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

5SHEIKH A. SAEED
Aga Khan University

NAHEED ANWAR
Aga Khan University

QAISER JABEEN
Aga Khan University

Anwar Hassan Gilani
Aga Khan University, anwar.gilani@aku.edu

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ORIGINAL ARTICLE

Aqueous Extract of Nigella sativa Seeds Suppress Testicular Steroidogenesis in Mice L vitro

SHEIKH A. SAEED, ENAAM 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 well studied for its biological activities. However, there is limited information reg male reproductive system. This study describes the effect of *Nigella sativa* aqueous (NSE) on testicular steroidogenesis from mice Leydig cells *in vitro*. Mice testicular cells were incubated in a containing either no treatment or NSE or LH alone or combination of LH carried out for three hours in a shaking water bath at 34°C. Testo 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 testo LH-stimulated testosterone, compared to control (0.32 ± 0.03 ng/ml and 0.83 ± 0.1 ng/ml) respectively. Thus, it is concluded that the basal and LH-stimulated secretion of testos from Leydig cells are suppressed significantly in the presence of NSE *in vitro*. However, different further studies are needed to explore the effect of chronic treatment with NSE be used as a contraceptive in male.

Keywords: Nigella sativa, Black seed, Male reproductive system, aqueou testosterone

The seeds of *Nigella sativa* Linn (Ranunculaceae), commonly, known as black seed, locally as Kalonji have been used in folk (herbal) medicine for centuries for treatment of various acute as well as chronic conditions. It has been used in the treatment of asthma, diarrhea, dizziness, influenza, dyslipidemia and conditions and as diuretic and immune modulator. The seeds contain 38% fixed oils, alkaloids, saponins, 0.5% essential oil, crude fiber, minerals, vitamins, aliphatic alcohols and ketones [1].

Many studies have been conducted on pharmacological activities of *Nigella sativa* extract or its active compound(s) in various body systems *in vivo* or *in vitro*. The herb has been extensively studied for its different biological activities which inc antioxidant [2], hepatoprotective, antihypertensive [4], smooth muscle relaxant, broncho [5,6], CNS depressant [7], antiepileptic [8], antidiabetic [9,10], anticancer [11,12], analgesic [13,14], antiinflammatory [15], antiulcer [16] and immunomodulatory effect [17,18]. Much of the biological activities of the seeds have been shown to be due to the thymoquinone, which is the major component of the essential oil and fixed oil. Nigellone, compound of *Nigella sativa*, which has been shown effective in inhibiting histamine relea antigen in sensitized [19]. However, the herb is not well studied

reproductive system. Moreover, the existing information in this regard is quite scanty and contradictory rather co... Significant abortificient activity of N. sativa seed powder, ethanolic and hexane extracts was demonstrated [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 150 and 200 mg/kg daily in rats on days 1-7 postcoitum schedule.

There is 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 contr...

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.

Fig. Effect of aqueous Nigella sativa extract (NSE) on testosterone by mice Leydig cells in vitr...
*significant difference between control a...

MATERIALS AND METHODS

Preparation of crude extract

Dried black seeds of Nigella sativa were purchased from the local market in Karachi. The plant seeds were cleaned of any adulterant material. Seeds were ground with an electric grinder. A measured quantity was soaked in 70% aqueous methanol (30:70) at room temperature for maceration for a total of 3 days. ... was collected through Whatman filter papers and the plant material was again subjected to the same treatment as the ... combined filtrate was concentrated ... evaporator at 40°C under reduced pressure. Extract was stored at 4°C until used for biological activity.

Leydig cell preparation

Three bulb c male mice (weight ± 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 Nigella sativa extract seeds (NSE) on testosterone secretion ... the incubation of Leydig cells as described by Van Damme et al [22], 1974 with minor modification. Mice were killed by cervical dislocation, ... dissected out immediately and decapsulated. Leydig cells were isolated as described earlier [22]. Leydig cells (80,000/tube) were cultured for 1 h to remove the endogenous steroid hormones. Then the media were repl... with either fresh medium or medium conta... doses of crude aqueous extract of NS se... (10-1000 µg/tube).

Moreover, to test the ability of the ... modulate stimulated testosterone secretio... were challenged with LH (500 µIU/tube) a... different doses of NSE (10-1000 µg). After 3 h incubation reaction was stopped by dippin... water bath at 60°C for 10 min. Samples were frozen and kep... until testosterone was measured by highly specific radioimmunoassay.

Radioimmunoassay

Testosterone was measured in the i... medium directly by using RIA according to WHO protocol using ^{3}H-labelled testosterone, as tracer. Highly specific antiserum ... from ... Guildford, UK. The reagents were directly add... incubation medium. After ... tubes were incubated for 30 ... °C. The bound and unbound fractions were separated by the addition of 0.1% activated charcoal. Radioactivity was measured in liquid scintillation counter. Testosterone concentration was calculated by logit log transformation [23].

The sensitivity of T assay was 0.0125 ... intra-assay coefficient of variation was less than 10%. The levels of testosterone in the media a... ng/ml.

Statistical analysis

Data was expressed as Mean ± S.E.M. Results were analyzed by ... statistical significance ... independent t test on SPSS. P < 0.05 was considered significant.

Fig 2. Effect of aqueous Nigella sativa seed extract (NSE) on LH-stimulated testosterone production by mice Leydig cells. *Significant difference between control a... Significant difference between LH-alone and treated grou...

RESULTS

Nigella sativa seeds extract was able to significantly (p<0.05) inhibit both basal and LH-stimulated testicular testosterone in vitro. Moreover, the inhibitory effect of NS seed extract was pronounced at the higher doses.

Effect on basal testicular steroidogenesis

As shown in Fig 1, the basal testosterone production in the cells treated with NS seed extract (100-1000 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 100 µg.

Effect on LH-stimulated testicular steroidogenesis

As shown in Fig 2, administration of different doses of NS seed extract (100-1000 µg) caused a significant (p < 0.05) and 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 (5-1000 µIU) (data not shown). Treatment with NSE caused dose-dependent inhibition of the LH stimulated testosterone production when compared to LH (500 µIU) response (Fig 2) with maximum effect (97% inhibition) obtained at 1000 µg dose).

DISCUSSION

This study provides the first evidenc effect of N. sativa seed extract on testic steroidogenesis indicating a potential c Our data suggest that N. sativa extract inhibits both b and LH-stimulated testosterone biosynthe pathways. The mechanism behind its ef and further studies are needed to elucidate role and mechanism of action. The crude extract of N. sativa seeds has been reported to possess calcium channel blocking activity [5] and there calcium may be involved in the signaling [24]. Significant abortificient activity of N. sativa powder, ethanolic and hexane extract, i in women [22, 25] and rats [21]. However, Prakas [26] did not find anti-fertility activity in aqu ethanolic and petroleum ether extracts Nigella sativa when administered at a dose of 150-200 mg/kg daily in rats on 1-7th postcoitum schedule. The volatile oil of Nigella seed inhibits the spontaneou movements of rat and guinea pig uterine sm and also the oxytocin-induced contractions [27]. A single report in male rats has suggeste treatment not only causes a general red of reproductive organs but also spermatogenesis at the spermatid stage. However, similar changes in the reproductive horm treated animals was not observed [28].

The testis is a complex male reproduc serves two crucial functions: synthesis and sec testosterone by Leydig cells and pro sufficient number of competent spermat by Sertoli cells, to attain fertility. It is the essential prerequisite for norm development and maintenance of spermato controlled secretion of Luteinizing ho (LH), Follicle stimulating hormone (FSH), and tes during fetal and postnatal life [29]. A deficiency of these hormones leads to hypogonadism and sterility a condition that can be treated with spec therapies [30]. Testosterone biosynthesis in the Leydig cells is primarily regulated by LH [31]. Deficiency of these hormones leads to hypogonadism, a condition that can be treated with specif therapies [30]. Reversible inhibition of these h by an external measure may be beneficial used as a contraceptive. Oral administration ethanol extracts showed significant con in male rats [20]. Since, no data about the effe seed extract on testicular steroidogenesis have been available, these results open new f exploration of possible effects of Nigella sativa reproductive functions. These data offer potential contraceptive effects of Nigella sativa the hormonal regulation of reproductive axis. T study has provided us with important i formulation of a new contraceptive pi temporarily stop spermatogenesis, th reversible infertility.

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Current Author Addresses

Sheikh A. Saeed, Department of Basic Sciences, College of Medicine, King Saud Bin Abdulaziz University of Health Sciences, King Abdulaziz Medical City, Jeddah 21423, Kingdom of Saudi Arabia. Email: saeeds@ksau-hs.edu.sa (Corresponding author)

Naheed Akhter, Dept. of Biological & Biomedical Scienc Khan University, Stadium Road, Karachi

Qaiser Jabeen, Dept. of Biological & Biomedical Sciences University, Stadium Road, Karachi

Anwar H. Gilani, Dept. of Biological & Biomedical Sciences, Aga Khan University, Stadium Road, Karachi

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