

Effect of the Oil Extract of *Ocimum Gratissimum* Leaves on the Reproductive Function and Fertility of Adult Male Rats

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Abstract

To study the effect of the oil extract of *Ocimum gratissimum* leaves on male reproductive function and fertility in of adult male rats. 18 Male rats, 3-4 months old, weighing 220-260 g were administered (by gastric intubation) the oil extract of *Ocimum gratissimum* leaves at two doses, 100 and 300 mg/kg for 60 days. The control group received distilled water for the same duration. After the end administration, male rats coupled with coeval female rats. Then animals were sacrificed and the blood, testes, epididymis, were collected for parameters analyses and fertility percentage. There was a significant decrease in the GSI ($P<0.01$), Sperm motility ($P<0.05$), Sperm viability ($P<0.001$), ESR ($P<0.001$), DSP ($P<0.05$), level of testosterone in the serum ($P<0.05$) and Fertility percentage ($P<0.01$) after days of treatment in treated rats with high dose. The oil extract of *Ocimum gratissimum* leaves at dose (300 mg/kg) had a negative impact on the male reproductive function and fertility

Key words: reproductive function, *Ocimum gratissimum*, oil extract, male fertility.

INTRODUCTION

Medicinal plants have for long been used as a source of relief either in the form of traditionally prepared concoctions or in the form of pure active principles [1]. Leaves, flowers, stems, roots, seeds, fruit, and bark of Medicinal plants can all be constituents of herbal medicines. The medicinal values of these plants lie in their component phytochemicals, which produce definite physiological actions on the human body. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds [2-3].

Previous studies have shown the anti-fertility effects, fertility-enhancing properties and spermatogenic effects of some plant extracts. *Melia Azadrach*, *Sarcostemma acidum*, *Malvaviscus conzattii*, *piper linn*, *Boswellia thurifera*, *Lepidium meyenii* and *Colebrokia oppositifolia* have mentioned effects [4-12].

Among all families of the plant kingdom, members of the Lamiaceae have been used for centuries in folk medicine. In Persian folk medicine, *Ocimum gratissimum* L. (Lamiaceae), commonly known as "Raihan-e- Soleiman and Jamesferm", is naturally used in the treatment of different diseases, e.g., rheumatism, Gonorrhoea, upper respiratory tract infections, fever and strengthening the stomach [13]. The leaf extract has been reported to be curative for respiratory disease, earache, vomiting and gastric upsets in children. [14], diarrhea, headache, ophthalmic and skin disease, and pneumonia

and els where [15-16]. The *Ocimum* oil is also active against several species of protozoa (*Trypanosomatid Herpetomonas samuelpessoai*), bacteria (*Escherichia coli*, *Shigella*, *Salmonella* and *Proteus*) and fungi (*Trichophyton rubrum* and *T. mentagrophytes*) [16-21].

The objective of this investigation was to determine Effect of the oil extract of *Ocimum gratissimum* leaves on the reproductive function and fertility of adult male rats.

MATERIAL AND METHODS

Plant material and stock solutions

Ocimum gratissimum was collected from Fars province, Iran and have been identified, and deposited in the herbarium of Biology department of Payamenoor university. Fresh leaves from the plant were cut into pieces and subjected to steam distillation. The distillate was then extracted with petroleum ether, which was removed carefully, and the essential oil was obtained. The oil was then stored at -20 (°C) until needed [16].

Animals

18 Adult male Wistar rats (240 ± 20 g body weight and 3- 4 months age) were provided by the animal house of Science Faculty of Tehran University. Animals were maintained in plastic cages, under controlled temperature (25 ± 2C) and light (12L, 12D).

Treatments

Eighteen Male rats of proven fertility were divided randomly into 3 groups of 6 animals each. Group A: treated 100 mg/kg extract oil for 60 days. Group B: treated 300 mg/kg extract oil for 60 days. Control group: received 1ml distill water for the same duration. All animals received oil extract and distill water through oral administration.

Evaluation of parameters

After the last administration, each male rat was caged separately with 2 coeval females of proven fertility in the evening for 6 days (mating test). After the evaluation of fertility by mating test, the animal were sacrificed by decapitation and testes and epididymis removed and weighted.

Body weight

Body weights of animals were recorded every week during treatment and before the experiments.

GSI (Gonadosomatic index)

This index indicates the testes weight/body weight ratio.

Sperm motility and sperm viability

To determine these parameters, 100 mg of cauda epididymides was minced into 5 ml of 0.9% NaCl. One drop of evenly mixed sample was applied to a Neubauer's counting chamber under coverslip. Quantitative motility expressed as percentage was determined by counting motile and immotile spermatozoa per unit area and quantitative viability expressed as percentage was determined by counting viable and inviable spermatozoa per unit area. Viable spermatozoa can't absorb the Negrosin stain but inviable spermatozoa can absorb the Negrosin stain. Cauda epididymal sperm counts were performed by routine procedure and expressed as percentage[5 and 22-24].

ESR and DSP

Epididymal sperm reserve (ESR) and daily sperm production (DSP) were assessed by method of Ribb et al. Each epididymis was divided into the caput, corpus, and cauda, and each part was processed separately. After mincing the tissue with a pair of scissors, it was transferred quantitatively to a Waring Blendor using 20 ml of homogenizing solution. This solution consisted of physiological saline with 0.05% Triton X-100 added. The tissue was homogenized for 1 min, and the homogenates were transferred quantitatively to glass jars. Additional homogenizing solution was added to dilute sperm concentrations to convenient levels for accurate hemacytometer counting (400-600 sperm per chamber). Sperm concentrations were determined by counting sperm present in 10 large squares of each of 8

hemacytometer chambers. Each chamber was filled with a different pipette, and the homogenates were mixed for 2 min with a magnetic stirrer just prior to filling pipettes. The DSP was determined from quantitative testicular histology. Testes after the weighting, transferred to 50 ml of solution consisted of physiological saline. The tissue was homogenized for 5 min, and the homogenates were transferred quantitatively to glass jars, then sperm concentrations were determined by counting of ESR method. Sperm counts were performed by routine procedure and expressed as million[5 and 25].

Fertility test(mating test)

This index was determined by Oberlander and et al. In each stages, each male rat was caged separately with 2 coeval females of proven fertility in the evening for 6 days. Presence of sperms in the vaginal smears examined on the next day morning indicated that the females had mated to the particular male and the day of mating was taken to be days 1 of pregnancy. Fertility test was considered positive when implantation sites were present[26].

Concentration of testosterone

After the evaluation of fertility by mating test, the animals were sacrificed by decapitation and blood was collected by cardiac puncture and serum was separated. Concentration of testosterone was determined by Radioimmunoassay(RIA)[27].

STATISTICAL ANALYSIS

Data are expressed as mean \pm ESM differences between control and test groups were analyzed using either Student's 't' test and INSTANT software, $P < 0.05$ was considered as significant difference.

RESULTS

The results showed that administration of extract oil of *O. gratissimum* leaves, at the doses of 100 and 300mg/kg/day, for 60days, caused no significant deference of body weight, Epididymis weight and GSI compared with control group (table 1). In addition, a significant decrease in sperm motility ($P < 0.05$) and Sperm viability ($P < 0.001$) were observed between high dose group (300 mg) in compared with control group (table 1).

The results presented in table 2 shows that oral administration of extract oil of *Ocimum gratissimum* leaves at dose (300 mg/kg body weight) for 60 days to male rats had significant decreased on ESR($P < 0.001$), DSP($P < 0.05$), Testosterone concentration($P < 0.05$) and fertility($P < 0.01$). The results also presented no significant deference in ESR, DSP, Testosterone concentration and fertility between low dose group(100 mg) in compared with control group (table 2).

Table 1. Effects of extract oil on body and organs weight and motility and viability

Group	Body weight(g)	Epididymis weight (1000 × g)	GSI(1000 × g)	Sperm motility(%)	Sperm viability (%)
Control	81.08±4.94	60.5±1.65	7.23±0.26	77.68±2.11	80.5±2.26
A	72.75±4.99	66.17±2.84	7.02±0.33	70.17±3.5	80.33±2.25
B	74.5±6.26	63.83±2.44	6.03±0.13**	62.33±3.68*	56.17±3.54***

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, compared with the control

Table 2. Effects of extract oil on ESR, DSP, testosterone and fertility.

Group	ESR(million)	DSP(million)	Testosterone concentration(ng/dl)	Fertility(%)
Control	219.83±7.73	25.83±1.17	580.5±21.1	77.67±1.99
A	202.33±3.89	23.5±1.33	584.33±14.7	75.5±2.75
B	172.67±7.96***	19.33±1.69*	505.67±23.79*	61.83±2.55**

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, compared with the control

DISCUSSION

The aim of the present study was to assess the effects of oil extract of *Ocimum gratissimum* leaves after oral administration on the reproductive function and fertility of adult male rats. The animal model used in this study has been used previously with minor changes in several studies to assess the adverse effects of different compounds on reproduction in laboratory animals [5, 10].

The decrease in GSI (testes weight/body weight) may be attributed to the increase level of damage on the seminiferous tubules of experimental rats because in previous study showed the lesion characterized by erosion of germinal tissue and interstitial edema after ingestion of *O. gratissimum* to rats [28]. In this study. In addition, oral administration of oil extract of *O. gratissimum* (300 mg/kg), for 60 days, reduced the hormone level of testosterone. The reduction in testosterone might be due to this oil, which altered androgen hormones synthesis of Leydig cells.

The reduction in the motility and viability of spermatozoa may be due to activity of its spermicidal. Earlier sperm abnormalities and a severe degeneration of spermatogenic element and reduction in activity of GTP, a marker of sertoli cell function were reported in experimental animals treated with extracts of *O. gratissimum* [28 and 29].

Reduction in ESR and DSP parameters may be due to altered androgenic synthesis and spermatogenesis as indicated by the decrease of GSI and sperm motility and sperm viability in this study.

Decrease in pregnancy in untreated females rats which were mated with treated males

may be due to failure of fertilization as indicated by the smaller number of sperm motility and sperm viability and sperm abnormalities in previous study [28].

In summary, this investigation confirm, that *O. gratissimum* (300mg/kg oral administration for 60 days) has an inhibitory effect on the reproductive function and

fertility of adult male rats. This study also showed that *O. gratissimum* (100mg/kg oral administration for 60 days) has no effect on the reproductive function and fertility of adult male rats.

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