

CONTRACEPTIVE EFFECT OF NEEM SEED OIL AND ITS ACTIVE FRACTIONS ON FEMALE ALBINO RABBITS

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Received: 28 June 2018, Revised and Accepted: 13 August 2018

ABSTRACT

Objective: In the present investigation, contraceptive efficacy of neem seed oil and its fractions have been examined in female albino rabbits to find out their contraceptive potential.

Methods: The experimental protocol was designed for intact control, Neem seed oil alone and its six active fractions in eight groups for 30 days. The estimation of serum biochemistry, histometry of reproductive organs, and hematological parameters were done.

Results: Oral administration of Neem seed oil alone and its Fraction IV and V causes significant reduction in weight of ovary and uterus which reflects antiestrogenic function of plant material. Significant increase in serum cholesterol and phospholipid in Neem seed oil alone and its Fraction IV and V-treated groups reflects that they may affect the intraovarian estrogen level either by inhibiting the ovarian cell function or by inhibiting follicle-stimulating hormone and luteinizing hormone secretion through hypothalamus-hypophysis axis and impaired estrogen synthesis.

Conclusion: From the ongoing work, it can be concluded that Neem seed oil alone and its Fraction IV and V cause functional sterility in female albino rabbits without causing any side effect on general metabolism.

Keywords: Contraceptive, Histometry, Intraovarian, Hypothalamus.

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INTRODUCTION

Today, alarming rate of population growth is one of the most imminent hurdles of country's development if the population is not controlled or checked it will lead to several problems [1]. To control the population, it is necessary that there will be availability of safe contraceptive. Natural products are a significant source of synthetic and traditional herbal medicine and are still the primary health-care system [2,3]. *Azadirachta indica* commonly known as Neem tree has also been called as multipurpose tree [4] because its all parts have been used in various medicinal preparations. >135 compounds have been isolated from different parts of Neem [5,6]. It has been reported to possess antiviral, antifungal, antibacterial, and anti-inflammatory properties. Similarly, antidiabetic and antiatherosclerotic effect of Neem plant was also reported [7]. Neem seed oil is known to have antifertility and antiovarian effect [8]. It has also reported that Neem seed oil inhibits folliculogenesis in albino rabbits and also the significant change in the levels of associated reproductive hormones [9,10]. The present study was undertaken to evaluate the effect of six active fractions of Neem seed oil and their contraceptive efficacy in female albino rabbits for developing antifertility agent from the indigenous plant sources.

METHODS

Extraction of plant material

The ripe fruit of Neem was collected in an around new campus of Jai Narain Vyas University, Jodhpur, and identification was done by the Department of Botany, Jai Narain Vyas University, Jodhpur. After collection, they were cleaned, depulped, and dried in bright sunlight for about 10-12 h and decorticated mechanically by double roller mills. The crushed kernels were initially blended with the hexane. The crushed material was packed into cellulose thimble and placed in extractor. Hexane was recovered by distillation and the residue left after distillation was collected and to prevent oxidation Neem seed oil was stored under refrigeration.

Fractionation

50 g of Neem seed oil was chromatographed over deactivated silica gel in glass column and six major fractions were eluted with EtOH/hexane in six different proportions (Table 1).

Experimental animals

Healthy adult female albino rabbits were housed in well-lighted air-conditioned room in metallic wire gauge cages under controlled environment with 24 h light/dark cycle. All the rabbits were fed on standard rabbit chow supplied by Hindustan Lever Ltd., India. The food was supplemented with green leafy and seasonal vegetables and water *ad libitum*.

Experimentation

Mature regular cyclic female albino rabbits were divided into eight groups of three animals each as given in Table 2. For this experiment, weight and age of animal were 1.25-1.75 kg and 10-12 months, respectively, and the route of drug administration was orally. The experimental protocol was approved by the Institutional Animal Ethical committee.

Serum biochemistry

At the end of experiments, all rabbits were sacrificed under prolonged anesthesia and blood were collected through direct cardiac puncture. Collected blood was stored in EDTA vials at 20°C, and hematological parameters were examined through standard method [11,12] and serum was separated and stored at -20°C and analyzed for different parameters by standard methods such as cholesterol [13] and phospholipids [14].

Histometry

Uterine epithelial nuclear diameter and vaginal epithelial nuclear diameter were traced at ×800 with camera lucida. The epithelial cell height of uterus and vagina was also traced at ×360. Follicular types such as primary secondary and mature were calculated by observing stained sections of ovary.

Statistical analysis

All the values of body/organ weights, biochemical estimation, and histometry and follicular numbers were expressed in terms of mean value±standard error. The different groups were compared among each other using Student's t-test [15].

RESULTS

Morphological study

Body weight (Figs. 1-2)

Non-significant changes occurred in body weight of experimental animals of all treatment groups. Administration of Neem seed oil (Group 2) and its chromatographic Fraction IV and V (Group 6-7)

Table 1: Weight of eluted six major fractions

| Fraction | Amount of EtOH (%) | Amount of hexane (%) | Weight of the fraction (g) |
|----------|--------------------|----------------------|----------------------------|
| I | 0 | 100 | 2.232 |
| II | 7 | 93 | 2.438 |
| III | 20 | 80 | 4.680 |
| IV | 30 | 70 | 7.128 |
| V | 80 | 20 | 2.844 |
| VI | 100 | 0 | 1.44 |

Table 2: Dose determination of experimental animals

| Experimental Groups | Dose in mg/1 kg body Wt. | Autopsy schedule |
|--------------------------|--------------------------|-------------------------|
| Group 1, (olive oil) | 1 ml | On 31 st day |
| Group 2, (neem seed oil) | 500 mg | On 31 st day |
| Group 3, (Fraction I) | 9 mg | On 31 st day |
| Group 4, (Fraction II) | 10 mg | On 31 st day |
| Group 5, (Fraction III) | 19 mg | On 31 st day |
| Group 6, (Fraction IV) | 28 mg | On 31 st day |
| Group 7, (Fraction V) | 10 mg | On 31 st day |
| Group 8, (Fraction VI) | 7 mg | On 31 st day |

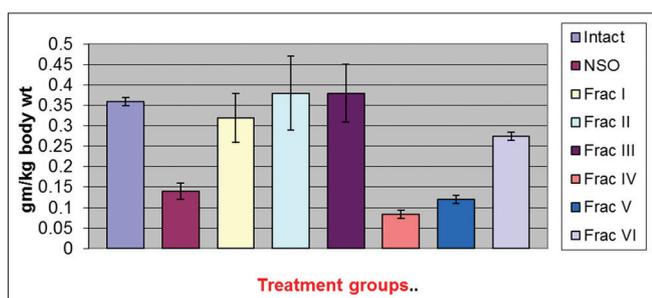


Fig. 1: Graphical representation of changes in the weights of ovary (mean of five values±standard error of the mean) of neem seed oil alone or various fractions treated intact female rabbits

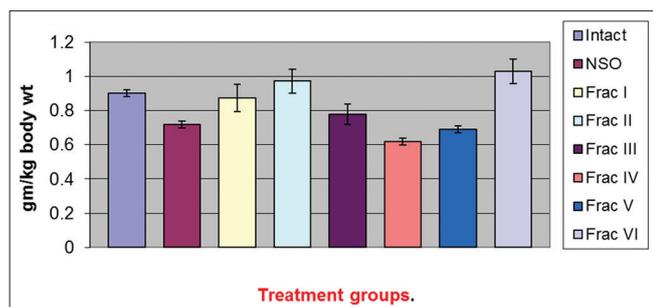


Fig. 2: Graphical representation of changes in the weights of uterus (mean of five values±standard error of the mean) of Neem seed oil alone or various fractions treated intact female rabbits

brought about significant reduction in the weight of ovary and uterus in relation to control.

Histometry (Figs. 3-5)

Histometry of uterus wall including epithelial cell height and its nuclear diameter shows the shrinkage in the Neem seed oil and its Fraction IV and Fraction V. Similarly, vaginal epithelial cell height and its nuclear diameter also show reduction in Neem seed oil alone (Group 2) and its Fractions IV and V (Group 6-7) treatment groups. The number of mature follicles reduces drastically.

Hematology

The hematological parameters such as blood sugar, red blood cells, white blood cells, and hemoglobin concentration of vehicle-treated control (Group 1), neem seed oil (Group 2), Fraction I (Group 3), Fraction 2 (Group 4), Fraction III (Group 5), Fraction IV (Group 6), Fraction V (Group 7), and Fraction VI (Group 8) values were all found in normal range in all treatment groups.

Serum biochemistry (Figs. 6 and 7)

The serum biochemistry such as cholesterol, phospholipids of vehicle-treated control, and all other experimented groups was represented in Fig. 6 and 7. The concentration of cholesterol when estimated in Neem seed oil (Group 2), Fraction IV (Group 6), and Fraction V (Group 7)-treated rabbits increased, which was statistically significant (p=0.001) while as in Fraction I, II, III, and VI treated shows no significant changes. Phospholipids concentration also increases in Neem seed oil (Group 2) and Fraction IV and V (Group 6-7)-treated intact rabbits.

DISCUSSION

A reduction in the weight of ovary and uterus suggests antiestrogenic nature of Neem seed oil alone and its Fraction IV and V. Isolated fraction of neem seed oil was found to be more effective in comparison

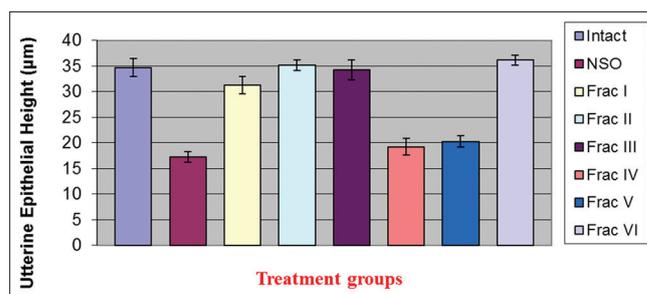


Fig. 3: Graphical representation of changes in the heights of uterus epithelial (mean of five values±standard error of the mean) of neem seed oil alone or various fractions treated intact female rabbits

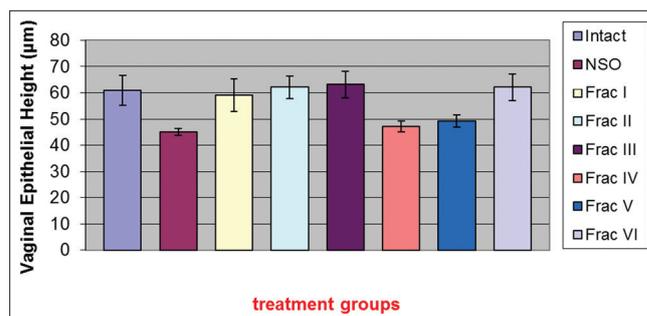


Fig. 4: Graphical representation of changes in the vaginal epithelial height (mean of five values±Standard error of the mean) of neem seed oil alone or various fractions treated intact female rabbits

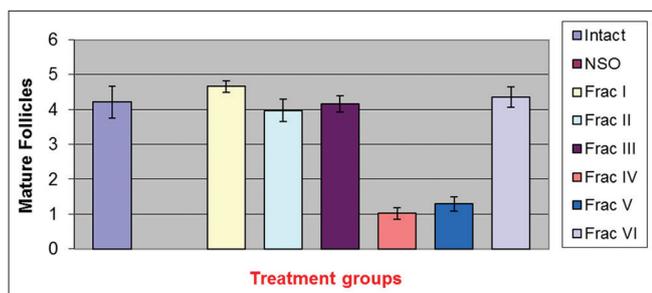


Fig. 5: Graphical representation of changes in the mature follicles number (mean of five values±standard error of the mean) of neem seed oil alone or various fractions treated intact female rabbits

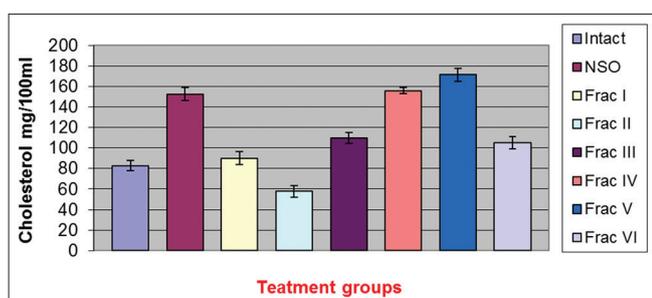


Fig. 6: Graphical representation of changes in the concentration of serum cholesterol (mean of five values ± standard error of the mean) of neem seed oil alone or various fractions treated intact female rabbits

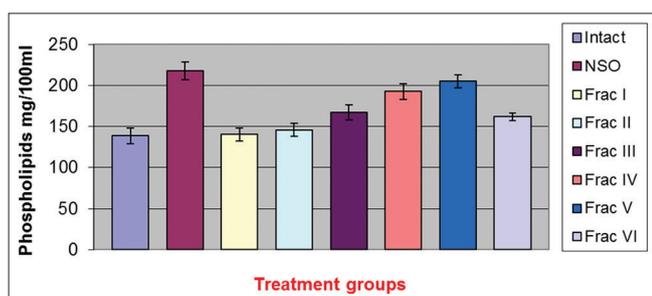


Fig. 7: Graphical representation of changes in the concentration of serum phospholipid (mean of five values ± standard error of the mean) of neem seed oil alone or various fractions treated intact female rabbits

to Neem seed oil alone and this is due to reduced estrogen level. Histomerty of uterus and vagina shows significant reduction in cell height and nuclear diameter in the Neem seed oil alone and its Fraction IV and V. As the functional status of these organs is mainly dependent on estrogen [16,17], whereas in other groups no significant changes were observed. It indicates that Neem seed oil and its Fraction IV and V may have antiestrogenic nature [18,19]. The essential prerequisite in the process of ovulation is complex sequence of hormonal events there includes timed preovulatory rise of threshold levels of estradiol followed by an ovulatory luteinizing hormone (LH) peak and subsequent ride in progesterone [20-22]. During the initial development, the ovarian follicles cells produce estrogen, and during the latter stage under the influence of LH, they produce progesterone [23,24]. Since the maturation of follicles has been affected by Neem seed oil and its Fraction IV and Fraction V. In the all above three treatment groups, the number of follicles reduces drastically which reflects inhibitory function of neem seed oil and its Fraction IV and V. Similarly, the increased level of serum cholesterol may show the antiestrogenic nature of plant material

because cholesterol is an important precursor in synthesis of steroid hormones [25-28]. The specific function of cholesterol in the ovary is to act as precursor molecule for synthesis of estrogen [29] so the increased level is thus due to non-utilization of cholesterol due to inhibitory ovary function so it is confirmed that Neem seed oil alone and its Fraction IV and V may affect the intraovarian estrogen level either by inhibiting the ovarian cell function or by inhibiting follicle-stimulating hormone and LH secretion through hypothalamus-hypophysis axis, and impaired estrogen synthesis has been achieved resulting infertility. Hematology and serum study shows that Neem seed oil and its all fractions have no toxic effect on general metabolism.

CONCLUSION

From the ongoing work, it can be concluded that neem seed oil alone and its Fraction IV and V cause functional sterility in female albino rabbits without causing any side effect on general metabolism.

ACKNOWLEDGMENT

We would like to acknowledge Head, Department of Zoology, Jai Narain Vyas University, Jodhpur, and Rajasthan, for providing all facilities.

AUTHORS' CONTRIBUTIONS

The complete research work was suggested and mentored by Prof. Ashok Purohit. All the experimental works were performed by Vijeyata Vyas. Author drafted and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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