

Histological and Hormonal Study about the Effect of Aqueous Extract of *Ocimum gratissimum* on Female Reproductive System in Albino Mice

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Abstract

Thirty female mice were used in this study to investigate changes in some hormonal and histological parameters after treatment with *ocimum gratissimum* aqueous extract at two doses 200_300 for 28 days. The animals were divided randomly into three groups. Group I (10 female mice) received 200 mg/kg/day, Group II (10 female mice) received 300 mg/kg, and the control group (10 female mice) received distilled water for the same duration. At the end of treatment, the animals were sacrificed and blood collected for the assay of the serum level of prolactin, progesterone, luteinizing hormone, estradiol (E2) and follicle stimulating hormone were determined using enzyme-linked immunosorbent assay (ELISA). In this study, the results show significant ($P > 0.05$) difference in the level of E2 in group I and a significant increase in group II. The results also show non-significant increase in group II in the level of luteinizing hormone in both groups. However, significant increases were also observed in prolactin and progesterone levels in the test group compared with the control group. The findings in this study indicate that *ocimum gratissimum* has an important implication for female contraceptive development.

Key words: Hormones, Elisa, Albino mice, Ovaries, *Ocimum Gratissimum*

INTRODUCTION

Herbal plants especially vegetable used as sources of medicines that may be traditionally prepared concoctions or in the form of pure active constituents. The most active substances in the medicinal plants such as alkaloids, tannins, flavonoids and phenolic compounds^{[1][2]}. Some herbal plants have anti-fertility enhancing properties and spermatogenesis effect of some plant extracts; amongst these plants is *ocimum gratissimum*^{[3][4]}. Which belongs to the Lamiaceae and known as Raihan-e-soleiman^[5], this plant is widely distributed in the tropical countries of the world^[6], *Ocimum gratissimum* is a plant highly praised for its nutritional and therapeutic benefits. Phytochemical screening has revealed many bioactive as well as toxic agents of plant extract that can affect the regulation of oestrous cycle, conception and reproduction. The most components of these plants are linaloleuol, thymol and in addition, they contain saponins, steroid, estragol and terpenes^[7]. The components of these plants have biological activities such as antidiabetic, antiseptic, antimicrobial activities^{[8][9]}. The leaf extract of this plant is used for the treatment of respiratory diseases, vomiting, gastric disorder in children^[10].

Female fertility is a biological process regulated by female hormones and these hormones used to regulate the reproductive cycle and used to test various types of disorder in females such as menopause, early or delayed puberty. In addition, one of the causes of female infertility are hormones commonly associated with ovulation, polycystic ovarian syndrome, damage to fallopian tube. Endocrine disorders result from increased or decreased production of hormones or absence of tissue that respond to nature circulation hormone^{[11][12]}. Estrogens inhibit mouse oocyte nest breakdown and follicle assembly. Estrogenic action reduces follicle assembly leading to fewer primary and subsequent developing follicles. Thus, the study of follicular populations provides important information about the function of the ovary, in particular the relationship between folliculogenesis and also environmental factors having estrogenic property that regulate it^{[13][14]}.

MATERIALS AND METHODS

The study is performed on (30 mature female Swiss-albino mice), their ages ranged between (9-10 weeks) with body weights ranged between (25-30g) mice were obtained from the colony of the animal house in Kut Technical Institute. They were kept in a room supplied with air conditioner to keep the temperature

between (18-24°C), the air of the room was changed continuously by using ventilating fan and the light was controlled with arrangement of 12 hours of light and 12 hours of darkness. The animals were housed in plastic. The bedding material used was fine sawdust and wood shaving which was changed every other day to prevent accumulation of urinary pheromones, feeding with standard diet and watering was ad libitum^[17].

Collection of plant materials:

Fresh *Ocimum gratissimum* leaves were obtained from supermarket in Kut. They were washed under tap water and then again with distilled water. The plant material was air-dried in the shade for 5 days and then homogenized to fine powder and stored in airtight bottles with proper labeling.

Preparation of extract:

Powdered plant materials were collected and weighed carefully. (15) grams of the dry powder were dissolved with 150 ml of cold distilled water in a closed vessel and were allowed to stand for 24 hours, shaking occasionally. The marc was strained and pressed and the liquids obtained were mixed. The extraction is performed by repeated maceration with agitation. The crude extracts were filtered using Whatman no.1 filter paper and the filtrates were then evaporated to dryness in a rotary evaporator under a reduced pressure at 90°C, each (15) grams of dry plant mixture yield 1.4129 grams of gummy residue, and the gummy residue is dissolved in an adequate amount of distilled water and stored in a labeled sterile screw-capped bottle at (-20) °C until use^[16].

Experimental protocol

Animals were divided into three main groups each having five mice

Group I received 200 mg/kg body weight of plant extract

Group II received 300 mg/kg body weight of plant extract

Group III (control) received standard feed and distilled water.

Collection of blood sample

Blood samples were collected under light ether anesthesia by heart puncture using a 22-19 mm gauge needle. Serum for hormonal assay was obtained by centrifugation for 10 minutes at 3000 rpm and kept at (-20) °C until use^{[17][18]}.

Statistical analysis

Data from treated and control groups were expressed as mean ± standard error (M±SE) and analyzed using student's t-test to compare values from experimental and control groups at individual time points. Differences between groups were considered significant at $p < 0.01$ and highly significant at $p < 0.001$ ^[19].

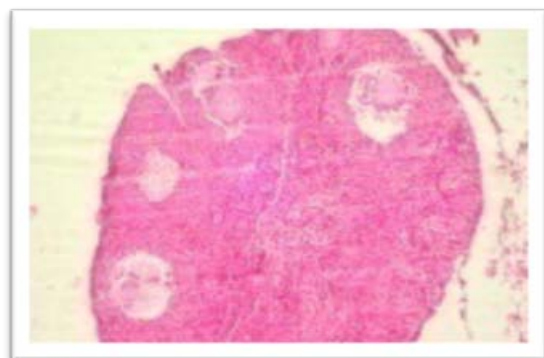
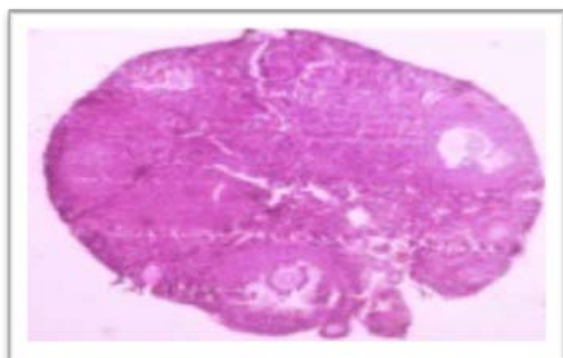
RESULT

Table 1: Effect of oral treatment of aqueous extract of *Ocimum gratissimum* (200mg/kg/day- 300mg/kg/day) for four weeks on some reproductive hormones in females mice.

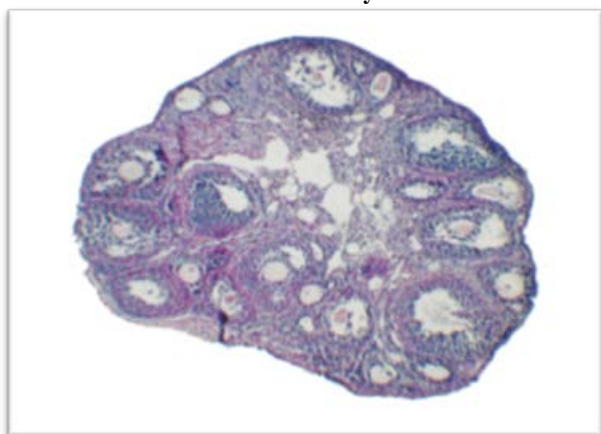
Parameters Groups	FSH (mIU/ml)	LH (mIU/ml)	E2 (pg/ml)	Progesterone (ng/ml)	Prolactin (ng/ml)
Group I 200mg/kg/day	9.75±0.82	8.54±0.62	2.04±0.01	7.54±0.93	7.93±1.07
Group II 300mg/kg/day	10.12±1.30	9.86±1.62	3.60±0.50*	7.16 ±0.86	7.48±1.12
Group III Control group	10.38±1.73	8.11±0.84	1.73±0.17	6.71±0.58	1.30±5.64*

Values= mean ± SE

P<0.05 significantly different from control group.



Real image (1): Image of hematoxylin and eosin stained after treatment of *Ocimum gratissimum* appear section of the ovarian tissue illustrating the interstitial cell masses, follicular residue and oocyte



Real image (2): Image of hematoxylin and eosin stained of ovarian section of a mature female mouse (control group)

DISCUSSION

Ocimum gratissimum is a plant important for its nutritional and therapeutic benefits. Phytochemical screening has discover many biological activities in addition toxic agent of plant extract that can affect the regulation of oestrous cycle, conception and reproduction [22] [23] [24]. The use of herbs in the management of disease has been a regular practice in Africa with considerable therapeutic success. Therefore the presence of these phytochemicals may account for the changes in the levels of the circulating hormones observed in this study [25].

Follicle stimulating hormone is the central hormone of mammalian reproduction, essential for gonadal development and maturation at puberty as well as gamete production during the fertile phase of life [26]. It stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulose cells. The reduction in the levels of follicle stimulating hormone by the extract may retardation folliculogenesis and delay maturation of the follicle in the pre-ovulatory phase [27]. It is possible that the extract have exerted its effect on the hypothalamus since the secretion of stimulating hormone is regulated by the gonadotropic releasing hormone secreted by the hypothalamus. The reduction observed in the level of this hormone may adversely affect on conception in female animals. Other researchers had observed the inhibitory effect of other plant parts on the release of the gonadotropins [28] [29] [30].

Lutenizing hormone stimulates secretion of sex steroids from the gonads. In females, ovulation of mature follicles in the ovary is induced by a surge of luteinizing hormone secretion during the pre-ovulatory periods. Several authors have demonstrated that lutenizing hormone release surges at the pre-estrous stage are responsible for ovulation [31] [32]. Any substance capable of inhibiting this release could provoke disruption of ovulation by decreasing the number of mature follicles or induce an oestrous cycle disruption at rest [30]. The reduction in level of serum lutenizing hormone indicates the inhibitory effect of the extract on the release of lutenizing hormone which may trigger disruption of ovulation. This may result in impairment of oestrous cycle, inhibit conception and normal reproduction in the females. It is therefore possible that *Ocimum gratissimum* contains anti-gonadotropic substance which may affect the oestrous cycle and obstruct reproduction in females.

Prolactin helps to initiate breast development by inducing labulo-alveolar growth of the mammary gland. It also stimulates lactogenesis. Dopamine serves as the major-inhibiting factor on prolactin secretion [33]. The enhanced level of prolactin observed in this study may be attributed to the effect of the extract probably acting as a dopamine antagonist. High prolactin level tend to suppress the ovulatory cycle by inhibiting the secretion of both follicle stimulating hormone and gonadotropic-releasing hormones (GnRH) [34], which are necessary for ovulation. Such

increase in prolactin may inhibit ovulation and stimulation the loss of menstrual periods which will prevent conception.

The result of this study indicates that aqueous extract of *Ocimum gratissimum* significantly ($p > 0.05$) increased serum progesterone in female mice. Phytochemical constituents of *Ocimum gratissimum* are alkaloids, saponin, tanins, phlobatannins, anthraquinones, steroids, flavonoids and cardiac glycosides^[34] ^[35]. Yu and others^[36] had reported that saponins lower serum androgens and 17β -estradiol, but elevate progesterone levels, suggesting that saponins modulate steroidogenesis in the ovary.

Estradiol stimulates the growth of the uterine lining, causing it to thicken during the pre-ovulatory phase of the cycle. It is well established that estradiol is directly responsible for growth and development of reproductive organs^[37]. Plants with estrogenic property can directly effect on pituitary action by peripheral modulation of lutenizing hormone and follicle stimulating hormone, decreasing secretion of these hormones and blocking ovulation^[38] thus, the reduction in the serum concentration of estradiol observed in the low dose group may be attributed to a decreased aromatase activity during estrogen synthesis. Consequently such decreased in estradiol levels may prevent ovulation, preparation of the reproductive tract for zygote implantation and the subsequent maintenance of pregnancy state Kadohama and others^[39] had reported that several plant alkaloids inhibit aromatase activity.

Thus it is possible that the aqueous extract of *Ocimum gratissimum* contain biologically active phytochemicals which may be effect on endocrine . Such substances in the plant extract may induce hormonal disorders such as anti-fertility and contraception in hormone dependent organs like the ovary and mammary glands. The findings in this study have important implications for female contraceptive development.

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