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 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 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 antioxidant, hepatoprotective [3], nephroprotective, locally as Kalonji have been used in folk (herbal) antihypertensive [4], muscle relaxant, bronchodilator medicine for centuries for treatment of many acute as [5,6], CNS depressant effects [7], antilipemic [8], well as chronic conditions worldwide [1,2]. It has been [9,10], antidiabetic [11,12], analgesic, used in the treatment of asthma, diarrhea, indigestion, [13,14], anti-inflammatory [13,15], antialcier [16] and dizziness, influenza, dyslipidemia, many dermatological [17,18], neuroprotective effects [17,18]. Much of the biological activities of the black seeds contain 36%-38% fixed oils, proteins, alkaloids, have been shown to be due to the presence of saponins, 0.4%-2.5% essential oil, crude fiber, minerals, thymoquinone, which is the major component of the vitamins, aliphatic alcohols and ketones [1]. Essential oil and fixed oil. Nigellone, is another compound of *Nigella sativa*, which has been shown to pharmacological action(s) of *Nigella sativa* seed extract be very effective in inhibiting histamine release induced or its active compound(s) on various body systems in by the secretagogues: antigen in sensitized cells [19]. *in vivo or in vitro*. The herb has been extensively studied 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 abortifacient activity of *N. sativa* seeds has been 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 of *N. sativa* 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 1.0% alcoholic extract was prepared and filtered. The organic solvent was removed by evaporation at 40°C. A 1.0% aqueous extract was prepared with fresh medium. The seeds were kept frozen 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 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).

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 (1.0-1000 μg). 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 1.0% alcoholic extract was prepared and filtered. The organic solvent was removed by evaporation at 40°C. A 1.0% aqueous extract was prepared with fresh medium. The seeds were kept frozen until used for biological activity.

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**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.
Nigella sativa and testicular steroidogenesis

**RESULTS**

*Nigella sativa* seeds extract was able to inhibit significantly (*p < 0.05*) both basal and LH-stimulated testosterone secretion in vitro. Moreover, the LH-stimulated testosterone by Leydig cells and production of a 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 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 seed extract (1.0–1000 µg) reduced LH-stimulated testosterone production by Leydig cells treated with NSE (500 µg) and also inhibited LH-stimulated (500 µU) testosterone production. The effect of NSE was able to inhibit 52% of the basal testosterone production and this inhibition was more pronounced at the higher doses.

**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 reduction in LH-stimulated testosterone production. Oral administration of crude seed extract (1.0–1000 µg) reduced LH-stimulated testosterone production by Leydig cells treated with NSE (500 µg) and also inhibited LH-stimulated (500 µU) testosterone production. The effect of NSE was able to inhibit 52% of the basal testosterone production and this inhibition was more pronounced at the higher doses.

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* seed 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 abortifacient activity of *N. sativa* seed powder, ethanolic and hexane extract, is demonstrated in women [20,25] and rats [21]. However, Prakash et al. 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].

**DISCUSSION**

The results from the current study provide evidence for the contraceptive potential of *N. sativa* seed extract. The extract was able to inhibit both basal and LH-stimulated testosterone production by Leydig cells in vitro, indicating its potential as a contraceptive agent. Further studies are needed to elucidate the mechanism of action and to determine the optimal dose for contraceptive efficacy.
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