observed. Right ovary was processed, embedded and stained for histological study. Number of cystic follicles were counted and noted.

**Results:** Significant number of cystic follicles was observed in ovaries of animals of group B as compared to group A. While their number decreased significantly in group C and D as compared to group B.

**Conclusion:** Nigella sativa seeds powder and its oil, both have a similar protective effect on histomorphology of ovary of polycystic ovarian syndrome (PCOS) in mice by decreasing the number of cystic follicles.

**Keywords:** Cystic follicles, Letrozole, Nigella sativa, Ovary.

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### INTRODUCTION

Polycystic ovarian syndrome (PCOS) is one of most common inheritable endocrine disorder. It influences almost 10% of women of reproductive age all over the world<sup>1</sup>. PCOS is characterized by multiple small follicular cysts located just beneath the surface of ovary and can be visualized by ultrasonography. These cysts are the immature follicles that could not mature to ovulate due to hormonal imbalance and leads to infertility in these women<sup>2</sup>. In recent years it has become apparent that PCOS patients present not only with reproductive features but they also

develop characteristic metabolic disturbances including insulin resistance and dyslipidemia<sup>3</sup>.

Letrozole is used for treatment of hormone receptive positive breast cancers in postmenopausal women. It is an aromatase inhibitor. Aromatase P450 is an enzyme present in placenta, ovaries and testis and in other human tissues and responsible for conversion of testosterone and androstenedione into estradiol and estrone respectively. Hence, Letrozole by inhibiting this aromatase enzyme, leads to hyperandrogenism and PCOS<sup>4</sup>.

Nigella sativa Linn, commonly known as black seeds, have a long history of therapeutic uses. Health benefits of Nigella sativa have been extensively reported. Effect of Nigella sativa on ovary has yet not been reported. The rationale of

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current study is to observe the protective effect of *Nigella sativa* seeds and its oil on histomorphology of ovary in PCOS mouse model.

## MATERIAL AND METHODS

The Laboratory based randomized control trial was conducted at Department of Anatomy, Army Medical College Rawalpindi, in collaboration with the National Institute of Health (NIH), Islamabad. It spanned for one year

Letrozole tablets (Femara) were purchased from Novartis Chemicals Import and Export Corporation (product no SO102). *Nigella sativa* seeds were purchased from National Agriculture Research Center. The plant material was identified and authenticated as *N. sativa* by Dr. Zafar, Botanist at Quaid - e - Azam University, Islamabad. Seeds were powdered in electrical grinder for group C and oil was extracted for group D.

**Table-1- showing Comparison of mean values of numbers of cystic follicles among group A, B, C and D.**

	Group A Mean±SD (n = 10)	Group B Mean ± SD (n = 10)	Group C Mean ± SD (n=10)	Group D Mean ± SD (n=10)	p -value
Number of cystic follicles	0.0 ± 0.0	5.2 ± 2.09	1.1 ± 0.3	1.30 ± 0.4	0.001

**Table-2- Intergroup comparison of p value of number of cystic follicle among groups A, B, C and D.**

	Group Avs B	Group B vs C	Group A vs C	Group B vs D	Group A vs D	Group C vs D
Number of cystic follicles	0.001	0.001	0.126	0.001	0.052	0.97

p value < 0.05 is statistically significant.

i-e Nov 2014 to Nov 2015. Forty, adult non pregnant females BALB/c mice, 6-8 weeks old, weighing 30- 35gm were used for the study. They were kept in the NIH animal house. Animals were divided into four groups and fed with NIH laboratory diet for two months.

Animals in group A served as control and were given plain water by oral gavage tube. Group B was given Letrozole at dose of 1milligram/kilogram body weight once daily for two months by oral gavage tube<sup>5</sup>. Group C was treated with Letrozole for eight weeks at dose of 1mg/kg body weight and *Nigella sativa* seeds powder at a dose of 10grams/kilogram body weight once daily starting at 22 day and continued up to eight weeks by oral gavage tube<sup>6</sup>. Group D was treated with Letrozole for eight weeks at dose of 1mg/kg body weight and *Nigella sativa* oil at a dose of 4milliliter/kilogram body weight once daily starting at 22 day and continued up to eight weeks by oral gavage tube<sup>7</sup>.

At the end of two months, the animals were dissected. Ovary was histomorphologically studied for size, shape, color and consistency. The right ovary was taken to maintain uniformity and was placed in 10 per cent formalin. The ovary was processed and embedded. Tissues were cut into 5-micron thick sections using rotary microtome. The sections were stained with haematoxylin and eosin (H&E) for routine histological study of ovary under light microscope.

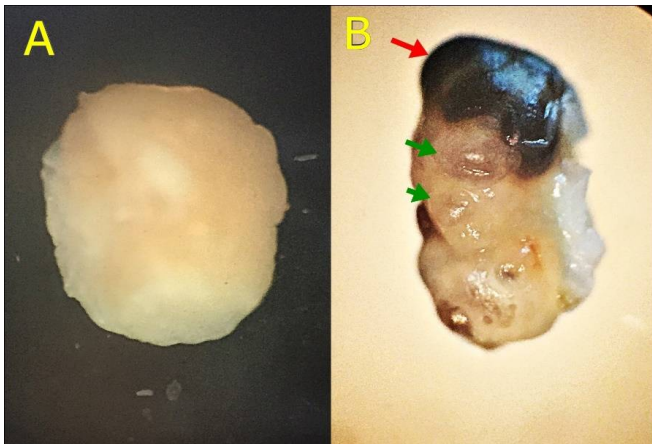
Cystic follicles, fluid filled, having a thickened theca cell layer and a thin granulosa layer, were identified and their number was counted<sup>8</sup>. One slide per specimen was observed. X4 objective was used to count number of cystic follicles.

Data was analyzed using statistical package for social sciences version 22. Descriptive statistics were used to describe the results. Quantitative parameters were expressed as

mean  $\pm$  standard deviation. Significant difference was determined using one way analysis of variance (ANOVA) followed by post Hoc Tuckey test. Results having  $p < 0.05$  were considered significant.

## RESULTS

Forty adult non-pregnant female BALB/c mice, 6-8 week old, with an average weight  $42.3 \pm 2.29$  were used in the experiment. After dissection of animals, ovary of animals of control group were light pink in color, elliptical in shape and firm in consistency with beaded appearance (fig-1). Histologically ovary was covered by

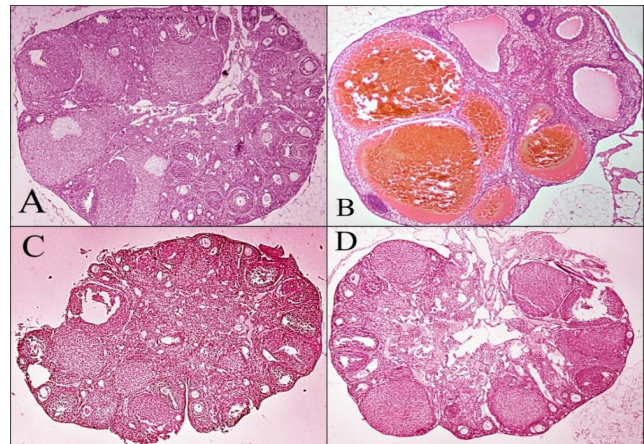


**Figure-1: Photograph showing ovary (A) of animal # A2 of control group and polycystic ovary (B) with hemorrhagic cyst (red arrow) and non-hemorrhagic cyst (green arrow) of animal # B3 of group B under dissecting microscope.**

simple cuboidal epithelium. Just beneath epithelium was tunica albugenia consisting of collagen fibers. Ovarian tissues was divided into outer cortex consisting of follicles in different stages of development and inner medulla containing stroma and blood vessels. There were no cystic follicles in ovary of control group (fig-2 A).

H&E stained section of group B showed slight changes in normal cytoarchitecture of ovarian tissues. Color was changed from pink to pale whitish having some black spots over it (fig-1 B). Histologically, epithelium was distorted at certain places. Stroma was apparently

increased. Multiple cystic follicles were found in the cortex (fig-2B). The mean number of cystic follicles was  $5.20 \pm 2.098$ . When compared with group A,  $p$  value was 0.001 which was statistically significant. Regarding ovaries of group C and D, color was light pink with no black spots and consistency was firm in both groups C and D. Ovarian histomorphology in group C and D was analogous to control group. The mean number of cystic follicles was  $1.1 \pm 0.3$  and  $1.30 \pm 0.4$  in group C and D respectively (fig-2 C,D) which was statistically insignificant when group A, C and D were compared with each other but significant when group B was



**Figure-2: Photomicrograph of histological section of ovary of control and experimental groups showing multiple cystic follicles in group B while no cystic follicles are seen in group A, C and D.**

compared with C and D.

## DISCUSSION

Polycystic ovarian syndrome is gaining a lot of attention these days for being affecting women of childbearing age and becoming a common cause of infertility<sup>9</sup>. Increased insulin resistance with hyperandrogenism is observed in obese and also in non-obese women with this syndrome<sup>10</sup>. The objective of the study is to see the protective effect of Nigella sativa in Letrozole induced polycystic ovarian syndrome.

Number of cystic follicles had been significantly increased in group B as compared to group A. It is due to androgen excess with

decreased estrogen within the ovary which leads to follicular atresia and development of sub capsular cystic follicles in ovaries of animals of group B<sup>11-13</sup>. Insulin resistance along with compensatory hyperinsulinemia develops in most of patients with PCOS. Nigella sativa, being a hypoglycemic agent improves insulin sensitivity and compensatory hyperinsulinemia<sup>14,15</sup>. Raised insulin levels stimulated ovaries to produce more testosterone which in turn induces formation of cystic follicles<sup>16</sup>. Hence, serum androgen concentration decreases in females in whom insulin concentrations are lowered by insulin sensitizing agents<sup>17,18</sup>. Due to this reason, number of cystic follicles had decreased significantly in animals of group C and D as compared to group B. Hence Nigella sativa improves insulin resistance and compensatory hyperinsulinemia in PCOS mice in oil as well as in seeds form, which in turn decreases the serum testosterone levels and number of cystic follicles in ovaries of animals of group C and D.

## CONCLUSION

Nigella sativa has a protective effect on ovary of Letrozole induced PCOS by decreasing number of cystic follicles in its oil as well as in seeds form.

## CONFLICT OF INTEREST

The study has no conflict of interest declared by any of the author.

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