

The Effect Of Aqueous Extract Of *Anastatica Hierochuntica* On Some Hormones In Mouse Females

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Abstract

Anastatica hierochuntica is believed to be very useful in Arab countries for treating various health disorders. The aqueous extract of *Anastatica hierochuntica* was investigated for its effects on hormones in mice females. the aqueous extract of plant at a dose level of 100mg/1mL showed significant increase ($p < 0.05$) on levels of hormones LH, FSH, PRL level and PRO level. Clomid treated group showd significant increase in LH, FSH, and PRL while there was non-significant increase in PRO. From the above results, it is concluded for the first time that aqueous *Anastatica hierchuntica* extract offers significant effect of LH, FSH, PRL, and PRO in this mice females scompared with control.

Keyword: *Anastatica hierochuntica*, Clomid, LH, FSH, PRL, PRO.

Abbreviations: LH, Luteinizing hormone; FSH, Follicle stimulation hormone; PRL, Prolactin; PRO, Progesteron.

Introduction

Anastatica is a monotypic genus with the type species *anastatica hierochuntica*. The genus is a member of the family Brassicaceae (formerly called Cruciferae), in the division Magnoliophyta of the class Magnoliopsida. The plant is a small gray annual herb that rarely grows above 15 cm high, and bears minute white flowers (figure 1A). It is a tumbleweed and a resurrection plant [1,2].

Anastatica is found in arid areas in the middle East and Sahara Desert, including parts of North Africa and regions of Iran, Egypt, Palestine, Israel, Iraq, Jordan, and Pakistan [3,4]. After the rainy season, the plant dries up, dropping leaves and curling branches into a tight ball, and dies. Within the ball, the fruits remain attached and closed, protecting the seeds and preventing them from being dispersed prematurely (see figure 1B) [4].

The process of curling and uncurling is completely reversible and can be repeated many times. The ability of the plant to do this attributes to the presence of trehalose, [5] a disaccharide involved in several mechanisms of cryptobiosis [6].

Anastatica hierochuntica is among the common medicinal plants widely used in Hijaz, Najd, and Al Rub'Al Kali. The plant is prescribed in folk medicine for difficult labor, uterine hemorrhage and to facilitate the expulsion of dead fetuses [2].

The German Commission recommends *Anastatica* for the treatment of menstrual problems, mastalgia, and premenstrual syndrome (PMS). [1]. It has been used to treat fibroid cysts and infertility (3), to stop miscarriages caused by progesterone insufficiency and to flush out the placenta after birth [[7- 9]. German health authorities recommend its use for corpus luteum insufficiency, menopausal symptoms and inadequate milk production in nursing mothers [10]. It is used to treat acne in teenagers [11]. The whole dried plant contains flavonoids: Quercetin (flavonol), Luteolin (flavone) [12].

Clomid

Clomifene or clomiphene (trademarked as Clomid) is a selective estrogen receptor modulator (SERM) that increases production of gonadotropins by inhibiting negative feedback on the hypothalamus. It is supplied as white, round tablets in 50 mg strength only [13]. It has become the most widely used fertility drug. It is used mainly in female infertility, in turn mainly as ovulation induction to reverse oligoovulation or anovulation such as in infertility in polycystic ovary syndrome, as well as being used for ovarian hyperstimulation, such as part of an *in vitro* fertilization procedure [14]. Structure of clomid is shown in fig (2).

The aim of our study is to evaluate the effect of the aqueous *Anastatica hierochuntica* extract on some hormones levels in female mouse, and to compare it with the effect of a well known drug (clomid).

Materials and methods

Samples of whole dried *Anastatica hierochuntica* were brought from Iraqi market in Baghdad, then aerial parts of the plant were isolated and kept in airtight glass containers till the time of the experiment. Then the dried plant was ground to fine powder. 25 gm of the powder, mixed with 250 mL of distilled water and were incubated for 3 hrs at (60) C°. Suspension was then filtered. Water extracts were prepared daily just before administration orally to the experimental animals in a dose of (10 mL/mouse of 250 g).

Preparation of clomid: Fresh solution of clomid was prepared (tablet dissolve in water or food) just before feeding. For the animals given the clomid (50 mg/kg body weight).

Experimental animals: Forty female micromys minutes (250 ± 10 g. each) were kindly supplied by city of Medicine, for the period from December 2011 to May 2012. And were used in this research. Mice were maintained with free access to water and diet (containing

multivitamins, protein, and wheat). Experimental animals were divided into four groups (13 mice each):

- 1- Control group: mouse were orally administered (using a feeding solution) with a daily dose of 10 mL distilled water for 1 month;
- 2- Plant – treated group: 10 mL of the plant extract (100 mg/mL) was orally administered daily to each mouse in this group for 1 month;
- 3- Clomid- treated group: 50mg of clomid was orally administered daily to each mouse in this group for 1 month;
- 4- Plant- treated clomaid group: 500mg of clomid was orally administered daily to each mouse in this group for 1 month, then 10 mL of the plant extract (100 mg/mL) was orally administered daily to each mouse in this group for 1 month.

Blood sampling: Blood samples were collected from the heart of mouse using heparinized capillary tubes. Serum was separated from blood samples, then frozen until used.

The levels of hormones (LH, FSH, PRL, and PRO) in the serum were measured by ELSA (Human Germany) using special kits for each hormone, provided from Monobind Inc (15-18).

Preliminary phytochemical screening: The tests were done to check the presence of the active chemical constituents such as alkaloids, phenolic content, carbohydrates, reducing sugar, and tanine by the following procedure:

- 1- Test for alkaloids, [19].
- 2- Test for phenolic content [20].
- 3- Test for carbohydrates [21].
- 4- Test for reducing sugar [9].
- 5- Test for tanine [23].
- 6- Test for amino acid and protein [24].

Statistical analysis: All statistical analysis of the study were done using SPSS version 15.0 for Windows (statistical Package for Social Science, Inc., Chicago, IL, USA).

Descriptive analysis was used to show the mean \pm standard deviation of variables. The significance of difference between mean values was estimated by Student T- test. The probability $p < 0.05$ = significant.

Results and discussion

Table(1) showed results of phytochemical screening of aqueous extract of *Anastatica hierochuntica*. Table(2) shows the hormone levels in the four studied groups. There was a significant increase in the levels of the hormone in mouse serum when compared with the control group. For G1 given the clomid (50mg/kg), the levels of these hormones were highly significant compared with G except for PRO.

The results in table (2) indicated that one month plant extract administration, shows a significant increase in the levels of hormones LH, FSH, PRL and PRO, (11.2 \pm 1.2), (5.5 \pm 1.8), (8.7 \pm 8.2) and (0.4 \pm 0.19) respectively compared to control groups (6.8 \pm 2.07), (3.8 \pm 1.6), (7.8 \pm 2.8) and (0.3 \pm 0.3) respectively. In the group where clomid was given, for 1 month the levels of LH, FSH, PRL and PRO (8.5 \pm 2.1), (26 \pm 3.3), (13.7 \pm 1.2), and (0.3 \pm 0.13) respectively were significant increased except PRO compared to control groups.

In the present study, the effect of the water extract of *Anastatica hierochuntica* was tested on normaly females mouse models. Clinical studies have shown its beneficial effects in the management of premenstrual stress syndrome (PMS) (Wuttke, et al., 2003)[25], and infertility and also for treatments disorders including corpus luteum insufficiency cyclic mastalgia as well as to treat hormonally induced acne (Mahady, et al., 2005)[26] Propping (1987) refers to a positive effect for plant extract use in regulation the menstrual cycle[27].

The effect of clomid on hormones is well

documented known. Our results agree with previous studies. That clomid works by "Kick-starting" the hormone system in to stimulation an egg to grow. It causes the pituitary gland to produce more follicle- stimulating hormone (FSH) and luteinizing hormone (LH), which, in turn, stimulate the ovaries in to producing more eggs and follicles[28]. If clomid does not stimulate production and ovulation of a follicle, progesterone levels won't rise. Neither clomid nor PRO alone, or both together, will fix the potential problems that can arise with embryo implantation[29].

PRL secretion is controlled by hypothalamic neurons that produce dopamine to inhibit its release. Disturbance of this inhibitory control results in hyperprolactinemia [30].

Previous investigators mentioned that serum PRL levels were not significantly changed by clomid in the patients who ovulated with the drug, they markedly decreased during and immediately after clomid treatment in patients who failed to ovulate with clomid[31].

However, no studies up to date have been able to demonstrate elevated LH, FSH, PRL and PRO levels in females mouse with aqueous extract of *Anastatica hierochuntica*.

In our study, the comparison between the effect of clomid and the effect of aqueous extract of the plant on hormones shows the rise in LH hormone levels after treating with aqueous extract of the plant more than treating with clomid. This raise could be attributed to aqueous extract of the plant stimulate pituitary gland, therefore return hormone balance for many members of the body. This aqueous extract of the plant reduces the side effect of estrogen hormone on the body and stimulates production LH hormone.

On the otherhand, the effect of clomid on FSH and PRL hormones was more than the effect of aqueous extract of the plant especially on the FSH hormone. This may be attributed to that Hormones are the body's way of communicating[32]. Hormones bind to hormone receptors to communicate. When estrogen binds to its receptor cell in the hypothalamus it communicates and lets the brain knows that estrogen levels are increasing[31]. Clomid has a similar structure to estrogen. Clomid binds to the receptor cell in the hypothalamus, which keeps the estrogen from attaching. Because the Clomid is blocking the receptors, they don't get the signal from the estrogen and this essentially tricks your body into thinking there is not enough estrogen. The body tries to kick up the production of estrogen by producing more GnRH. GnRH is what causes the release of FSH and LH. And FSH is what makes the follicles ripen and produces more estrogen. If the Clomid does what it is supposed to, the body will be tricked into producing more FSH and LH, which will hopefully cause mouse body to ovulate[33].

Conclusion

The aqueous of extract of the plant (*Anastatica hierochuntica*) has increased the levels of some hormones such as FSH, PRO, PRL and LH.

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Table(1): Result of phytochemical screening of aqueous extract of plant.

Pytochemical class	Aqueous extract
Alkaloids	+
Phenolic content	+
Carbohydrates	+
Reducing sugar	+
Tannins	+
Proteins	+

Table(2): Illustrate values of LH, FSH, PRL, and PRO level in blood mouse females.

Control n= 13	Clomid n= 13 G1	Plant extract n= 13 G2	Clomid+ plant extract n= 13 G3	p-value			
				G ₁ vs. C [*]	G ₂ vs.C [*]	G ₃ vs.C [*]	G ₁ vs.G ₂
Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D				
6.8± 2.07	8.5 ± 2.1	11.2± 1.2	12.5 ± 1.9	0.05	0.05	0.05	0.05
3.8 ±1.6	26± 3.3	5.5± 1.8	6.1 ± 7.5	0.05	0.05	0.05	0.05
7.8±2.8	13.7±1.2	8.7± 8,2	11.0±3.08	0.05	0.05	0.05	0.05
0.3± 0.13	0.3± 0.13	0.4 ±0.19	0.4± 0.19	No.sig ^{**}	0.05	0.05	0.05

C^{*}=Control, No.sig^{**}= No significant



(A)



(B)

Fig. (1): green plant (A), Anastatica hierochuntica dry curled skeleton (B) (4).

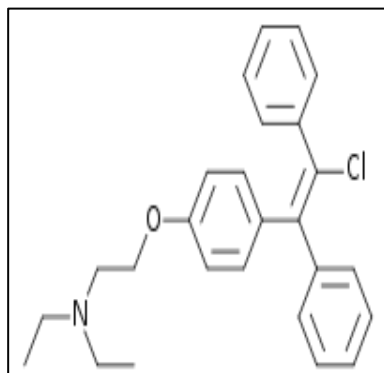


Fig.(2): 2-(4-(chloro-1,2-diphenylethenyl)phenoxy)-N,N-diethyl-ethanamine(14).

تأثير المستخلص المائي لنبات كف مريم في بعض الهرمونات لإناث الفئران

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الخلاصة

يعد نبات كف مريم من النباتات التي تمتلك العديد من الخصائص العلاجية المعروفة التي تستعمل على نطاق واسع في الوطن العربي. تضمن البحث الحالي دراسة تأثير المستخلص المائي لنبات كف مريم في بعض الهرمونات (LH,FSH,PRL,PRO). أظهرت نتائج الدراسة وجود تغير معنوي ملحوظ ($p<0.05$) عند استعمال تركيز 100mg/mL من المستخلص المائي للنبات، في حين أظهرت نتائج الدراسة في المجموعة المعاملة بالكلواميد تغيراً معنوياً ملحوظاً ($p<0.05$) في (LH,FSH, PRL) فيما عدا PRO. نستنتج بان المستخلص المائي لنبات كف مريم أعطى تأثيراً ملحوظاً ولأول مرة في نشاط بعض الهرمونات لإناث الفئران مقارنة بمجموعة السيطرة .

الكلمات المفتاحية: نبات كف مريم، كلوميد، الهرمونات (LH,FSH) ،هورمون البروجستيرون، هورمون البرولاكتين