Aqueous Extract of *Nigella sativa* Seeds Suppresses Testicular Steroidogenesis in Mice Leydig Cells in Vitro

SHEIKH A. SAEED, NAHEED ANWAR, QAISER JABEEN, and ANWAR H. GILANI

*For author affiliations, see end of text.*

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**ABSTRACT**

*Nigella sativa* (black seed) is an important medicinal herb with folkloric use in wide range of diseases. It is well studied for its biological activities. However, there is limited information regarding its effect on the male reproductive system. This study describes the effect of the aqueous extract of *N. sativa* (NSE) on testicular steroidogenesis from mice Leydig cells in vitro. Mice testicular cells were incubated in a media containing either no treatment or NSE or LH alone or combination of LH and NSE. Incubations were carried out for three hours in a shaking water bath at 34°C. Testosterone was measured by radioimmunoassay. At all doses, NSE significantly (p < 0.05) inhibited both basal and LH-stimulated in vitro testosterone secretion. At a dose of 1000 µg, NSE inhibited 52% of basal testosterone and 97% of LH-stimulated testosterone, compared to control (0.32 ± 0.008 ng/ml) and LH alone (0.33 ± 0.01 ng/ml) respectively. Thus, it is concluded that both the basal and the LH-stimulated secretion of testosterone from Leydig cells are suppressed significantly in the presence of different doses of NSE in vitro. However, further studies are needed to explore the effect of chronic treatment with NSE in male and its potential to be used as a contraceptive in male.

**Keywords:** Nigella sativa, Black seed, Male reproductive system, aqueous extract, Leydig cell, testosterone
Materials and Methods

Preparation of the crude extract

Dried black seeds of Nigella sativa were purchased from the local market in Karachi. The plant seeds were cleaned of any adulterant materials. NS seeds were ground with an electric grinder into a coarse powder. A measured quantity was soaked in 70% aqueous methanol (30:70) at room temperature for 3 days and then filtered. The combined filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure. Extract was stored at -4°C until used for biological activity.

Leydig cells preparation

Three bulbe male mice (weight 36 ± 2 g) were used for each experiment. Animals were obtained from the AKU animal facility, where they were maintained under standard conditions of 14-hour light and 10-hour dark cycle. Direct effect of aqueous extract of Nigella sativa seeds (NSE) on testosterone secretion was studied by dissection out immediately and de-capsulated. Leydig cells were isolated as described earlier.[22] Leydig cells (80,000/tube) were pre-incubated for 1 h to remove the endogenous testosterone, the media were replaced with either fresh medium or medium containing graded doses of crude aqueous extract of NS seeds (1.0-1000 µg/tube).

Radioimmunoassay

Testosterone was measured in the incubation medium directly by a highly-sensitive RIA according to the WHO protocol, using 3H-labeled testosterone, as tracer. The bound and unbound fractions were separated by addition of 0.1% activated charcoal. Radioactivity was measured in a scintillation counter. Testosterone concentration was calculated by logit-log transformation [23]. The sensitivity of T assay was 0.0125 ng and the intra-assay coefficient of variation was less than 10%.

Statistical analysis

Data were expressed as mean ± S.E.M. Results were analyzed for statistical significance using an independent t test on SPSS. A p value < 0.05 was considered significant.

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Fig 2. Effect of aqueous extract of Nigella sativa (NSE) on LH-stimulated testosterone production by mice Leydig cells in vitro.

*Significant difference between control and treated groups (p<0.05)
†Significant difference between LH alone and treated groups (p<0.05)
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