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

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Received May 12, 2012; Revised August 27, 2012; Accepted October 9, 2012

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

The seeds of *Nigella sativa* Lin. (*Ranunculaceae*), for its different biological activities which includes commonly, known as black seed or black cumin and 41 locally as Kalonji have been used in folk (herbal) 42 antioxidant, hepatoprotective [3], nephroprotective, 43 antihypertensive [4], muscle relaxant, bronchodilator 44 [5,6], CNS depressant effects [7], antilipidemic [8], 45 antidiabetic [9,10], anticancer [11,12], analgesic 46 [13,14], anti-inflammatory [13,15], antiulcer [16] and 47 dizziness, influenza, dyslipidemia, many dermatological 48 neuroprotective effects [17,18].

Much of the biological activities of the black seeds 49 contain 36%-38% fixed oils, proteins, alkaloids, 50 have been shown to be due to the presence of 51 saponins, 0.4%-2.5% essential oil, crude fiber, minerals, 52 thymoquinone, which is the major component of the 53 vitamins, aliphatic alcohols and ketones [1].

Many studies have been conducted on the 54 compound of *Nigella sativa*, which has been shown to 55 be very effective in inhibiting histamine release induced 56 or its active compound(s) of *Nigella sativa* seed extract 57 by the secretagogues: antigen in sensitized cells [19]. 58 However, the herb is not well studied for its effect on
A growing demand for men to share the reproductive system. Moreover, the existing information in this regard is quite scanty and rather contradictory. Significant abortifacent activity of *N. sativa* seed powder, ethanolic and hexane extracts was demonstrated in rats [20]. However, Prakash et al. [21] did not find any anti-fertility activity in aqueous, ethanolic and petroleum ether extracts of the seeds of *N. sativa* when tested at a dose of 150-200 mg/kg daily in rats on the days 1-7 post-coitum schedule. There is a growing demand for men to share the burden of responsibility and risks of contraception because of growing population pressures and the increasing dissatisfaction of women in assuming almost all the risks of adequate contraception. A major challenge in this field is that the most of the male contraceptive agents currently in use offer little promise and about 15% of the 200 most commonly prescribed drugs can have adverse effects on male reproduction, either by influencing its hormonal profile or impairing their sexual performance. The discovery of key regulators of gonadal hormones and gametogenesis from black seeds may provide opportunities to alter our approaches towards management of contraception. Since, no data on the effect of *N. sativa* on testicular steroidogenesis is available, we designed this *in vitro* study to investigate the direct effect of crude aqueous extract on basal and LH-stimulated testicular steroidogenesis by mice Leydig cells.

**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 mixture of 1:3:2 was prepared by mixing *N. sativa* seeds and 20% ethanolic and petroleum ether extracts of the seeds of *N. sativa*. The mixture was then homogenized. The mixture was then stored at 4°C for 24 h. The next day, the mixture was again homogenized. This process was repeated for a total of 3 days. The filtrate was collected through Whatman’s qualitative grade 1 filter papers and the plant material was again subjected to the same treatment as the first macerate. 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 bulbce male mice (weight 36 ± 2) were dissected 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).

Moreover, to test the ability of the extract to modulate stimulated testosterone secretion, samples were challenged with LH (500 µIU/tube) alone or with different doses of NSE (1.0-1000µg). After 3 h, the incubation reaction was stopped by dipping the tubes in water bath at 60°C for 10 min. Samples were kept frozen until testosterone was measured by highly-specific radioimmunoassay.

*Radioimmunoassay*

Testosterone was measured in the incubation ground with an electric grinder into a coarse powder. A medium directly by a highly-sensitive RIA according to WHO protocol, using ³H-labeled testosterone, as tracer. Methanol (30:70) at room temperature for cold. Highly specific antiserum for testosterone was acquired maceration for a total of 3 days. The extract was collected through Whatman’s qualitative grade 1 incubation medium. After addition of filter papers and the plant material was again subjected to the same treatment as the first macerate. The combined filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure. Extract 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%.

The levels of testosterone in the media are expressed as 41 ng/ml.

*Statistical analysis*

Data are 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.
**RESULTS**

*Nigella sativa* seeds extract was able to inhibit testicular testosterone secretion in vitro. Moreover, the inhibitory effect of NS seed extract was more pronounced at higher doses.

**Effect on basal testicular steroidogenesis**

As shown in the Fig 1, basal testosterone production in the cells treated with NS seed extract (1.0–1000 µg/tube) was significantly (*p* < 0.05) reduced compared to the control in a dose-dependent manner. The inhibition was more pronounced at the higher doses. A condition that can be treated with specific replacement therapies [30]. Reversible inhibition of these hormones by any external measure may be beneficial as it can be used as a contraceptive. Oral administration of crude ethanol extracts showed significant contraceptive effect on stimulated (500 µU) testosterone production. The inhibition was more pronounced at higher doses of NS seed extract on testicular steroidogenesis have yet been available; these results open new fronts in the exploration of possible effects of *Nigella sativa* on the reproductive functions. These data offer insights into the potential contraceptive effects of *Nigella sativa* on the LH/testosterone dose–response curve to variable doses of LH (16-500 µU) [data not shown]. The study has provided us with important insight towards formulation of a new contraceptive pill that would temporarily stop spermatogenesis, thus producing reversible infertility.
ACKNOWLEDGMENTS

This work was supported by funds provided by the Department of Biological & Biomedical Sciences, Aga Khan University. We are grateful to National Hormone and Pituitary Programme California, USA for providing a gift LH (NIDDK-hLH-B-SIAFP2).

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Published online: January 31, 2013