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

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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 l000 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 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. (Ranunculaceace), commonly, known as black seed or black cumin and locally as Kalonji have been used in folk (herbal) medicine for centuries for treatment of many acute as well as chronic conditions worldwide [1,2]. It has been used in the treatment of asthma, diarrhea, indigestion, dizziness, influenza, dyslipidemia, many dermatological conditions and as a diuretic and immune modulator. The seeds contain 36%-38% fixed oils, proteins, alkaloids, saponins, 0.4%-2.5% essential oil, crude fiber, minerals, vitamins, aliphatic alcohols and ketones [1].

Many studies have been conducted on the pharmacological action(s) of *Nigella sativa* seed extract or its active compound(s) on various body systems *in vivo* or *in vitro.* The herb has been extensively studied for its different biological activities which includes antioxidant, hepatotprotective [3], nephroprotective, antihypertensive [4], muscle relaxant, bronchodilator [5,6], CNS depressant effects [7], antlipidemic [8], antidiabetic [9,10], anticancer [11,12], analgesic [13,14], anti-inflammatory [13,15], antiulcer [16] and neuroprotective effects [17,18].

Much of the biological activities of the black seeds have been shown to be due to the presence of thymoquinone, which is the major component of the essential oil and fixed oil. Nigellone, is another compound of *Nigella sativa*, which has been shown to be very effective in inhibiting histamine release induced by the secretagogues: antigen in sensitized cells [19]. However, the herb is not well studied for its effect on

reproductive system. Moreover, the existing information in this regard is quite scanty and rather contradictory. Significant abortificient 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 seed 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 measured quantity was soaked in 70% aqueous-methanol (30:70) at room temperature by cold maceration for a total of 3 days. Thereafter, 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 -4ºC until used for biological activity.

Leydig cells preparation

Three bulbce male mice (weight 36 ± 2) 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 the incubation of Leydig cells as described by Van-Damme et al,1974 [22], with minor modifications. Mice were killed by cervical dislocation. Testes 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).

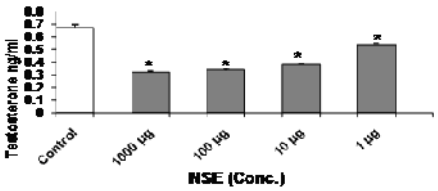

Fig 1. Effect of aqueous extract of *Nigeel sativa* seeds (NSE) on basal testosterone by mice Leydig cells in vitro
*Significant difference between control and treated groups ($p < 0.05$)

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 medium directly by a highly-sensitive RIA according to WHO protocol, using ^{3}H-labeled testosterone, as tracer. Highly specific antiserum for testosterone was acquired from Guildhay UK. RIA reagents were directly added to tubes containing incubation medium. After addition of all the reagents, tubes were incubated for 30 min. at 4°C. The bound and unbound fractions were separated by the 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%. The levels of testosterone in the media are expressed as 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.

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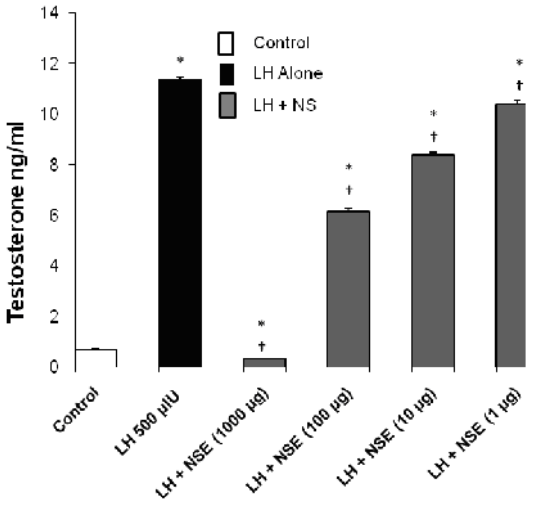

Fig 2. Effect of aqueous extract of *Nigeel sativa* seeds (NSE) on LH-stimulated testosterone production by mice Leydig cells in vitro
*Significant difference between control and treated groups ($p<0.05$)
†Significant difference between LH alone and treated groups ($p<0.05$)

RESULTS

Nigella sativa seeds extract was able to inhibit significantly ($p < 0.05$) both basal and LH-stimulated testicular testosterone secretion *in vitro.* Moreover, the inhibitory effect of NS seed extract was more pronounced at the 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 with the control in a dose-dependent manner. The inhibition was more pronounced at the higher doses. Inhibitory effect of NSE was able to inhibit 52% of the basal testosterone production and this inhibition was still present at the lowest NS dose of 1.0 µg.

Effect on LH-stimulated testicular steroidogenesis

As shown in Fig 2, administration of different doses of NS seed extract (1.0– 1000 µg) caused a significant ($p < 0.05$) and a dose-dependent inhibition of LH-stimulated (500 µIU) testosterone production. The inhibition was more pronounced at higher doses of NSE with maximum effect (97% inhibition) obtained at 1000 µg dose of NSE. LH (500 µIU) was used for maximal stimulation. This dose was selected from LH/testosterone dose–response curve to variable doses of LH (16-500µIU) (data not shown). Treatment with *NSE* caused dose-dependent inhibition of the LH-stimulated testosterone production when compared to LH 500 µI response (Fig 2, with maximum effect (97% inhibition) obtained at 1000 µg dose).

DISCUSSION

This study provides the first evidence for a strong effect of *N. sativa* seed extract on testicular steroidogenesis indicating a potential contraceptive role. Our data suggest that *N sativa* extract inhibits both basal and LH-stimulated testosterone biosynthesis signaling pathways. The mechanism behind its effect is not clear and further studies are needed to elucidate its further role and mechanism of action. The crude extract of *N. sativa* seeds has been reported to possess calcium channel blocking activity [5] and there is evidence that calcium may be involved in the signaling mechanism [24]. Significant abortificient activity of *N. sativa* seed powder, ethanolic and hexane extract, is demonstrated in women [20,25] and rats [21]. However, Prakash et al. [26] did not find any anti-fertility activity in aqueous, ethanolic and petroleum ether extracts of the seeds of *Nigella sativa* when tested at a dose of 150-200 mg/kg daily in rats on the days 1-7 post-coitum schedule. The volatile oil of Nigella seeds inhibits the spontaneous movements of rat and guinea pig uterine smooth muscle and also the oxytocin-induced contractions [27]. A single report in male rats has suggested that seed extract treatment not only causes a general reduction in the size of reproductive organs but also suppresses spermatogenesis at the spermatocyte stage. However, similar changes in the reproductive hormones of the treated animals was not observed [28].

The testis is a complex male reproductive organ that serves two crucial functions: synthesis and secretion of testosterone by Leydig cells and production of a sufficient number of competent spermatozoa supported by Sertoli cells, to attain fertility. It is well known that the essential prerequisite for normal testicular development and maintenance of spermatogenesis is the controlled secretion of Luteinizing hormone (LH), Follicle stimulating hormone (FSH), and testosterone during fetal and postnatal life [29]. A deficiency of these hormones leads to hypogonadism and sterility, a condition that can be treated with specific replacement therapies [30]. Testosterone biosynthesis in the Leydig cells is primarily regulated by LH [31]. Deficiency of these hormones leads to hypogonadism and sterility, 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 in male rats [20]. Since, no data about the effect 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 potential contraceptive effects of *Nigella sativa* on the hormonal regulation of male reproductive axis. This study has provided us with important insight towards formulation of a new contraceptive pill that would temporarily stop spermatogenesis, thus producing reversible infertility.

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