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Detection of Active Compounds in the Water Extract of *Foeniculum Vulgare* L. and Its Effects on Serum Estrogen and Prolactin Levels in Female Albino Rats

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Abstract

The present study was designed to estimate the active ingredients in the aqueous extract of fennel *Foeniculum vulgare* L. fruits and test the effects of different concentrations of the extract on serum estrogen and prolactin levels in female rats. The work was conducted to prepare the aqueous extract in the laboratory, while the secondary active substances in the extract were estimated using High-Performance Liquid Chromatography (HPLC) technology. The experiments were conducted in the animal house of the College of Science, Tikrit university, on a total of 12 adult albino virgin female rats divided into four groups, each having three rats. The aqueous extract of the fruit plant was administered orally to animals at three concentrations (50%, 75%, and 100%) per day for 45 days. The fourth group was the control group that was left without treatment. Blood samples were obtained from the corner of the eye with a capillary tube and the serum was extracted to determine the hormone levels. The results showed that the aqueous extract of fennel fruits contains eleven active secondary metabolites, namely Eucalyptol, Terpinene, Anisole, Camphor, Anethole, Anisaldehyde, Apiole, α -pinene, Estragole, Fenchone, and Limonene, which seemed to play a role in regulating the levels of prolactin and estrogen hormones in rats treated with the extract. The results showed a direct relationship between the concentration of the fruit extract given to the animals and their serum levels of estrogen and prolactin. Increases in the levels of estrogen (44.56 ± 0.90 ng/ml) and prolactin (134.66 ± 0.80 ng/ml) were recorded using the concentration of 100% of the watery extract of *Foeniculum vulgare* L., as compared to the control group (126.33 ± 1.18 ; 6.37 ± 0.45 ng / ml, respectively). The results demonstrated the effective role of the aqueous extract of fennel fruits in stimulating estrogen and prolactin in female rats through their content of active compounds, which can be of great importance in stimulating the process of milk secretion in animals.

Keywords: effective compounds, *Foeniculum vulgare*, Estrogen, Prolactin, albino rats, HPLC.

الكشف عن المركبات الفعالة في المستخلص المائي *Foeniculum vulgare* وتأثيره على هرموني الحليب Prolactin والاستروجين Estrogen في اناث الجرذان البيض albino rats

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الخلاصة

الهدف: صممت الدراسة لتقدير المكونات الفعالة في المستخلص المائي لثمار نبات الشمر *Foeniculum vulgare* واختبار تأثيره على مستويات هرموني الاستروجين و البرولاكتين (هرمون الحليب) من خلال مقارنة التراكيز مع المجموعة الضابطة . **control group**

المواد وطرائق العمل: حضر المستخلص المائي في المختبر وقدرت المواد الفعالة (الايضات الثانوية) في المستخلص باستخدام تقنية كروماتوغرافيا الطور السائل عالي الالاء (HPLC) واجريت المعاملات في البيت الحيواني التابع لكلية العلوم – جامعة تكريت على مجموعة من الجرذان العذاري البيضاء البالغة وعددها 12 جرد قسمت الى اربعة مجاميع لكل مجموعة ثلاثة جردان ، عوملت بالمستخلص المائي لثمار نبات الشمر بثلاث تراكيز 50%، 75%، 100% وبالتجرع الفموي يوميا و لمدة 45 يوما اما المجموعة الرابعة فهي المجموعة الضابطة تركت بدون تجرع ، سحبت عينات الدم من زاوية العين بأنيوية شعرية لتقدير مستويات الهرمونات فيها .

النتائج: اظهرت النتائج ان المستخلص المائي لثمار نبات الشمر احتوى على احد عشر مركبا فعالا من مركبات الايض الثانوي وهي Eucalyptol, Terpinen, Anisole, Camphor, Anethole, Anisaldehyde, Apiole, Estragole, Fenchone, Limonene, a-pinene هذه المركبات لعبت دوراً في تنظيم مستويات هرموني البرولاكتين والاستروجين .اما بالنسبة لمستويات هرموني الاستروجين و البرولاكتين فقد ازدادت بالمقارنة مع مجموعة السيطرة عند التركيز 100% للمستخلص المائي لنبات الشمر وكانت على التوالي هو (134.2- 135.6 بيكومول / مل) و(43.7-45.5 بيكومول / مل) .

الاستنتاج : اثبتت النتائج الدور الفعال للمستخلص المائي لثمار نبات الشمر في تحفيز وتنشيط هرموني الاستروجين والبرولاكتين لدى اناث الجرذان من خلال ما تحتويه ثمار هذا النبات من مركبات فعالة ذات اهمية كبيرة في تحفيز الهرمونات الانثوية وافراز الحليب.

Introduction

Medicinal plants have been used since ancient times as a treatment for many diseases. One of these medicinal plants is fennel, which is a well-known perennial herb from the Umbelliferae family. The stems of this plant have many filamentous leaves which hang downward. Umbel inflorescences carry a large number of flowers, and there is a vial of each flower and the fruit contains inside the seed [1].

Several names of fennel have been mentioned in the literature, including cinnabar, raising, cabbage, cumin, fennel, bitter fennel, sweet fennel, and sweet seed [2]. The portions used are roots, stems, leaves, fruits, and seeds [3]. Fennel contains volatile oils, including anethole which has a pharmacological values, and give the distinctive smell of the plant. It also contains estragole, vitamins A, B, and C, phosphorous minerals, calcium, sulfur, iron, and potassium [4].

High-performance liquid chromatography is a technique used for separating the components of a mixture. Usually, HPLC is preferred over other methods used in quantitative analysis for obtaining an accurate qualitative separation of the components of the material to be identified [5].

The pituitary gland is the most important endocrine gland in the body. It is responsible for the regulation of a wide range of endocrine glands and organs, through the various hormones it produces. Prolactin is one of the most important hormones secreted by the pituitary gland. Normally, prolactin works to stimulate the breast of a woman to secrete milk from the first months of pregnancy, and then the hormone remains high in the blood [6]. High levels of prolactin cause infertility and disturbances in the menstrual cycle in women; the period between session's increases or the amount of blood changes from month to month. In addition, some menstruation may be absent from the menstrual cycle forever. Milk secretion increases from the breasts even though the woman is neither pregnant nor breastfeeding [7].

Estrogen is a female hormone and, although it is found in both women and men, it is existing in women in higher proportions. This hormone helps women to initiate sexual development in addition to another hormone that is important to women, which is progesterone. Estrogen and progesterone are the main pregnancy hormones that regulate the menstrual cycle of women and affect the entire reproductive process. During pregnancy, a woman produces more estrogen than she does during her lifetime when she is not pregnant. Increased estrogen during pregnancy enables the uterus and placenta to improve the

formation of blood vessels and transport nutrients to the developing fetus. Additionally, it is believed that estrogen plays an important role in helping the fetus grow and mature [8].

Fennel has been used to enhance milk production in breastfeeding women without knowing its effective chemicals [9]. The current study aims at determining the effects of fennel aqueous extract on prolactin and estrogen levels in female rats.

Material and Methods

Collection of fruits: The fruits of the fennel plant were collected and kept away from light and heat. The fruits were placed in dark boxes with a temperature of 12° C until they were used to estimate the active compounds and to prepare the aqueous extract for them.

Preparation of the extract: The aqueous extract of fennel fruits was prepared according to the method mentioned earlier [10] and the extract was kept in the refrigerator at 12 ° C until it was used.

Determination of the active compounds in *Foeniculum vulgare*

1. The aqueous extract of the fennel fruits was cooled.
2. The extract was dissolved in a solution representing the mobile phase, consisting of distilled water and ethanol alcohol at a ratio of 70:30 ml. Acetic acid (1 ml) was added to reduce the pH of the solution.
3. A volume of 10 ml of the sample was injected into a Shimadzu HPLC device equipped with a c-18 separation column (with specifications of 30 mm * 0.25 mm * 0.25 mm) and a UV detector at 280 nm.
4. The flow rate of the mobile phase was 1 ml/min at a temperature of 25 ° C.
5. The concentration of the standard solution was 50 mm / ml.

Concentrations preparation: Fennel leaves and fruit extract were prepared according to a method reported previously [11] with some modifications. A specific weight of the leaves was taken with distilled water. Mixed to homogenized at a rate of 4 g leaves and 100 ml of distilled water for half an hour with an electric mixer (Blender). The resulting solution was filtered. The filter was distributed to test tubes and a centrifuged at a speed of 5000 rpm for 50 minutes. The supernatant was collected and poured into glass dishes and then placed in an incubator at 50 M for 48 hours for the leachate to dry. The dry extract was collected and crushed to form a fine powder and kept in tightly sealed tubes until use.

Experimental blood samples: Twelve virgin female albino rats aged 2-3 months and weighting 175-195 g were taken from the animal house for the period from 1-9-2019 to 01-11-2019 and placed in plastic cages for rearing. The cages were supplied with sterile water bottles, as well as a vegetable diet consisting of brown flour. The aqueous extract concentration of the fennel fruit was given to the animals as a daily diet, consistent with the American Constitution method of medicinal herbs [12].

Treatment with extract: The twelve adult female rats were divided into four groups. Each group consisted of three animals. The first, second, and third groups were administrated orally with one of the prepared concentrations of the fennel fruit aqueous extract (50%, 75%, 100%, respectively) for 30 days. The fourth control group remained un-dosed for a duration of 45 days.

Physiological study: Before beginning the experiments, the animals were weighed and their weights were stabilized. A blood sample from all animals was drawn from the corner of the eye with the capillary tube [13]. The blood sample was separated by a centrifuge and placed under freezing at -50 ° C in a deep freeze device. A dosage of 1 ml of the aqueous extract solution of 50 %, 75 %, and 100 % of fennel plants was prepared daily and administered orally to all groups using a syringe except for the control group. After 30 days, a second blood sample was drawn once again [14].

Hormonal study: After centrifugation and discarding of the blood components of the treated animals and the control group, the blood serum was used to measure the levels of the concentrations of prolactin (PRL) and estrogen (Estradiol; EST). The working method was based on the principle of the enzyme-linked immunosorbent assay (ELISA) [15].

Statistical analysis

A statistical analysis of the results was performed to compare the result of different treatments using one-way ANOVA. The mean, standard deviation and standard error were calculated for all hormonal measures using SPSS program. The data were presented as mean + S.E and the values of $p < 0.05$ were considered statistically significant.

Results

Determination of effective compounds

The current study involved the identification and evaluation of some effective compounds in the water

extract of fennel fruits using the HPLC technique which included the detection of eleven active compounds and their retention time as well as the concentrations, as explained in Table- 1 . These active compounds in the extraction of fennel plants might possibly have a role in regulating the levels of prolactin and estrogen hormones.

Table 1-Effective compounds identified in fennel fruit extract using HPLC , with the values of retention time and concentrations.

NO.	The active compounds	Retention time Rt	Concentration mg / ml
1	Limonene	2.571	9.5
2	Eucalyptol	7.212	11.5
3	Terpinen	8.537	9.3
4	Anisole	11.018	6.7
5	Camphor	14.194	10.0
6	Anethole	19.610	6.9
7	Anisaldehyde	20.135	10.2
8	Apiole	23.826	9.1
9	a-pinene	26.924	18.3
10	Estragole	29.577	4.1
11	Fenchone	31.102	4.0

Estimating the level of hormones in fruit extract treated rats

Prolactin hormone: The results of the current research demonstrated a statistically significant increase in prolactin hormone levels in female rats upon treatment with the three concentrations (50%, 75%, and 100%) of the aqueous extract of fennel fruits, as compared to the control group ($p \leq 0.05$). The highest levels of prolactin hormones were reported at the concentrations of 100% aqueous fennel extract (134.66 ng/ml) (Table- 2).

While the other two concentrations of 50% and 75% had lower effects on the level prolactin, reaching 131.66 ng/ml and 133.36 ng/ml, respectively, as compared to the control group (126.33 ± 1.18 ng/ml). Thus, the concentration of 100% of the aqueous extract showed the best effect when compared to the 50% and 75% concentrations.

Estrogen hormone: This study showed a rise in estrogen levels with different concentrations of aqueous fennel fruits extract, relative to the control group, with a highest increase in the hormone levels with the highest concentrations of aqueous extract (Table- 2).

Table 2- Levels of estrogen and prolactin hormones in response to the treatment with different concentrations of water fennel fruit extract.

Categories	No. animal	Prolactin hormone ng/ml	Estrogen hormone pg/ml
Control	3	126.33 ± 1.18 a	6.37 ± 0.45 a
50 % of the extract	3	131.66 ± 0.90 b	13.06 ± 0.72 b
75 % of the extract	3	133.36 ± 0.83 b	29.80 ± 1.10 c
100 % of the extract	3	134.66 ± 0.80 c	44.56 ± 0.90 d

Data represents Means \pm S.E. Different small letters mean statistically significant differences at the value of $p < 0.05$

Discussion

It is believed that some effective compounds the aqueous extract of fennel fruits, as diagnosed by using liquid-phase chromatography technology, such as estragole and apiole and other compounds have a great role in raising the level of estrogen. These compounds act like hormone-stimulating compounds and stimulants. Fennel has been used since ancient times to stimulate hormones and release milk for lactating women [16]. Thus, our results are consistent with those and other previous reports[17]. In the current research, the results showed a high increase in the level of estrogen by using the concentration of 100% of the aqueous extract of the fennel fruit. This result is different from those of a previous study [18]. which showed a decrease in the level of the hormone estrogen by increasing

the concentration of the extract. This is due to the fact that the authors used the roots of fennel, which have much lower content of active compounds than that in the fruits.

This study demonstrated the effective role of the aqueous extract of fennel fruits in stimulating prolactin release in female rats. This could theoretically lead to increased milk secretion by treated animals, since fennel has been reported in traditional and alternative medicines as a stimulator of milk generation in breastfeeding females. In fact, phenols and flavonoids, such as α -pinene, limonene, and anisole, are all proven compounds as having roles in affecting female hormones.

This research proves the existence of these effective compounds in fennel fruits extract as being identified with HPLC technology. This was further proven by the effective role of the extract in increasing the serum levels of the female hormones. These results agree with those reported by a previous study [19]. The current study used fruits instead of leaves which have been tested in previous works [20], suggesting the presence of active substances in both leaves and fruits.

As shown, due to the nature of certain components and their ability to dissolve in water, the water extracts of some medicinal plants are more effective than alcoholic extracts. The current outcomes are different from those reported by other authors [21], who used alcohol extract to suppress female sex hormones secretion in female rats. The reason for this is that the nature of the plant compounds changes differently using the alcohol extract as compared to the water extract. As far as prolactin is concerned, the current results demonstrated an increase in the secretion of the hormone in females, and this is consistent with other studies, including a previous work [22] which reported an increase in the levels of prolactin in animals treated with a high concentration of 100% of watery extract of *Foeniculum vulgare* L. fruit.

Conclusions

From the abovementioned results, it is concluded that the aqueous extract of *Foeniculum vulgare* fruits with the concentration of 100% played an important role in the stimulation of the female hormones (prolactin and estrogen), which could possibly stimulate milk secretion in females. In comparison, the aqueous extract concentrations of 50% and 75% of fennel fruits caused similar, but lower, increases in hormone levels in treated animals.

References

1. Joe, A. **2012**. Description of some medicinal plants. *Journal of Medicine*. UK.
2. AL-Katib, Y. **2000**. *Taxonomy of seed plant*. university of Baghdad .Iraq.(in Arabic).
3. Kathrin, D. and Edward, T. **2018**. *Alternative medicine by medical plants*. The University of Nottingham, united kingdom.124-133.
4. Folen, S. **2019**. chemical compounds in plants . *Journal Botany*. Toronto. Canada .354-380p.
5. Oliveira, S. G. D.; de Moura, F. R. R.; Demarco, F. F.; Nascente, P. D. S; D. Pino, F. A. B., and Lund, R. G. **2012**. "An ethnomedicinal survey on phytotherapy with professionals and patients from Basic Care Units in the Brazilian Unified Health System," *Journal of Ethnopharmacology*, **140**(2):428–437.
6. Jones, A.J. **2018**. "effects of alcoholic extraction for *Foeniculum vulgare* to more hormones in female rabbit ", *biochemical journal*. **23**: 89-112.
7. Jevery, S.E. **2016**. Changes in Milk hormones by some medicinal plants. *Physiological and hormonal journal*, American. **4**: 122-132.
8. Marine, S.C., and Porland, M.T. **2019**. " prolactin hormones and many hormones in pregnant female rats ". *The Journal of human physiology*. **120**(1): 145–158.
9. Cathrin, E.R. **2015**. Effect some herbal remedies in Asexual and sexual hormones in female rabbits. University of adhenbarah. Ph.D. thesis. pp.220.
10. Martin H. Johnson (14 December **2012**). Essential water extraction in the plants. John Wiley & Sons. pp. 40.
11. Morreto, S.I., Deunly, R.A., and Firby, K.C. **2018**. Effect of some plant extracts on prolactin and FSH in albino rats under stress. *Amer, jour.*, **3**: 118-130.
12. Watson, S.F. **2017**. The herbal remedies in alternative medicine . 2 ed .734-758.
13. Al-hussaini, S.J. **2014**. The physiological and biochemical changes in sexual hormones by herbal remedies in female rats in Kufa. The University of Kufa.PHD.234-242.

14. Vitt, U. A.; Kloosterboer, H. J.; Rose, U. M.; Mulders, J .W.; Kiesel, P.S, Bete, S.and Nayudu, P.L.(1998)." Isoforms of human recombinant Follicle–Stimulating in vitro". *Biol Reprod* , **59**: 854-861.
15. Sumaly, B.S. **2013**. *Textbook of Physiology of glands*. 2nd ed. British, London: pp.350.
16. Horrobin, D.F. **2012**. *Prolactin and Estrogen: Physiology and Clinical Significance*. Springer Science & Business Media. pp. 13–.
17. Kreml, E.S. **2019**. Study of some medicinal plants in the pituitary gland and all hormones from its. University of Ankara. *Journal of Physiology*. 1: 85-95.
18. Carlose, A.F. **2015**. *Hormones from Pituitary and Hypothalamus under stress* ". New York: McGraw-Hill Medical Publishing Division.
19. Dunne. M.P. **2012**. " *effect of deficiency mineral and Prolactin in female rate under stress* ". University of turkey. **21**(2): 212-234.
20. Albajar, A. **2016**. Some herbal extraction by saxuolate rotary evaporator. The University of Mosul. Thesis. 137-139.
21. Albarazangi, S. **2018**. Effect of alcoholic extraction of fennel seeds in some hormones in female rats. The University of Kalar. sulimanya. Ph.D. thesis. 322-325.
22. Algubori, G. **2013**. Effect some herbal remedies in sexual hormone in female albino rate in Anbar governorate. The University of Anbar. Ph.D. thesis. 322-325.