

## ORIGINAL ARTICLE

# Impact of *Nigella Sativa* on Body Weight, Volume and Weight of Ovary in Letrozole Induced Polycystic Ovarian Syndrome in Mice

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## ABSTRACT

**Background:** Polycystic ovary syndrome (PCOS) influences almost 18% of women in their child bearing age and responsible for reproductive, metabolic and psychological dysfunctions.

**Aim:** To examine remedial effect of *Nigella sativa* on body weight, weight, volume and gross appearance of ovary in polycystic ovarian syndrome induced by Letrozole in mice.

**Study Design:** Randomized control trial.

**Methodology:** Mature female BALB/c mice (n=40) were placed in four groups having ten in each. Group-1 was fed with normal diet. Rests of three were treated with Letrozole at dose of 1mg/kg body weight once daily for eight weeks. Groups (3&4) were also given *Nigella sativa* seeds powder and oil at dose of 10g/kg body weight and 4ml/kg body weight respectively, once daily commencing at 23 day and remained at end of eight weeks. Ovaries were identified, shape and color were recorded then weighed using a digital precision balance and volume in mm<sup>3</sup> by water displacement method.

**Statistical analysis:** Data analyzed by SPSS 21.0v.

**Results:** Body weight of group-2 animals, weight and volume of their ovaries was increased in comparison to group-1 but reduced to group-3 and group-4 animals. Blackish spots on group-2 animal ovaries were observed. Ovaries of groups (3&4) animals were normal in appearance, volume and weight

**Conclusion:** This study concluded that *Nigella Sativa* seeds in both solid and liquid state leave identical curative impact on body weight, weight, volume and gross appearance of ovaries in polycystic ovarian syndrome in mice.

**Keywords:** Body Weight, Letrozole, *Nigella Sativa*, Ovaries and Polycystic Ovarian Syndrome

## INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of frequently occurring reproductive problem influencing almost 18% of women in their child bearing age and responsible for reproductive, metabolic and psychological dysfunctions. However, two out of three domains, when present then it confirms the development of PCOS. They are anovulation, raised androgen levels and presence of cysts in the ovaries<sup>3</sup>. In PCOS, raised androgen levels are responsible for increased estrogen and progesterone production in the follicles. These females are mostly overweight. Obesity has negative impact on oocyte development and implantation<sup>4</sup>.

In healthy females, enzyme P450 aromatase is responsible for conversion of testosterone to estradiol. Reduced action of this enzyme is responsible for hyperandrogenism and development of PCO<sup>5</sup>. In current years, multiple researches have proved that females having polycystic ovarian syndrome present with fertility problems or pregnancy complications as well as they evolve certain metabolic derangements including dyslipidemia and resistance to insulin hormone<sup>6</sup>.

Letrozole is an effective, nonsteroidal, aromatase inhibitor, originally used for postmenopausal breast cancer therapy<sup>7</sup>. Therefore Letrozole, by obstructing the enzyme, is responsible for preventing estrogen synthesis and more androgen production that in turn leads to development of polycystic ovarian syndrome<sup>8</sup>. Herbal drugs have definitive role in treatment of PCOS. They show gradual curative effect with minimal side effects<sup>9</sup>. *Nigella sativa* Linn, frequently appreciated as black seeds, historically used for treatment of several diseases. *Nigella sativa* oil can significantly decrease the raised blood glucose level in experimental rats as well as it also has antioxidant properties. Hence, it has a beneficial role in female reproductive problems<sup>10</sup>. It also has a positive effect on lowering serum cholesterol levels as it decreases cholesterol

synthesis by hepatocytes and also decreases its absorption from small intestine<sup>9</sup>. Therefore, due to its hypolipidemic activity, oil of *Nigella sativa* has a protective role in atherosclerosis<sup>10</sup>. The rationale of present study was to monitor ameliorative action of powder as well as oil of *Nigella sativa* seeds on body weight, weight, volume and gross appearance of ovary in mouse model of PCOS.

The objective of the study was to examine remedial effect of *Nigella sativa* on body weight, weight, volume and gross appearance of ovary in polycystic ovarian syndrome induced by Letrozole in mice.

## METHODOLOGY

This randomized experimental study was carried out for 12 months at Anatomy Department of Army Medical College Rawalpindi, in team work with National Institute of Health (N1H) Islamabad. Forty, mature, non-pregnant females, BALB/c mice of age 8-10 weeks and weight 35- 40gm were chosen. They were selected by non-probability consecutive sampling technique and grouped by random number method in four groups. Mice were retained in N1H animal house and nourished with lab feed for eight week<sup>11</sup>. Group-1 (control): Animals were fed plain water with diet. Group-2: was treated with Letrozole 1 mg/kg body weight once daily for eight weeks<sup>12</sup>. Group-3 was given 1 mg/kg body weight Letrozole once a day for eight weeks and 10grams/kilogram body weight of *Nigella sativa* seeds powder daily that starts at day 22 and continues at end of eight weeks<sup>13</sup>. Group-4 was dealt with 1 mg/kg body weight Letrozole once a day for eight weeks and 4milliliter/kilogram body weight of *Nigella sativa* oil once daily that also starts at day 22 till end of eight weeks<sup>14</sup>. All mice were fed by oral gavage tube. Letrozole tablets (Femara) were bought from Novartis Chemicals Import and Export Corporation (product no S0102). The seeds of herb were picked from National Agriculture Research Center. They were driven in electrical grinder to make a powder for group-3 and its oil was squeezed out for group-4. Animals were weighed at

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commencement and termination of trial just before the sacrifice of animals to observe any change in weight gain. At termination of trial, animals were sacrificed. Ovaries lying at fimbriae of fallopian tube were identified. Weight of the ovary was recorded in mg using a digital precision balance<sup>1</sup>. Volume of ovary was recorded in mm<sup>3</sup> by water displacement method<sup>2</sup> (fig-1). Shape of the ovary was observed and recorded as ellipsoid or round. Color of ovary was observed and recorded as light pink or white. Any other change as presence of hemorrhages or cyst on gross examination if present was recorded.

Fig-1: Measurement of ovarian volume by water displacement method



Table-1: Comparison of parameters between control group and other groups (n=40)

	Group-1	Group-2	Group-3	Group-4	P-value
Animal Weight gain (gm)	9.40±0.964	13.26±2.07	10.59±1.14	11.50±1.06	0.001*
Ovary weight(mg)	15.85±1.26	19.85±1.66	16.48±1.13	16.72±2.03	0.001*
Volume of Ovary (mm <sup>3</sup> )	10.0±0.0	14.0±2.1	1.0±2.1	11.0±2.1	0.001*

\*Statistically significant

Table-2: Comparison of parameters between control group and other groups (n=40)

	G-1vs G-2	G-1 vs G-3	G-1 vs G-4	G-2 vs G-3	G-2vsG4	G-3vsG-4
Animal Weight gain (gm)	0.001*	0.246	0.009*	0.001*	0.035*	0.467
Ovary weight(mg)	0.001*	0.721	0.447	0.001*	0.001*	0.978
Volume of Ovary (mm <sup>3</sup> )	0.001*	0.616	0.616	0.004*	0.004*	1.0

\*Statistically significant

## DISCUSSION

Polycystic ovarian syndrome is becoming a subject of high interest now a days as it is influencing more than 18% females in their reproductive age and responsible for fertility problems. Females having symptoms of PCOS are mostly obese, presenting commonly with hyperandrogenism and insulin resistance. The insulin increases unbound and active androgen level by ameliorating ovarian androgen production. In addition, it also arrests sex hormone binding (SHBG) formation by liver. Then raised insulin concentration which occurs in compensation to insulin resistance is mostly responsible for increased androgen concentration. The main point of this research was to monitor ameliorative impact of *Nigella sativa* seeds and oil on body weight gain, weight, volume and gross appearance of ovaries of animals in whom polycystic ovarian syndrome is induced experimentally by the drug Letrozole.

In present research, animals of all groups were active and healthy throughout the study. The body weight of animals was statistically significant when group-1 was compared with group 2. Symptoms of PCOS are interconnected with raised androgen levels and weight gain. In fact, raised androgen levels would increase the amount of adipose tissues and it might be responsible for weight gain in these mice<sup>15</sup>. Insulin resistance and compensatory hyperinsulinemia develops in most of PCOS patients. The raised insulin levels might stimulate appetite center in brain hence, resulting in increase of weight<sup>16</sup>. On the other hand, increase in body weight of animals of group 3 and 4 was comparatively low when compared with group 2 and it was statistically significant, but it was insignificant when compared with group 1, it was close to the normal. Another study on patients diagnosed as a case of metabolic syndrome showed that intake of *Nigella sativa* oil results in reduction in body weight. According to

**Statistical analysis:** The data was summarized and analyzed on Statistical package for social sciences (SPSS) version 21.0. Descriptive statistics was applied for interpretation of results. Quantitative parameters were elicited as mean ± SD. One-way analysis of variance (ANOVA) followed by post Hoc Tuckey test was applied to find out remarkable difference. Results with p<0.05 were noteworthy.

## RESULTS

Parameters like body weight gain, weight of ovary and volume of ovary for animals of all groups was presented as mean ± SD in table -1.

Intergroup comparison of p-values for parameters like weight gain, weight and volume of ovaries among control group and experimental groups was shown in table-2.

this study, over weight and obesity are associated with insulin resistance and *Nigella sativa* oil as well as its seeds results in weight loss in these patients with metabolic syndrome by improving their insulin resistance<sup>17</sup>. *Nigella sativa* is useful in patients having diabetes and glucose intolerance because it not only decreases appetite but it also reduces intestinal absorption of glucose. Then by decreasing gluconeogenesis by liver it maintained normal glucose and also cholesterol levels. In addition, *Nigella sativa* increases insulin secretion from beta cells of pancreas<sup>13</sup>. This effect of *Nigella sativa* might be related to its action on lipid metabolism as well as alteration in plasma insulin levels<sup>18</sup>.

The gross appearance of ovary of animals in all groups was noted. Color of ovary of animals in group-2 was different as compared to groups (1,3 and 4). It changes from normal pinkish white to pale white along with blackish spots on surface. The ovarian weight of animals of group-2 was increased as compared to group-1 and it was statistically significant. This increased ovarian weight was due to increased stromal tissue and increase in number of follicles including cystic follicles<sup>19</sup>. Ovarian weight of animals of group-3 and 4 was reduced as compared to group-2 and it was statistically significant. In PCOS, insulin resistance and compensatory hyperinsulinemia was the main culprit. It is responsible for hyperandrogenemia caused by theca cells of ovary. In this way, *Nigella Sativa*, being an insulin sensitizing agent played a role in decreasing ovarian weight, improves ovarian morphology and function<sup>20</sup>. Oil of *Nigella sativa* also improved polycystic ovarian morphology as well as metabolic disorders by its antiandrogenic action and also ameliorate PCOS related dyslipidemia<sup>21</sup>.

**Limitations:** Our limitations included time with financial constraints and limited resources. No genetic workup was done.

## CONCLUSION

This study concluded that *Nigella Sativa* has an ameliorative impact on body weight gain, weight, volume and gross appearance of ovaries of animals in Letrozole induced PCOS by decreasing fasting blood glucose levels and improving insulin resistance in its liquid as well as in its solid state.

**Author's contribution: AN&NA:** Conceptualized the study, analyzed the data, and formulated the initial draft, **KN&SAJ:** Contributed to the histomorphological evaluation, **MSA&AA:** Contributed to the analysis of data and proofread the draft, **TL:** Contributed to the proofreading the manuscript for intellectual content.

**Conflict of interest:** None

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