

Research Article**Investigating the Effect of Aqueous Extract of Olibanum on Folliculogenesis in Rats****Hossein Kargar Jahromi¹ and Hojatollah Karimi Jashni¹**¹Research center for non-Communicable Diseases,
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ABSTRACT

Background and Objective: reproductive parameters in males and females can be affected by herbal extracts. *Boswellia serrata* also known as the Indian version of frankincense is an aromatic resin extracted from a tree and belongs to *Boswellia*, which improves and increases fertility indices in males. The present study aimed to evaluate the effect of aqueous extract of Olibanum on folliculogenesis in rats.

Materials and Methods: in this experimental study, 24 mature female Wistar rats were used. The rats were divided into three experimental and control groups consisting of eight individual rats. The first and the second experimental groups were respectively given 250mg/kg and 500mg/kg aqueous extract of Olibanum. All extracts were administered by gavage for 21 days. At the end of the experiments, blood samples were collected from the rats to evaluate serum levels of estrogen and progesterone. The ovaries were also isolated and weighed. Tissue sections were cut from the ovaries and examined by a light microscope. One-way analysis of variance was used to analyze the results. *Findings:* mean weight of ovaries in the first and the second experimental groups was significantly different compared to the control group ($p < 0.05$). Average number of primary follicles and tertiary follicles significantly increased in the second experimental group compared to the control group ($p < 0.05$). Mean serum concentration of estrogen in the two experimental groups significantly increased compared with the control group ($p < 0.05$). Mean serum concentration of progesterone in the first and the second experimental groups significantly increased compared with the control group ($p < 0.05$).

Conclusion: The results showed that aqueous extracts of Olibanum in doses prescribed in the study significantly increased folliculogenesis in rats.

Keywords: Olibanum, folliculogenesis, rat**INTRODUCTION**

Regular and natural ovarian cycles are determinants of fertility in females. Normal ovarian cycle consists of two follicular and luteal phases. Growth and development of ovarian follicles occur at follicular phase. Growth of corpus luteum occurs at luteal phase (1). The ovaries produce and release not only eggs, but also two groups of sex hormones as estrogen and progesterone. Estrogen is mainly involved in proliferation and growth of specialized cells in the body. This hormone is responsible for

development of secondary sexual traits in females. Progesterone also prepares the uterus for pregnancy and the breasts for lactation (2).

Several studies have shown that reproductive parameters in males and females can be affected by herbal extracts (3). Accordingly, herbs significantly contribute to treatment of infertility or birth control in both traditional medicine and modern medicine.

Olibanum is an aromatic resin obtained from trees of the genus *Boswellia* (4). Olibanum in Iranian

market originates from two species as follows: (a) *B.carteri* as an indigenous plant to shores of the Red Sea and particularly northeastern Africa that originates from Somalia and Ethiopia; (b) *B.serrata* grows in different parts of India. Chemical composition of Olibanum depends on plant species and origin as well as the season and time of collection of plant specimens. Nevertheless, chemical composition of this species contains 25%-35% alcohol-insoluble resin, 60%-70% alcohol-soluble resin and the rest consists of a kind of essential oil (5). Boswellic acids are a series of pentacyclic triterpene molecules that appear in Olibanum resin. These compounds are structurally similar to steroids. These are found either in singular forms or in combination with other materials (6). Other ingredients such as incensole, pinene, cymene, sabinene, limonene and carene were also identified in Olibanum (7). Scientific studies on pharmacological effects of Olibanum showed anti-inflammatory activity (8), immunomodulatory effect (9), antitumor properties (10), fat and blood glucose lowering effects (11) and memory booster (12) features of Olibanum. The results showed that aqueous extract of Olibanum improves and increases fertility indices in males. Various studies have shown that aqueous extract of Olibanum increases secretion of follicle-stimulating hormone, testosterone, the number and percentage of sperm motility as well as weights of sexual organs in males (13 and 14). It was also shown that aqueous extract of Olibanum increases the number of transferred embryos to the uterus in female rats (14). Thus, the present study aimed to investigate the effect of aqueous extract of Olibanum on folliculogenesis in rats because no study has examined the effects of Olibanum resin on reproductive physiology of females and according to presence of Boswellic acids with steroid-like properties in Olibanum resin.

MATERIALS AND METHODS

In this study, 24 healthy and mature adult female rats were used who were from two to three months

old and averagely weighted from 170 grams to 200 grams. The rats were kept in 12 hours of light and 12 hours of darkness during the experiment. Compressed food and water were given to them without any restrictions during this period. The rats were kept at in animal breeding room of Jahrom University of Medical Sciences for one week to adapt to the environment.

A certain amount of Olibanum resin was powered using a porcelain mortar in order to prepare aqueous extract of the herb. The powered extract was weighted proportional to desired dose and dissolved in distilled water as a solvent (14). Aqueous extract of Olibanum resin was daily given to the experimental groups using a special needle gavage in following doses: 250mg/kg and 500mg/kg with respect to body weight (14).

The rats were divided randomly into three groups. Each group consisted of eight animals. The control group was given enough compressed food during the experiment. The first and the second experimental groups were orally given respectively 250mg/kg and 500mg/kg aqueous extracts of Olibanum resin with respect to their body weight. Blood sample was taken from all groups after 3 weeks under anesthesia. Blood serum was isolated. Serum concentrations of estrogen and progesterone hormones were measured with special kits using ELISA method. The ovaries were isolated by dissection and weighed using a digital scale. The ovaries were placed in 10% formalin. After casting, 5 μ serial sections of tissues were prepared. Then, three fields from each slide were examined under a light microscope using a scaled graticule. The number of primary, secondary and tertiary follicles was counted and average number of these follicles was calculated. The collected data was analyzed using ANOVA. Statistical analysis was performed using SPSS version 21. Significance level was considered as $p < 0.05$.

Findings

Contents of Table 1 showed that average weight of ovaries in the experimental groups that received 250mg/kg and 500mg/kg doses of aqueous extract

of Olibanum significantly increased compared with the control group ($P < 0.05$). Average number of primary follicles in the experimental groups that received 250mg/kg and 500mg/kg doses of aqueous extract of Olibanum increased but a significant increase was only observed in the experimental group that received 500mg/kg doses of aqueous extract of Olibanum ($P < 0.05$). Average number of secondary follicles increased in the experimental groups that received 250mg/kg and 500mg/kg doses of aqueous extract of Olibanum compared to the control group. But this increase was not statistically significant. A statistically significant increase was also observed in average number of tertiary follicles only in the experimental group that received 500 milligrams

per kg doses of aqueous extract of Olibanum compared to the control group ($P < 0.05$).

Contents of Table 2 showed that mean serum concentration of estrogen increased in the experimental groups that received 250mg/kg and 500mg/kg doses of aqueous extract of Olibanum compared with the control group but a significant increase was only observed in the experimental group that received 500mg/kg doses of aqueous extract of Olibanum ($P < 0.05$). Mean serum concentration of progesterone hormone significantly increased in the experimental groups that received 250mg/kg and 500mg/kg doses of aqueous extract of Olibanum compared with the control group ($P < 0.05$).

Table 1: Average weight of ovaries and average number of ovarian follicles in different groups

Variable Groups	Weight of ovaries (mg)	Average number of primary follicles	Average number of secondary follicles	Average number of tertiary follicles
	Mean \pm standard deviation	Mean \pm standard deviation	Mean \pm standard deviation	Mean \pm standard deviation
Control group	29.8 \pm 4	5.5 \pm 1	4.5 \pm 1.3	1.6 \pm 0.5
The first experimental group (250mg/kg Olibanum extract)	35 \pm 5 *	6.2 \pm 1	5 \pm 1	1.8 \pm 0.6
The third experimental group (500mg/kg Olibanum extract)	38.8 \pm 3 *	7 \pm 1 *	5.3 \pm 1	3 \pm 0.7 *

Table 2 - mean serum concentration of estrogen and progesterone hormones in different groups

Variable Groups	Estrogen hormone (nano gram)	Progesterone hormone (nano gram)
	Mean \pm standard deviation	Mean \pm standard deviation
Control group	53.5 \pm 7.3	16.1 \pm 2.8
The first experimental group (250mg/kg Olibanum extract)	65 \pm 10	24.2 \pm 3 *
The second experimental group (500mg/kg Olibanum extract)	107 \pm 12 *	28 \pm 4.6 *

DISCUSSION

The results showed that oral administration of 250mg/kg and 500mg/kg doses of aqueous Olibanum extract to rats increased ovarian weight. Body weight gain and increased weight of sexual organs were also reported under the influence of aqueous extract of Olibanum in other studies (13 and 14).

Aqueous extract of Olibanum also increased the number of primary, secondary and tertiary follicles. This reflects positive effects of Olibanum extract on folliculogenesis as well as significant growth and differentiation of ovarian follicles under control of follicle-stimulating hormone secreted by pituitary gland (15). However, gonadotropin hormones were not measured in this study. Nusier *et al.* found out

that oral administration of 250mg/kg and 500mg/kg doses of aqueous extract of Olibanum significantly increased secretion of follicle-stimulating hormone in males in 2007 (14). Given the similarity of method of administration as well as doses of aqueous extract of Olibanum used in this study and the study conducted by Nusier *et al.*, it can be concluded that number of ovarian follicles significantly increases under the influence of Olibanum extract on follicle-stimulating hormone.

In addition, the results showed strong antioxidant properties of the compounds found in Olibanum, which eliminate free radicals from the body (16). The amount of antioxidants in follicular fluid is increased by reducing oxidative stress at follicular phase in ovarian cycle, which inhibits follicular atresia and consequently increases ovarian follicles (17).

An increase in ovarian hormones of estrogen and progesterone was consistent with an increase in the number of follicles under the influence of aqueous extracts of Olibanum in the present study. In normal ovarian cycle, estrogen is secreted by granulosa cells at follicular stage and progesterone is secreted by corpus luteum during ovulation (1 and 2). Boswellic acids are the most important ingredients of Olibanum resin with therapeutic properties. These compounds inhibit lipoxygenase enzyme (5 and 18). These enzymes play a key role in metabolism of arachidonic acid. Many therapeutic effects of Olibanum are manifested through inhibition of this enzyme (5). Inhibition of arachidonic also inhibits production of prostaglandins. Given the role of prostaglandins in production of gonadotropin (19), Boswellic acids found in Olibanum inhibit negative self-regulating effects of gonadotropin on sex hormones.

In addition, aqueous extract of Olibanum can directly affect ovarian steroidogenesis. The results showed that Boswellic acids found in Olibanum resin acids have steroid-like properties (6). As a result, Boswellic acids increase ovarian

hormone levels. Various studies have also shown that Boswellic acids and Boswellia extract activate protein kinase (18 and 20). Protein kinase causes phosphorylation of key enzymes involved in synthesis of steroid hormones (21). Thereby, increased activity of protein kinase by compounds found in Olibanum extract also contribute to increased steroidogenesis under the influence of Olibanum extract.

CONCLUSION

In the present study, the effects of aqueous extract of Olibanum on ovarian follicular phase and ovarian hormones was studied. The results showed boosting effect of this extract on folliculogenesis. However, further studies should be conducted on possible effects of aqueous extract of Olibanum on gonadotropin hormone in order to identify the exact mechanism of the extract due to proven effect of gonadotropin hormones secreted by pituitary on folliculogenesis.

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Conflict of interests:

The authors have no conflicts of interest with regard to the compilation and/or publication of this article.

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