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# POST-COITAL CONTRACEPTIVE EFFICACY OF THE SEEDS OF *NIGELLA SATIVA* IN RATS

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**Abstract:** Hexane extract of the seeds of *Nigella sativa* L. prevented pregnancy in Sprague-Dawley rats treated orally at 2 g/kg daily dose on days 1-10 *post-coitum*. Significant antifertility activity was also observed in its column fractions and subfractions. At contraceptive dose, the active hexane extract exhibited only mild uterotrophic activity comparable almost to 0·002 mg/kg dose of 17 ∝-Ethinylestradiol, but was devoid of any estrogenicity in the immature rat bioassay.

**Key words:** contraception *Nigella sativa*
antifertility agent post-coital contraceptive

## INTRODUCTION

*Nigella sativa* L. is a small herb, widely cultivated in Punjab, Himachal Pradesh, Bihar and Assam. Ethnomedically, seeds of this plant have been used as abortifacient/emmenagogue in Brazil, Ethopia, India, Nepal and several other countries (1). Claims of significant carminative, diuretic, galactagogue activities and in the treatment of mild cases of puerperal fever (2) are also available in the literature.

This study, aimed to develop newer contraceptive agents from natural sources, describes systematic evaluation of the seeds of this plant for post-coital contraceptive and estrogenic activities using standard biossays.

## METHODS

Colony-bred immature (25-35 g) and adult (180-200 g) Sprague-Dawley rats of the Institute, maintained in air-conditioned quarters (24±1°C) under standard husbandry conditions with alternate 12 h light/dark periods were used. Animals were given standard pellet diet and tap water *ad libitum*.

Two kg seeds of *N. sativa* were purchased from the local market at Lucknow, dried, powdered and defatted with hexane (4×5 L). The combined hexane extract was concentrated below 50°C under reduced pressure in a rotavapour to get 200 g of dark brown oily mass. This was dried at room temperature and then further extracted with 95% EtOH (4×5 L) and the combined ethanolic extract was concentrated under reduced pressure below 50°C to get 123 g of the ethanolic extract. The hexane extract was chromatographed over a column of silica gel and three fractions A, B and C were collected. The most active fraction 'C', showing 50% antifertility activity at 400 mg/kg dose, was refractionated into subfractions $C_1$, $C_2$ and $C_3$ by preparative layer chromatography using

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chloroform as the solvent.

Each extract was macerated with gum acacia or PVP (Mol. wt. 10,000) and suspended in glass distilled water. 17∝-Ethinylestradiol (EE; Sigma Chemical Co., U.S.A.) was dissolved in 2-3 drops of redistilled ethanol and diluted to the required concentrations with glass distilled water. All treatments were done by the oral route.

For antifertility activity, female rats were caged overnight with males (3 : 1) of proven fertility. Their vaginal smears were examined on the following morning and the day of presence of spermatozoa was taken as day 1 of pregnancy as described previously (3). Mated rats were randomly distributed into various groups (Table I) and treated orally with the test extracts/ vehicle on days 1-10 *post-coitum*. At autopsy on day 16, number and status of implantations and corpora lutea in each animal were recorded.

Estrogenic profile of the active hexane extract was determined before further fractionation. For this purpose immature female rats were bilaterally ovariectomized under light ether anaesthesia through lateral incisions in the skin just below the last rib (4). After a post-operative rest period of 7 days, these rats were treated orally with the hexane extract for 3 consecutive days. At autopsy 24 h after the last treatment, uterine weight, premature opening of vagina and extent of vaginal cornification were recorded (3).

The data was statistically subjected to analysis of variance.

## RESULTS

*Antifertility efficacy :* Hexane extract of dried seeds of *Nigella sativa* prevented pregnancy in 100% rats at a dose of 2 g/kg. Ethanolic extract obtained after defatting was, however, devoid of any significant antifertility activity upto 2·0 g/kg dose (Table I).

TABLE I : Post-coital antifertility activity of the seeds of *Nigella sativa* in adult female rats.

| *Treatment (days 1-10 post-coitum)* | *Extract/Fraction* | *Daily dose (mg/kg)* | *Total rats pregnant/treated* | *Implantations on day 16 post-coitum (Mean±SEM)* |
|---|---|---|---|---|
| Vehicle | | | 9/10 | 8·9 ± 1·3 |
| Seeds of *Nigella sativa* | Hexane ext. | 2000 | 0/10[c] | — |
| | Ethanolic ext. | 1250 | 7/10 | 5·8 ± 1·5 |
| | | 2500 | 6/10 | 6·0 ± 1·7 |
| Vehicle | — | — | 10/10 | 7·0 ± 0·6 |
| Column fractions of hexane extract | Col. fr. A | 1000 | 4/10[c] | 3·7 ± 1·6 |
| | Col. fr. B | 700 | 8/10 | 6·5 ± 1·3 |
| | Col. fr. C | 400 | 5/10[a] | 3·7 ± 1·3[a] |
| Vehicle | — | — | 10/10 | 7·9 ± 0·6 |
| PLC fractions of column fraction C | PLC fr. $C_1$ | 400 | 6/10[a] | 5·5 ± 1·5 |
| | PLC fr. $C_2$ | 400 | 3/10[c] | 1·8 ± 0·9[c,b] |
| | PLC fr. $C_3$ | 400 | 7/10 | 5·6 ± 1·0 |

$P < 0·05$ - [a] v/s corresponding control,
[b] v/s PLC fractions $C_1$, $C_2$.
$P < 0·01$ - [c] v/s corresponding control.

Hexane extract on fractionation exhibited promising antifertility activity in column fraction 'C' at 400 mg/kg dose ($P < 0.05$, versus corresponding vehicle control group, Table I). Even in animals that became pregnant, the average number of implantations was significantly lower ($P < 0.05$) than the corresponding vehicle control group. Amongst the preparative layer chromatography fractions of the column fraction 'C', significant antifertility activity ($P < 0.01$, versus corresponding vehicle control group) was recorded in the major PLC fraction $C_2$ when administered orally at 400 mg/kg daily dose. The 3 out of 10 treated rats that became pregnant had an average of only $1.8 \pm 0.9$ implantations, which was markedly lower when compared to the corresponding vehicle control group ($P < 0.01$) as well as the groups treated with PLC fractions $C_1$ and $C_3$ ($P < 0.05$).

were not observed. Higher dose 0.02 mg/kg of EE however, induced marked ($P < 0.01$, versus vehicle controls) increase in uterine weight and premature opening and cornification of the vagina in all the treated animals.

## DISCUSSION

Results of the present study clearly demonstrate post-coital contraceptive property of the hexane extract of the seeds of *N. sativa* and its column and preparative layer chromatography fractions. Pertinently, the available information in this regard is quite scanty and rather contradictory. Siddiqui *et al* (5) have reported significant (70%) abortifacient activity after administration of two doses (30 g each) of the *N. sativa* seed powder at an interval of 48 hr in women. However, Prakash *et al* (6) did not find any

TABLE II : Estrogenic activity of hexane extract of *Nigella sativa* seeds in ovariectomized immature female rats.

| *Particulars* | *Treatment (once daily for 3 days)* | | | | |
|---|---|---|---|---|---|
| | *Vehicle* | *Nigella sativa* | | *17∝- Ethinylestradiol* | |
| | | *2 g/kg* | *4 g/kg* | *0·002 mg/kg* | *0·02 mg/kg* |
| Total rats | 6 | 6 | 6 | 7 | 7 |
| Weight of uterus (mg ± SEM) without fluid | 17·4±0·6 | 27·3±1·5[a,c] | 45·3±2·0[a,b] | 24·5±2·0 | 72·3±6·4 a,b |
| Vagina (No. open) | 0/6 | 0/6 | 0/6 | 0/7 | 7/7 |
| Vaginal smear (No. of rats with predominantly epithelial or cornified cells) | 0/6 | 0/6 | 0/6 | 0/7 | 7/7 |

$P < 0.01$ - [a] v/s corresponding control,
- [b] v/s corresponding lower dose group.
$P > 0.05$ - [c] v/s 0·002 mg/kg 17∝- Enthinylestradiol treated group.

*Estrogenic activity :* When adminstered orally to ovariectomized immature female rats once daily for three consecutive days, 2 g/kg dose of the hexane extract of the seeds of *N. sativa* caused only mild uterotrophic effect. It, however, failed to induce premature opening of the vagina or cornification of the vaginal epithelium (Table II). The uterine weight gain induced at the contraceptive dose of the extract was almost similar to that induced by 0·002 mg/kg dose of EE ($P > 0.01$, Table II). Even at twice the contraceptive dose (i.e. 4 g/kg), premature opening of vagina or vaginal cornification

antifertility activity in the aqueous, ehtanolic and petroleum ether extracts of the seeds of *N. sativa* when tested at 150-200 mg/kg daily doses in rats in the days 1-7 *post-coitum* schedule. This might be related to the use of much lower doses of the test extracts. In the present study, the active hexane extract exhibited only very weak uterotrophic activity, comparable almost to 0·002 mg/kg dose of 17 ∝-ethinyl estradiol, but was devoid of any estrogenic activity. The reported lack of antiovulatory activity in the seed extract of this plant (7) confirms absence of significant estrogenic activity in them. This, together with the

observed pregnancy interceptive activity in rats, opens up new possibilities towards the generation of a contraceptive agent of plant origin and might warrant its further evaluation for identification of the active moiety.

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