Iraqi Journal of Science, 2024, Vol. 65, No.4, pp: 1870-1876
DOI: 10.24996/ijs.2024.65.4.7

ISSN: 0067-2904

Effect of Anise Seeds (*Pimpinella anisum*) Oil Extract on Some Fertility Parameters in Albino Male Mice

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Received: 29/8/2022 Accepted: 25/4/2023 Published: 30/4/2024

Abstract

Anise is an aromatic plant which is used in traditional medicine and pharmaceutical industry. The main components of anise seed are anise alcohol, p-anisaldehyde, estragol, acetophenone, limonene and pinene, while the volatile oil found in anise is anethole. The antioxidant, antimicrobial, anticonvulsant and analgesic properties of anise seeds and oil have been confirmed by various studies. The current study was designed to study the effects of oral administration of anise oil on the reproductive capacity of adult male mice by examining certain physiological parameters such as measuring the serum testosterone level, counting the number of sperm, sperm motility and abnormal sperm morphology. A total of 36 male mice were used and randomly divided into three equal groups. Group1 received corn oil (control), T1 received anise oil in a dose of 0.043 gm/kg B.wt , and T2 received anise oil in a dose of 0.086 gm/kg B.Wt orally. The above mentioned parameters were examined at the end of three weeks of administration for six animals of each group. As well as, the examinations were repeated at the end of six weeks of administration for the rest of the animals. The results exhibited that treatment with anise oil led to a significant increase in the level of testosterone, sperm count and motility. Furthermore, abnormal morphology of the sperms decreased significantly. In conclusion, it can be said that the administered doses of anise oil could have led to improve the fertility in male mice.

Keywords: Anise oil, sperm count, testosterone, fertility, traditional medicine.

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تأثير مستخلص زيت بذور اليانسون (*Pimpinella anisum*) في بعض معايير الخصوبة في ذكور الفئران البيض

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الخلاصة:

اليانسون نبات عطري يستخدم منذ فترة طويلة فى الطب التقليدي والصناعة الدوائية. المكونات الرئيسية لبذور اليانسون هي كحول اليانسون ، أنيسالديهايد ، استراغول ، أسيتوفينون ، ليمونين و بينين ، بينما الزيت المتطاير الموجود في اليانسون هو الأنثول. تم تأكيد الخصائص المضادة للأكسدة ومضادات الميكروبات ومضادات الاختلاج والمسكنات لبذور وزيت اليانسون من خلال العديد من الدراسات. صممت هذه الدراسة لدراسة تأثير تناول زيت اليانسون عن طريق الفم على القدرة التناسلية لذكور الفئران البالغة من خلال فحص بعض المعايير الفسيولوجية مثل قياس تركيز هرمون التستوستيرون في الدم ، حساب عدد الحيوانات المنوية ،حركة الحيوانات المنوية والمظهر الخارجي غير الطبيعي للحيوانات المنوية. تم استخدام 36 من ذكور الفئران وقسمت عشوائيا إلى ثلاث مجموعات متساوية ،مجموعة عوملت بزيت الذرة (السيطرة)، مجموعة T1 و T2 تلقتا زيت الينسون بجرعة 0.043 و 0.086 :T2 غم / كغم وزن الحيوان فمويا، على التوالي. تم فحص المعلمات المذكورة في نهاية ثلاثة أسابيع من من التجريع لستة حيوانات من كل مجموعة ، وأعيدت الفحوصات في نهاية ستة أسابيع العلاج لبقية الحيوانات. أظهرت النتائج أن العلاج بزيت اليانسون أدى إلى زيادة معنوية في مستوى هرمون التستوستيرون وعدد الحيوانات المنوية وحركتها. علاوة على ذلك ، انخفض المظهر غير الطبيعي للحيوانات المنوية بشكل ملحوظ . وفي الختام ، ان الجرعات المعطاة من زيت اليانسون ادت ربما الى تحسن في خصوبة ذكور الفئران. يمكن ان يعزى ذلك الى تأثير زيت اليانسون عن طريق التنظيم الهرموني عبر محورماتحت المهاد – الغدة النخامية – الخصية.

1. Introduction

Herbs have been used as therapy and in other medicinal uses for thousands of years. Herbal medicines are complex mixtures of about 50 chemical constituents [1, 2].

Anise is an aromatic plant which has a long tradition of being used in conventional medicine and pharmaceutical industry. The main components of anise seed are anise alcohol, p-anisaldehyde, estragol, acetophenone, limonene, and pinene. Whereas the volatile oil found in anise is known as anethole. Different studies indicate that *Eruca sativa* and anise seeds and oil have antioxidant, antimicrobial, anticonvulsant, analgesic properties [3]

It has been shown that *Pimpinella anisum* have many valuable effects on health. These effects are due to its ingredients, namely essential fatty oils, protein, choline and sugar. It also contains anethole, one of the main constituents of essential oil and anisaldehyde, anisic acid and estragole [4]. *Pimpinella anisum* has been used for millennia as estrogenic agent and has been specifically supposed to enhance milk secretion, encourage menstruation and facilitate

the process of birth [5]. In addition, Özcan and Chalchat in 2006 confirmed that *Pimpinella anisum* oil has antifungal effects [6]. Other characteristics such as antioxidant and antimicrobial and other different solvent extracts (ethanol and water) from *Pimpinella anisum* were also confirmed [7]. Essential oil residuals of the *Pimpinella anisum* have a high inhibitory effect on the pathogenic bacteria, including *Enterococcus faecalis, Salmonella typhi, Micrococcus Iuteus, Staphylococcus aureus* and *Escherichia coli* [8]. Extracts from the plant are mainly taken from the seeds and its aerial parts [9]. In addition, Bekra *et al*., [10] concluded that oral treatment with anise aqueous extract is effective in falling the level of some biochemical parameters that are related to neurobehavior in rats that were exposed and intoxicated by minerals such as lead. Review of reports of folk medicine explains that anise seeds are used for different conditions, for example, in migraine used as pain-relieving agents, as carminative, aromatic, disinfectant, diuretic, lessen the symptoms of the male climacteric and surges libido [11]. However, there is insufficient information on the effects of *Pimpinella anisum* oil in male mouse reproductive function. Therefore, this study was conducted to show the effects of anise oil on certain physiological parameters related to the male mouse fertility.

2. Materials and Methods

1- Preparation of anise oil.

The seeds of star anise, purchased from the local market and certified at the Iraqi National Herbarium, were cleaned and ground in a grinder and pressed by mechanical, hydraulic press without heating under pressure of 400 Bar. The yield was 20 ml of anise oil from 2 Kg of anise.

2- Animals used

The current research was carried out at the College of Veterinary Medicine, University of Baghdad with cooperation of Sulaimani University in 2019. A total of 36 healthy adult male mice were obtained from the Animal House of the Biotechnology Research Center/Al-Nahrain University. The age of these mice was between 8 to 10 weeks, and each animal weighed around 25 to 35 grams. The mice were reserved under appropriate environmental conditions of 20-25°C in a photoperiod room for 12 hours per day. They were also preserved for two weeks for adaptation before the commencement of the examination. Tap water and pellet were given to animals.

3- Experimental design

The mice were divided into 3 equal groups indiscriminately; each group had 12 mice and was handled as follows:

- Group (1) (T1): mice received anise oil orally 0.043 gm/kg B.wt; this volume of the oil was diluted in corn oil and given at 0.1 ml/ animal daily [12].
- Group (2) (T2): mice received anise oil orally 0.086 gm/kg B.wt; this volume of oil was diluted in corn oil and given at 0.1 ml/ animal daily [12].
- Group (3) (C): mice served as the control under similar conditions and administered 0.1 ml corn oil orally/ animal daily.

At the end of three weeks treatment, half number of the animals from each group (6 animals) were sacrificed. The second half of the animals (6 animals) were sacrificed at the end of 6 weeks treatment. Tissue and blood samples were taken for analysis.

4- Blood sample collection and serum testosterone measurement

After three and six weeks of treatment, animals were anesthetized by diethyl ether. Using disposable insulin syringes, the process of cardiac puncture was used to obtain about 1 ml of the blood samples . The samples were centrifuged at 2500 rpm for 15 minutes before being

stored in a freezer at -18°C till use. The hormone testosterone was measured (ng/ml) by Radioimmunoassay (RIA).

5- Tissue sample analysis

Mice were subjected to cervical dislocationand then the epididymis of the left side were reserved and embedded in one ml of normal saline at 37°C. The tails were then cut into small pieces by surgical scissors to perform the following microscopical inspection on some characters of sperm.

a- The sperm count
b- The percentage of the motility of sperm.
c- Abnormal sperm morphology (%) [13].

6- Statistical analysis

Data is shown as the Mean± SE. Two-way analysis of variance (ANOVA) was used to analyze the data. Group differences were considered significant at a probability level of $p<0.05$.

3. Results

The results in present study show that three weeks aniseed oil treatment produced a significant ($p< 0.05$) increase ($P<0.05$) in testosterone level of the T2 group but not T1 group (Table 1). However, at six week period, both doses (T1 and T2) of the anise oil caused a significant ($P<0.05$) increase in the testosterone level as compared to the control. At three-week period, the hormone level increased significantly ($P<0.05$) in the T2 compared to T1 group. Whereas, at six-week period, statistically there were no significant differences between the two dose levels of the anise oil (Table 1).

Table 1: Effects of different oral doses of anise seeds oil on the level of testosterone (ng/ml) in the serum of male mice.

DDose of Anise (gm/kg B.Wt)	Number Replication (n)	Testosterone Level (ng/ml) Mean ±SE	
		3 Weeks Treatment	6 Weeks Treatment
Control (zero)	6	0.83±0.06 Aa	0.74±0.07 Aa
T1 (0.043)	6	1.51±0.19 Ba	4.22±0.39 Cb
T2 (0.086)	6	3.97±0.54 Db	4.02±0.50 Db

- Capital letters represent significant differences among groups (time dependent) ($P<0.05$).
- Small letters indicate significant differences among groups (dose dependent) ($P<0.05$).

The results in Table 2 represent that the number of sperm (10^6 /ml) increased significantly ($P<0.05$) after 3 weeks treatment in the T2 group, However, after 6 weeks treatment, both T1 and T2 groups showed a significant ($P<0.05$) increase in the sperms number as compared to the control. (Table 2).

Table 2: Effects of oral doses of anise seed oil on the number of sperm (10^6/ml) in male mice.

Dose of Anise (gm/kg B.Wt)	Number Replication (n)	Sperm Number (10^6/ml) Mean ±SE	
		3 Weeks Treatment	6 Weeks Treatment
Control (zero)	6	18.76±0.94 Aa	15.60±1.00 Aa
T1 (0.043)	6	20.67±1.39 Ba	26.10±1.72 Cb
T2 (0.086)	6	24.93±1.29 Db	24.50±2.13 Db

- Capital letters represent significant differences between groups (time dependent) (P<0.05).
- Small letters indicate significant differences between groups (dose dependent) (P<0.05).

The percentage of sperm motility increased significantly after 3 weeks treatment in the T2 group,. However, after 6 weeks treatment, both T1 and T2 groups showed a significant (P<0.05) increase in the percentage of the sperm motility as compared to the control (Table 3).

Table 3: Effects of different oral doses of anise oil on the percentage (%) of sperm motility in male mice.

Dose of Anise (gm/kg B.Wt)	Number Replication (n)	The percentage of sperm motility Mean ±SE	
		3 Weeks Treatment	6 Weeks Treatment
Control (zero)	6	76.67±3.57 Aa	75.83±3.27 Aa
T1 (0.043)	6	83.33±3.33 Ba	87.50±1.70 Cb
T2 (0.086)	6	84.17±2.38 Db	89.17±2.00 Eb

- Capital letters symbolize significant differences between groups (time dependent) (P<0.05).
- Small letters designate significant differences between groups (dose dependent) (P<0.05).

Table 4 shows that the percentage (%) of abnormal sperm morphology decreased significantly (P<0.05) after 3 weeks treatment in the T2 group, but not in T1 as compared to the control group. However, after 6 weeks treatment, both T1 and T2 groups showed a significant (P<0.05) decrease in the percentage of abnormal sperm morphology as compared to the control.

Table 4: Effects of different oral doses of anise oil on the percentage (%) of sperm motility in male mice.

Dose of Anise (gm/kg B.Wt)	Number Replication (n)	The percentage of abnormal sperm morphology (Mean ±SE)	
		3 Weeks Treatment	6 Weeks Treatment
Control (zero)	6	23.33±1.13 Aa	26.33±1.30 Aa
T1 (0.043)	6	19.92±0.91 Bb	14.75±1.17 Cb
T2 (0.086)	6	19.17±0.88 Db	15.58±2.17 Eb

- Capital letters mean substantial differences between groups (time dependent) (P<0.05).
- Small letters indicate major differences between groups (dose dependent) (P<0.05).

4. Discussion

The current study was done to assess the beneficial effects of anise oil on testosterone level and certain sperm factors such as sperm counting, sperm motility and abnormal sperm morphology in male mice. The animals used in this study showed normal activities and viabilities during the study period, thus indicating natural, healthy state and the safety of the anise oil administration at 0.043, 0.086 gm/kg B.wt. doses at each of three and six weeks. Anise oil treatment led to a significant increase of testosterone level in serum. This augmentation of testosterone level might be due to the effects of anise oil on one or certain enzymes that participate in testosterone synthesis [14]. In another study, it has been shown that anise prevented the estrogen deficiency in rats [15]. It is known that testosterone, produced in the Leydig's cells from cholesterol, is also formed from androstenedione which is secreted by the adrenal gland. The enzyme 17-beta-hydroxysteroid dehydrogenases are

responsible for the conversion of androstenedione to testosterone [14]. Testosterone is produced by Leydig cells under the effect of two hormones, pituitary-secreted luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [16]. Therefore, the increased testosterone concentration produced by anise oil treatment could be explained either by stimulation of the enzymatic pathways of its production in the testes or adrenal cortex. Or another factor, this might be due to the stimulation of LH and FSH release, which in turn resulted in increased testosterone synthesis and release.

The data of this study also exhibited the statistically significant increase in sperm level (Table 2). The cause of this raise could be due to the effects of the high level of testosterone. According to Saito *et al.* (the process of spermiation is very sensitive to the hormone suppression with testosterone and FSH. After a week of combined FSH and testosterone removal, fifty percent of the spermatids in the testis were unsuccessful to be released in adult rats [17]. Moreover, this study explained the effects of the anise oil on significantly increased sperm motility and significantly decreased abnormal sperm morphology. These effects of the anise oil could be due to the antioxidant properties of the anise [18, 19]. Another study explained that testosterone level increases due to antioxidant activity of *Nigella sativa* plant oil, thus increasing number of sperm motility in rats [20]. In addition, another study found improvement of sperm motility, count, morphology and testosterone level in infertile men [21]. This is because sperm cell membrane mainly consists of polyunsaturated fatty acids and phospholipids which are highly vulnerable to be damaged by oxidation. Sperm production is under the control concentrations of reactive oxygen species, such as nitric oxide, the superoxide anion and hydrogen peroxide which are necessary for conception. However, higher levels of these free radicals can damage sperm cells directly [13]. There are many other plants that affect male fertility, for example, one study found that the administration of ethanol extract of *Cistanche tubulosa* (0.4 and 0.8 g/kg) increased sperm count (2.3 and 2.7 folds) and sperm motility (1.3 and 1.4 folds) and decreased the abnormal sperm (0.76 and 0.6 folds) respectively. In addition, a separate study established that significant increase was found in epididymal sperm density, percent of morphologically normal sperm and serum testosterone levels in rats that received 200 and 400 mg/kg/day of alcoholic extract of *Fumaria parviflora* [22].

5. Conclusion

This study concluded that the administered doses of anise oil led to marked improvement of some parameters of fertility in male of mice. This was accounted for by the effects of anise oil on spermatogenesis, probably due to hormonal regulation across hypothalamic-pituitary-testicular axis.

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