Role of aqueous extract of *Crocus sativus* to improvement of sexual dysfunctions induced by imipramine in male mice

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Summary

The present study was carried out to shed light on the possible improvement and enhancement of functional performance of male mice sexual activities by using the aqueous extract of *Crocus sativus*. Several pharmaceutical drugs affect sexual activities and upset the sexual desire sequence that can lead to impotence or loss libido; an example of these drugs are tricyclic antidepressant such as imipramine. To achieve this goal, twenty adult albino male mice were used, which were randomly divided into equal four experimental groups. The first group was given 10 mg/kg B.W. imipramine, the second group was given 100 mg/kg B.W. aqueous extract of *Crocus sativus*, and the third group was given 100 mg/ kg B.W. *Crocus sativus* aqueous extract and 10 mg/ kg B.W. imipramine, while the fourth group served as control, which was given D.W. In all groups the doses were given orally by stomach tube at 0.1 ml/10 g. B.W. for 30 days. The recorded parameters were; latency of copulation (second), frequency of copulation (per-hour), and duration of copulation (second). At day thirty blood samples were collected to determine the hormonal levels of luteinizing and testosterone hormones and the testis were obtained for histopathological processing .The results of sexual activity measured parameters showed significant decrease ($P<0.05$) in latency of copulation and significant increase in both frequency of copulation, and duration of copulation in the groups given aqueous extract of *Crocus sativus* and that given aqueous extract of *Crocus sativus* and imipramine as compared with imipramine and control groups. While results of hormonal levels showed significant increase ($P<0.05$) in both groups of *Crocus sativus* aqueous extract and imipramine as compared with imipramine only and control groups. On bases of these results, it can be concluded that the sexual activities of male mice given imipramine tend to be improved and returned when administered with aqueous extract of *Crocus sativus*.

Keyword: Aqueous extract, *Crocus sativus*, Imipramine, Sexual activities, Mice.

Introduction

Many medical conditions and their treatments contribute to sexual dysfunction. The commonly implicated drugs include antihypertensive (1), tricyclic antidepressants, which inhibit sexual desire and orgasm (2 and 3). A variety of strategies have been tried to reverse drug-induced sexual dysfunction. Imipramine (Tofranil) is a tricyclic antidepressant which is used to treat symptoms of depression but it may cause undesired effects, such as mood or behavior changes and sexual drive decrease that may lead to impotence in males (4). The dried stigma of *Crocus sativus* (saffron) is commercially available; it is popular because of its delicate aroma and attractive colour. Saffron is used as food additive (5), it is also used in folk medicine for various purposes as aphrodisiac, antispasmodic (6), antidepressant (7), anti inflammatory (8) and for treating various human disorders such as heart and blood disorders (9). *Crocus sativus* aqueous extract consist of many compounds such as α-crocetin, a water soluble carotenoid , crocins , picrocrocin and safranal (10). However, little attention has been given to the physiological effects and sexual activity of *C. sativus* extract on reproductive system (11). Thus it was an interest to study the possible effects of aqueous extract of *Crocus sativus* to improve the sexual dysfunction induced by subacute administration of imipramine in male mice.

Materials and Methods

The powder of *Crocus sativus* (saffron) L-stigma was purchased from Novin Zeferan Co. Mashhad, Iran. To prepare *Crocus sativus* aqueous extract stigma, powder of 40 g were macerated in 1500 ml of distilled water for 72

hours. The mixture was subsequently filtered and concentrated under reduced pressure at 40°C (12). The extraction yield was 45%.

This study was carried out on 20 adult albino male mice, weighting 25-30 g. were procured from the animal house of the College of Veterinary Medicine, University of Baghdad. They were housed in suitable environmental conditions: 20-25 °C in an air conditioned room, 12 hours light: 12 hours dark cycle and provided with standard pellet diet and tap water *ad libitum*. Animals were randomly divided into four equal groups (5mice in each). First group was given 10 mg/kg B.W. imipramine (13), and served as appositive control. Second group was given *Crocus sativus* aqueous extract at a dose of 100 mg/ kg B.W. (14). Third group was given aqueous extract of *Crocus sativus* at a dose of 100 mg/ kg B.W. and then after 30 minutes imipramine was given at a dose of 10 mg/ kg B.W., while the fourth group was given D.W. and served as a negative control. All experimental groups were given orally by using stomach tube for 30 days.

To estimate the sexual activity in experimental groups, 20 female mice were introduced to the four tested groups 1 male: 1 female in estrous phase, and the sexual activities of the all males were observed directly for 3 hours. The recorded parameters were; latency of copulation (second), frequency of copulation (per-hour), and duration of copulation (second) (15). At the end of the experiment, male mice were anesthetized by diethyl ether, and blood samples were taken via cardiac puncture and isolated serum were stored in a freezer at-18°C till analyzed to determine the concentrations of luteinizing and testosterone hormones by using radio immunoassay kits (16). Manufacture of these immunoassay kits is Abbott laboratories, Abbott park II, Diagnostic product Corp., Los Angles, CA, USA. The analysis of hormones concentrations were done in clinical laboratory of Radio Active Isotope, Baghdad, Iraq.

Furthermore, the male mice were sacrificed after 30 days of experiment and testis were obtained and preserved by 10% formalin then sent to the Medical City Hospital Laboratory, Baghdad for histopatho - logy processing as described by (17). For statistical analysis, results were expressed as mean ± SE. Data were analyzed by using completed randomized design in factorial experimental (one-way) ANOVA. All the data were analyzed according to (18) and the probability of ($P<0.05$) was considered as significant differences.

Results and Discussion

The results of sexual activity parameters of the treated mice (Table, 1) show a significant ($P<0.05$) decrease in latency of copulation (second), significant increase in frequency (per-hour) and duration of copulation (second) in both groups 2 and 3 in comparison to groups 1 and 4. The levels of luteinizing and testosterone hormones (Table, 2) showed a significant ($P<0.05$) increase in both saffron aqueous extract and saffron and imipramine groups, whereas they were significant ($P<0.05$) decreased in imipramine treated group as compared to control group. The body weight changes of the treated mice were recorded (Table, 3), body weights of groups 2 and 3 were significantly ($P<0.05$) increased in greater as compared with groups 1, pretreatment and control. The testicular histological sections of saffron and imipramine treated group showed regeneration of affected seminiferous tubules and regeneration of spermatogonia lining beside the appearance of spermatids in the lumen (Fig. 1 - 4). Although many pharmaceutical agents are available for treatment of mental disorders, it is believed that many patients do not have adherence to pharmacotherapy because of their side effects (19). Side effects of the antidepressants such as sleep disturbances and sexual dysfunction represent one of the important reason for which the antidepressants are often not taken in their appropriate doses (19 and 20).

Herbal medicines are the integrative approaches to treat these disorders. The herbal medicines can also be used in combination with other treatments to create beneficial effects and to reduce their potential side effects (21). Saffron is a herbal medicine which has been widely used in traditional therapy due to its potential effect for treating a variety of diseases in human and animal studies (22).

Table, 1: Effects of *Crocus sativus* aqueous extract and imipramine on male mice sexual activity.

Groups \ Parameters	Latency of copulation (second)	Frequency of copulation (per-hour)	Duration of copulation (second)
First group: Given 10 mg/kg B.W. imipramine (positive control)	100.00 ± 20.11^{C}	0.66 ± 0.04^{d}	2.26 ± 0.15^{d}
Second group: Given 100 mg/ kg B.W. aqueous extract of *C. sativus*	9.22 ± 0.32^{a}	21.01 ± 0.49^{b}	30.20 ± 0.52^{b}
Third group: Given *C. sativus* aqueous extract 100 mg/ kg B.W. and imipramine 10 mg/ kg B.W.	10.20 ± 0.28^{a}	23.00 ± 0.46^{a}	33.20 ± 0.45^{a}
Fourth group: Given D.W. (negative control)	28.20 ± 0.89^{b}	8.00 ± 0.26^{C}	15.00 ± 0.32^{C}

-Values are presented as mean ± SE. Small letters denoted to significant difference between treated groups (P<0.05). n =5 mice /group.

Table, 2: Effects of aqueous extract of *Crocus sativus* and imipramine on luteinizing and testosterone hormones in male mice.

Groups \ Hormonal levels	Luteinizing hormone (ng/ml)	Testosterone hormone (ng/ml)
First group: Given 10 mg/kg B.W. imipramine (positive control)	8.40 ± 1.40^{C}	0.33 ± 0.01^{c}
Second group: Given 100 mg/ kg B.W. *C. sativus* aqueous extract	51.00 ± 4.01^{a}	24.33 ± 0.02^{a}
Third group: Given *C. sativus* aqueous extract 100 mg/ kg B.W. and imipramine 10 mg/ kg B.W.	52.00 ± 3.84^{a}	23.42 ± 0.10^{a}
Fourth group: Given D.W. (negative control)	22.60 ± 2.77^{b}	2.33 ± 0.04^{b}

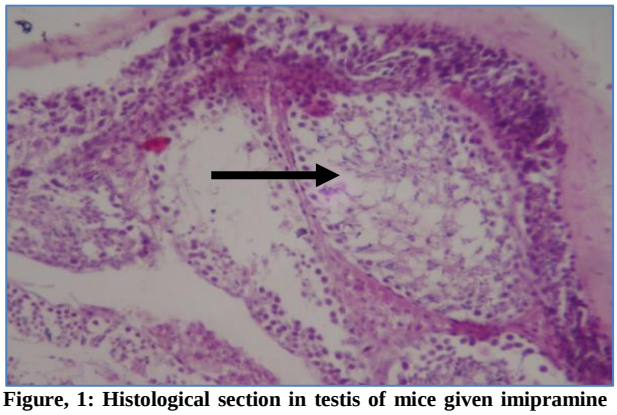
-Values are presented as mean ± SE. Small letters denoted to significant difference between treated groups (P<0. 05). n = 5 mice/ group .

Table, 3: The effects of aqueous extract of *C. sativus* and imipramine on male mice body weights.

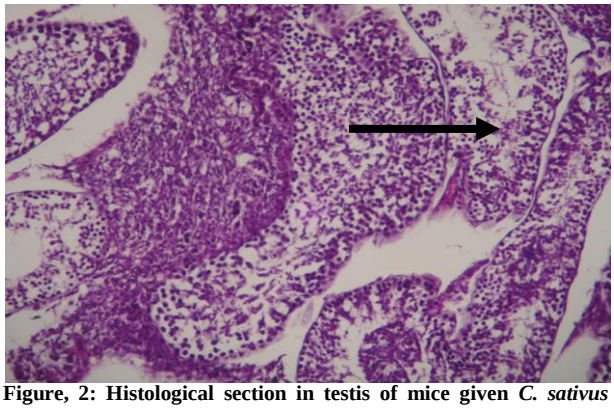
Groups	Pretreatment (Day Zero)	Body weights (g) post treatment			
		1 week	2 weeks	3 weeks	4 weeks
First group: Given 10 mg/kg B.W. imipramine (positive control)	Aa 27.00±1.37	Aa 26.42±1.20	Aa 25.40±0.82	Bab 23.20±0.60	Bb 22.77±2.81
Second group: Given 100 mg/ kg B.W. aqueous extract of *C. sativus*	Aa 27.20±1.37	Aa 27.99±1.27	Ba 29.60±1.10	Cab 31.00±0. 70	Ab 33.20±0.42
Third group: Given *C. sativus* aqueous extract 100 mg/ kg B.W. and imipramine 10 mg/ kg B.W.	Aa 26.92±1.40	Aa 27.82±1.33	ABa 28.72±1.20	Cab 30.00±0.83	Ab 32.33±0.50
Fourth group: Given D.W. (negative control)	Aa 25.60±1.70	Aa 26.00±1.27	Aa 26.60±1.20	Aa 27.00±1.07	Aa 28.25±0.57

- Values are presented as mean±SE. n= 5 mice/ group. Different capital letters denoted significant (P<0.05) difference between treated groups. Different Small latters denoted significant (P<0.05) difference within treated groups.

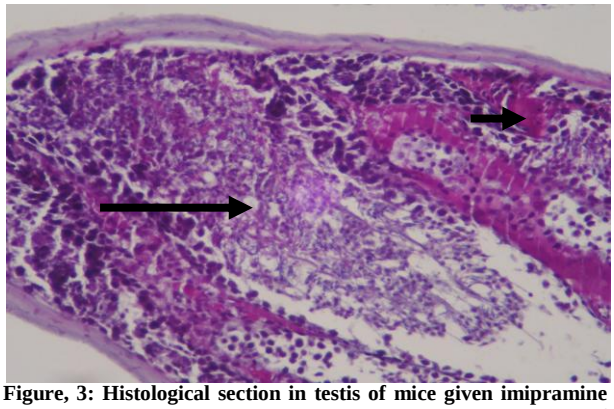
The present study revealed the ability of aqueous extract of saffron stigma to act as aphrodisiac agent. The increments of sexual activity in both saffron and saffron and imipramine treated groups might be related to the role of crocetin, the constituent of saffron, which increase the activity of endothelial nitric oxide synthase in blood vessels elevating nitric oxide production (23), thus it may act similarly to sildenafil (phosphodiesterase - 5 inhibitor) which relaxes the corpus cavernosum leading to an erection (24). In the present study, saffrons treatment at low dose 100 mg/ kg B.W. resulted in an increase in the epithelium cells thickness of the seminiferous tubules and sperm numbers, and this is an agreement with (25), which reported that consumption of saffron at low dose resulted in stimulation of testicular tissue and spermatognesis processes. The majority of changes were seen on testicular tissue histological sections following the administration of saffron extract and imipramine for 30 days may be related to the increase of testosterone levels (26-29). Saffron caused significant growth in anteriopituitary secretion cells numbers and their secretion and increases the mating and ejaculation numbers in mice (26 and 30). Also histological examination tissue of anterior pituitary cleared distinct accretion in basophil cells which are responsible for the secreted amount of luteinizing and testosterone hormones (26).



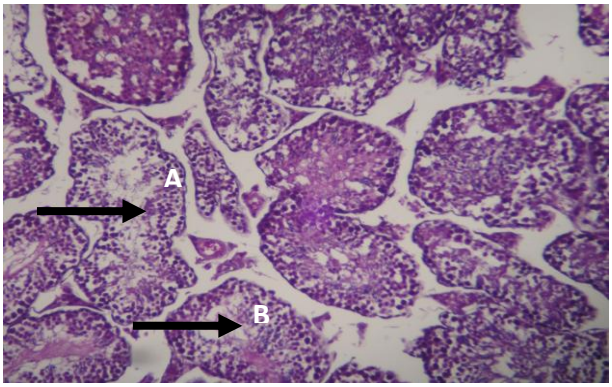
Figure, 1: Histological section in testis of mice given imipramine shows of unarrangement of seminiferous tubule with sloughing of its epithelial lining cells and loss of spermatid (H and Ex40).



Figure, 2: Histological section in testis of mice given *C. sativus* aqueous extract shows regeneration of seminiferous tubules and spermatogonia lining (H and E X40).



Figure, 3: Histological section in testis of mice given imipramine and *C. sativus* aqueous extract shows odema and regeneration of affected seminiferous tubules with appearance of spermatid in the lumen (H and E X40).



Figure, 4: Histological section in testis of mice given D.W. shows normal seminiferous tubules with appearance of spermatid (A) and sperms (B) in the lumen (H and E X40).

In the present study the data showed significant ($P<0.05$) increase in body weight of the group given saffron and imipramine as compared to pretreatment, imipramine and control treated groups, this may be due to the effect of saffron which help digestion and increase appetite. As related to amplify synaptic serotonin which increase food intake as the selective serotonin reuptake inhibitors such as (fluoxetine) do (11). It can be concluded that the male mice given imipramine tend to be less sexual activity, which can be improved and returned as near as possible to the normal situation by the concomitant administration imipramine with saffron.

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دور المستخلص المائي للزعفران لتحسين النقص الوظيفي الجنسي المستحدث بالامبرامين في ذكور الفئران

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الخلاصة

أجريت الدراسة الحالية لتسليط الضوء على إمكانية تحسين الفعاليات الجنسية وتسريعها لذكور الفئران باستعمال المستخلص المائي للزعفران. العديد من المستحضرات الصيدلانية تؤثر في الفعاليات الجنسية وتقلل الرغبة الجنسية نتيجة للعجز الجنسي او فقدان الشهوة الجنسية، كالأدوية المضادة للكآبة الثلاثية الحلقات مثل (الامبرامين). للوصول لهذا الهدف استعمل عشرون فأراً ذكراً حيث قسمت عشوائياً لأربع مجاميع متساوية تجريبية. المجموعة الأولى أعطيت امبرامين جرعة 10 ملغم/ كغم من وزن الجسم، المجموعة الثانية أعطيت المستخلص المائي للزعفران بجرعة 100 ملغم/ كغم من وزن الجسم، المجموعة الثالثة أعطيت المستخلص المائي للزعفران 100 ملغم/ كغم من وزن الجسم والامبرامين 10 ملغم/ كغم من وزن الجسم، بينما المجموعة الرابعة استعملت كسيطرة حيث أعطيت الماء المقطر. أعطيت الجرع لكل هذة المجاميع عن طريق الفم باستعمال الأنبوب المعدي بمقدار 0.1 مل /10غرام من وزن الجسم لمدة 30 يوم. المعايير التي سجلت: كمون التزاوج (ثانية) وهو الوقت منذ ادخال الانثى لحين حصول التزاوج، تكرر التزاوج (عدد مرات التزاوج - ساعة) ومدة التزاوج (ثانية). بعد 30 يوما جمعت عينات الدم لتحديد مستويات الهرمون اللوتيني والهرمون الذكري واخذت الخصية لغرض الفحص النسجي. أظهرت نتائج اختبارات معايير الفعاليات الجنسية نقصاناً معنوياً على مستوى ($P<0.05$) في كمون التزاوج (ثانية)، زيادة في تكرر التزاوج وزيادة في مدة التزاوج في كلا مجموعتي المستخلص المائي للزعفران والمستخلص المائي للزعفران والامبرامين بالمقارنة مع مجموعتي الامبرامين والسيطرة، في حين اظهرت نتائج المستويات الهرمونية زيادة معنوية على مستوى ($P<0.05$) الهرمون اللوتيني والهرمون الذكري في كلا مجموعتي المستخلص المائي للزعفران والمستخلص المائي للزعفران والامبرامين مقارنة بمجموعتي الامبرامين لوحدة والسيطرة. على ضوء النتائج لهذه الدراسة نستنتج أنّ الفعاليات الجنسية لذكور الفئران التي أعطيت الامبرامين تميل إلى التحسن والعودة الى الحالة الطبيعية عند اعطائه مع المستخلص المائي للزعفران.

الكلمات المفتاحية: مستخلص مائي، زعفران، امبرامين، فعاليات جنسية، فئران.