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1 ORIGINAL ARTICLE

2 Aqueous Extract of *Nigella arvensis* Suppresses 3 Testicular Steroidogenesis in Mice *In Vitro* 4

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10 ABSTRACT

11 *Nigella arvensis* (black seed) is an important medicinal herb with folkloric use in
12 well studied folkloric activities. However, there is limited information regarding
13 male reproductive system. This study describes the effect of NSE on aqueous
14 testicular steroidogenesis from mice Leydig cells. Leydig cells were incubated in a
15 containing either no treatment or NSE or LH alone or combination of LH
16 carried out for three hours in a shaking water bath at 34°C. Testosterone
17 radioimmunoassay. At all, NSE (5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml) inhibited both basal and LH
18 *in vitro* testosterone secretion. At a dose of 1000 µg, NSE inhibited 52% of basal testos-
19 LH stimulated testosterone, compared to control (0.32 ± 0.008 vs 0.33 ± 0.010 µg/ml) respectively.
20 respectively. Thus, it is concluded that treatment with NSE significantly inhibited the
21 from Leydig cells are suppressed significantly in the presence of LH. Further
22 further studies are needed to explore the effect of chronic treatment with NSE
23 be used as a contraceptive in male.

24 **Keywords:** *Nigella arvensis*, Black seed, Male reproductive system, aqueous
25 testosterone

26 The seed of *Nigella arvensis* (Ranunculaceae), for its different biological activities which include
27 commonly, known as black seed, is a natural antioxidant, antiparasitic, antiproliferative, antitumor, and
28 locally as Kalonji have been used in Ayurvedic medicine for the treatment of various ailments such as
29 medicine for centuries for treatment of various ailments such as asthma, bronchitis, hypertension, diabetes,
30 well as chronic conditions such as asthma, bronchitis, hypertension, diabetes, and cancer [1, 2]. It is also
31 used in the treatment of asthma, bronchitis, hypertension, diabetes, and cancer [1, 2]. It is also
32 dizziness, influenza, dyslipidemia, and other conditions [3, 4]. The biological activities of the
33 conditions such as asthma and immune modulation. The biological activities of the
34 seeds contain 3-6% of saponins, 2-5% essential oil, crude protein, iron, and other minerals, which is the main
35 saponins, 2-5% essential oil, crude protein, iron, and other minerals, which is the main
36 vitamins, aliphatic alcohols and ketones, essential oil and fixed oil. *Nigella arvensis*
37 Many studies have been conducted on the pharmacological activities of the seed which has been shown
38 pharmacological activities of the seed have been shown to be effective in inhibiting histamine release
39 or its active compound(s) in various systems [5, 6]. The seed is also a good source of antioxidants
40 vivorin *in vitro*. The herb has been extensively studied. However, the seed is not well studied *in*

reproductive system. Moreover, the existing information in this regard is quite scanty and rather controversial. Significant abortifacient activity of powder, ethanolic hexane extracts was demonstrated. However, Pralish et al did not find any anti activity in aqueous, ethanolic and ether extracts of the seeds of sati when tested at 1500 mg/kg daily in rats on a 71000 schedule.

There is growing demand for men to share the burden of responsibility and risks of contraception because of growing population, increasing divorce rates for women in assuming a major role in the risks of adequate contraception. A major challenge is that the most of the male contraceptive agents currently in use offer little promise and about 15% of the 200 commonly prescribed drugs can have adverse effects on male reproduction either by influencing its hormonal or by affecting their sexual performance. The regulators of gonadal hormones and gametogenesis from black seed may provide opportunities for alternative approaches towards management of sterility.

Since, no data on the effect of crude aqueous extract of black seed on steroidogenesis is available, it is necessary to study to investigate the direct effect of crude aqueous extract on basal-stimulated testicular steroidogenesis by mice Leydig cells. Different doses of crude aqueous extract were challenged with LH (500 μ U/tube) after incubation reaction was stopped by dipping in water bath for 10 min. Samples were kept until testosterone was measured by radioimmunoassay.

MATERIALS AND METHODS

Preparation of extract

Dried black seed were purchased from the local market in Karachi. The plant seeds were cleaned of any adulterant material. Seeds were measured in the ground with an electric grinder. A known quantity was soaked in methanol (30:70) at room temperature for 3 days. The maceration was collected through Whatman filter papers and the plant material was extracted by the combined filtrate was concentrated in a rotary evaporator at 40°C under reduced pressure. Extracts were stored at 4°C until used for biological activity.

Leydig cell preparation

Three bulbhead male mice (weight 18-20g) were used for each experiment. Animals were obtained from the AKU animal facility, where they were maintained under standard conditions (12h light-dark cycle).

Statistical analysis

Direct effect of aqueous extract of black seed (NSE) on testosterone secretion was studied by the introduction of Leydig cells as described by Damme et al (1974) with minor modifications. Independent t test was used to compare the data. Results were considered significant if $P < 0.05$ was achieved by cervical dislocation.

178 This study provides the first evidence
 179 effect of sativaeed extract on testic
 180 steroidogenesis indicating a potential c
 181 Our data suggest that sativaeed extract inhibits both b
 182 and stimulates testosterone biosynthe
 183 pathways. The mechanism behind its ef
 184 and the studies are needed to elucidate
 185 role and mechanism of action. Target of
 186 sativaeeds has been reported as poss
 187 channel blocking activity [5] and there
 188 calcium may be involved in the signaling
 189 [24]. Significant abortifacient activity of
 190 powder, ethanolic and hexane extract, is
 191 in women [20, 5] and rats [21]. However, Prakas
 192 [26] did not find any activity in aqu
 193 ethanolic and petroleum ether extracts
 194 Nigella sativa tested at 500 mg/kg
 195 daily in rats on 7 days schedule. The
 196 volatile oil of Nigella sativa has the spontaneou
 197 movement at and guinea pig uterine sm
 198 and also the inhibitory effect on A

Fig Effect of aqueous Nigella sativa extract (NS.E) on
 stimulated testosterone production by mice Leydig cells
 *Significant difference between control
 Significant difference between treated group

RESULTS

147

148 Nigella sativa extract was able to inhibit testis a complex male reproduc
 149 significant (p < 0.05) both basal and stimulated testes functions: synthesis and sec
 150 testicular testosterone and more over, the testosterone by Leydig cells and pro
 151 inhibitory effect of NS seed extract was more pronounced in the Sertoli cells, to attain fertility. It is
 152 pronounced at the higher doses by Sertoli cells, to attain fertility. It is
 153 Effect on testicular steroidogenesis
 154 As shown in Fig 1, basal testosterone secretion of Luteinizing hormone (LH) and tes
 155 in the cells treated with NS seed extract (100 µg/dose) during fetal and postnatal life
 156 µg/tube) was significantly reduced during fetal and postnatal life
 157 with the control-dependent manner. The hormones by a direct manner and sterility
 158 inhibition was more pronounced in the condition that case. The treated with spec
 159 inhibitory effect of NSE was able to inhibit the synthesis of testosterone in the
 160 basal testosterone production and this is primarily by LH deficiency of
 161 still present at the lowest NS dose of 100 µg/dose. The deficiency of
 162 Effect on stimulated testosterone production
 163 As shown in Fig 2, administration of NS seed extract (100 µg/dose) caused a significant
 164 of NS seed extract (100 µg/dose) caused a significant decrease in the testosterone
 165 (p < 0.05) and dependent inhibition of the extract showed significant con
 166 stimulated (500 µg/dose) testosterone production. Since the data about the effe
 167 inhibition was more pronounced in the extract of NS seed extract (100 µg/dose)
 168 with maximum efficiency (97%) obtained at 1000 µg/dose. These results open new f
 169 µg dose of NS seed extract was used as a preliminary exploration of possible Nigella sativa
 170 stimulation. This dose was used as a preliminary exploration of possible Nigella sativa
 171 LH testosterone response curve to a variable dose of NS seed extract (100 µg/dose).
 172 of LH (500 µg/dose) (data not shown) showed a dose-dependent inhibition of the
 173 NS caused a dose-dependent inhibition of the LH provided us with important i
 174 stimulated testosterone production when compared to that of a new contraceptive pi
 175 LH (50 µg/dose) (with maximum efficiency of 97%) primarily stop spermatogenesis, th
 176 inhibition) obtained at 1000 µg/dose) reversible fertility

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